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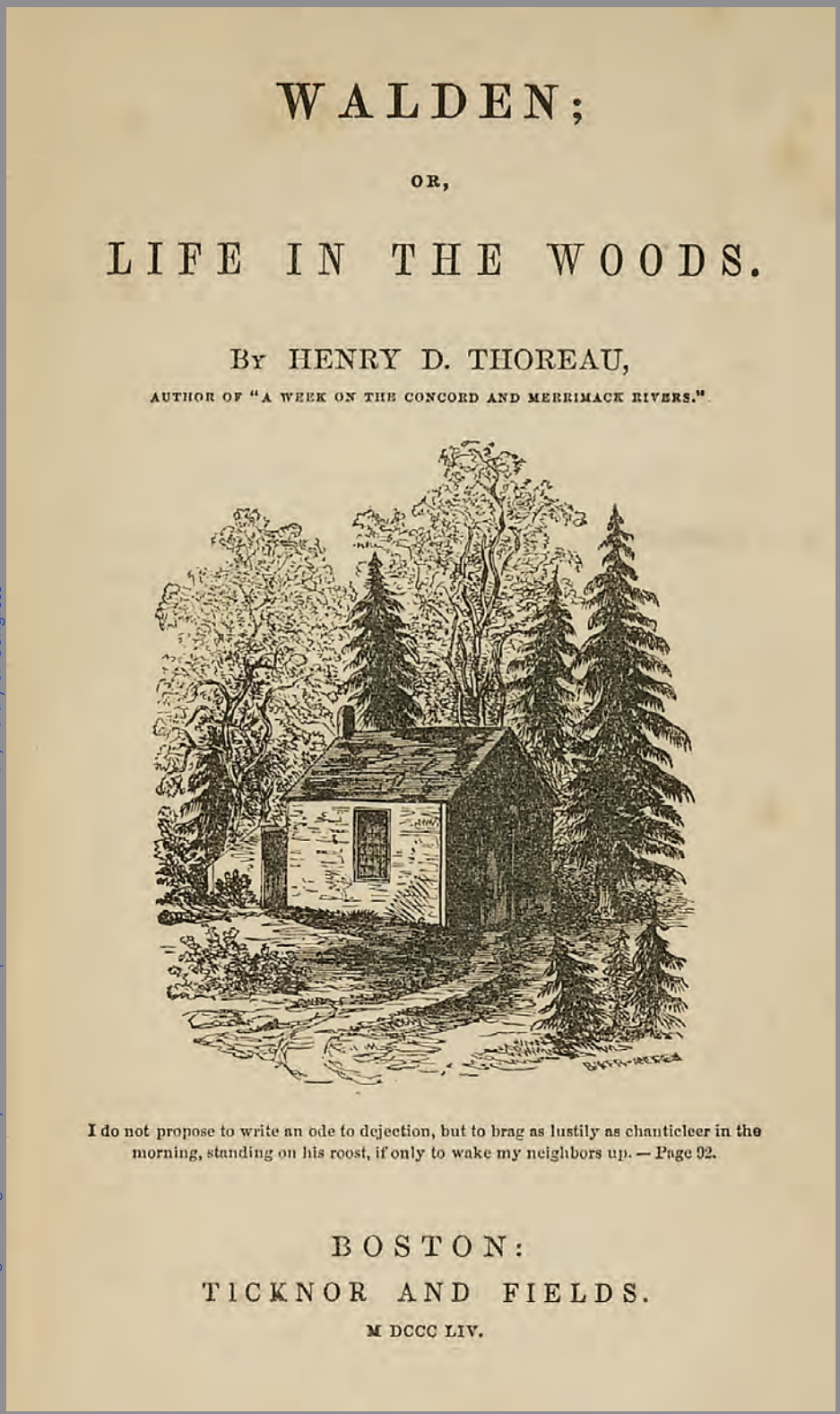
Bacterial Pathogens

November 2016



WALDEN;

OR,

LIFE IN THE WOODS.

By HENRY D. THOREAU,

AUTHOR OF "A WEEK ON THE CONCORD AND MERRIMACK RIVERS."

I do not propose to write an ode to dejection, but to brag as lustily as chanticleer in the morning, standing on his roost, if only to wake my neighbors up. — Page 92.

BOSTON:

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Sophia Thoreau (1819–1876) Title page illustration for Walden; or, Life in the Woods (1854) Wood engraving. 7 1/16 in x 4 1/2 in/17.94 cm x 11.43 cm. Public domain digital image courtesy Rare Book and Special Collections Division, Library of Congress

EMERGING INFECTIOUS DISEASES

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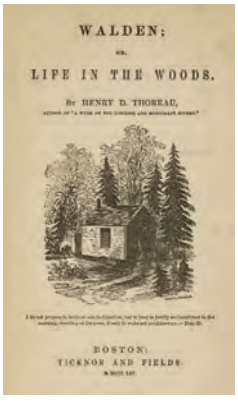

On the Cover

Sophia Thoreau (1819–1876)

Title page illustration for *Walden; or, Life in the Woods* (1854)

Wood engraving. 7 1/16 in x 4 1/2 in/17.94 cm x 11.43 cm. Public domain digital image courtesy Rare Book and Special Collections Division, Library of Congress

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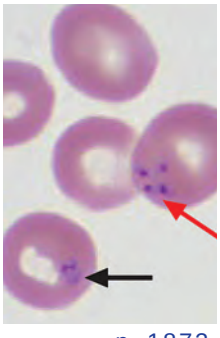

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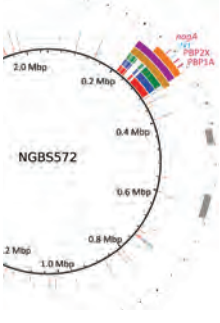

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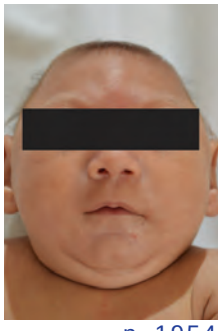

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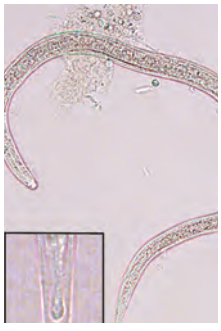

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About the Cover

Transmission of *Babesia microti* Parasites by Solid Organ Transplantation

Meghan B. Brennan, Barbara L. Herwaldt, James J. Kazmierczak, John W. Weiss, Christina L. Klein, Catherine P. Leith, Rong He, Matthew J. Oberley, Laura Tonnetti, Patricia P. Wilkins, Gregory M. Gauthier

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Release date: October 12, 2016; Expiration date: October 12, 2017

Learning Objectives

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

- Identify the presentation and likely mode of transmission of babesiosis after organ transplantation, based on 2 case reports
- Determine treatment of babesiosis in patients receiving organ transplantation
- Assess the clinical implications of findings from these case reports of babesiosis.

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Babesia microti, an intraerythrocytic parasite, is tickborne in nature. In contrast to transmission by blood transfusion, which has been well documented, transmission associated with solid organ transplantation has not been reported. We describe parasitologically confirmed cases of babesiosis diagnosed ≈8 weeks posttransplantation in 2 recipients of renal allografts from an organ donor who was multiply transfused on the day he died from traumatic injuries. The organ donor and recipients had no identified risk factors for tickborne infection. Antibodies against *B. microti* parasites were not detected by serologic testing of archived pretransplant specimens. However, 1 of the organ donor's blood donors was seropositive when tested postdonation and had risk factors for tick exposure. The organ donor probably served as a conduit of *Babesia* parasites from the seropositive blood donor to both kidney recipients. Babesiosis should be included in the differential diagnosis of unexplained fever and hemolytic anemia after blood transfusion or organ transplantation.

Babesia microti, an intraerythrocytic parasite, is the most common cause of human babesiosis in the United States and is endemic to the Northeast and upper Midwest regions, including parts of Wisconsin and Minnesota (*1*–*4*). *B. microti* infection can range from asymptomatic to severe. Common manifestations include hemolytic anemia and nonspecific influenza-like symptoms (*2*). Persons who are asplenic, elderly, or immunocompromised are at increased risk for symptomatic infection and for severe complications, such as multiorgan dysfunction and death (*5*).

The primary route of transmission of *B. microti* parasites is by the bite of an infected *Ixodes scapularis* tick (*6*). Transmission of *Babesia* parasites by blood transfusion also is well documented (*7*–*11*). In contrast, transmission associated with solid organ transplantation has not been reported. We investigated 2 cases of babesiosis for which transmission probably occurred when renal allografts were transplanted from a multiply transfused organ donor.

Materials and Methods

Diagnosis of babesiosis in 2 persons who received kidney transplants from the same donor prompted multifaceted, collaborative investigations, which were conducted by the authors and acknowledged persons and agencies (e.g., transplant, transfusion, and public health organizations). The Organ Procurement Organization (Madison, WI, USA) identified the disposition of all organs and tissues recovered from the organ donor and notified the United Network for Organ Sharing (Richmond, VA, USA) about the possibility of donor-derived transmission.

Only kidneys and corneas had been transplanted; the bilateral iliac arteries and veins had been recovered but discarded 14 days later, whereas the liver and other tissues that had been donated for research were embargoed. Medical and transfusion records of the organ donor and transplant recipients were reviewed, as were procedures and records for organ/tissue recovery, handling, and transplantation. The transplant recipients, the seropositive blood donor identified in the transfusion investigation, and surrogates for the organ donor were interviewed regarding risk factors for and potential clinical manifestations of *Babesia* infection.

Specimens from the transplant recipients, the organ donor, and the organ donor's blood donors were tested for evidence of *Babesia* infection. Evaluations of the transplant recipients included light microscopy of Giemsa- or Wright-stained thick and thin blood smears for *Babesia* parasites. The Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) conducted reference diagnostic testing of specimens from transplant recipients and organ donor. CDC also conducted serologic testing by using an indirect fluorescent antibody (IFA) assay for total immunoglobulin against *B. microti* antigens (*12*). Serum and plasma specimens were tested in serial 4-fold dilutions, and a reciprocal dilution titer of 64 was considered positive. CDC conducted PCR analysis of whole-blood specimens from the transplant recipients by using primers specific for the *B. microti* 18S rRNA gene (*13*) and a previously described 2-step nested PCR (*7*). CDC also conducted *B. microti* PCR analysis of fresh-frozen hepatic tissue from the organ donor. No fresh-frozen renal tissue or whole-blood specimens from the organ donor were available for testing. However, paraffin-embedded, pretransplantation specimens from both kidneys were available and were tested by using a *B. microti* immunohistochemical (IHC) assay (*14*); CDC also conducted IHC testing of hepatic tissue.

The American Red Cross obtained blood/serum specimens from all 33 blood donors who had contributed components transfused into the organ donor. No segments or components from original donor units were available for testing. The American Red Cross tested postdonation specimens by using a *B. microti* IFA assay for IgG and a *B. microti* real-time PCR. IFA testing was conducted with serial 2-fold dilutions of samples.

Case Reports

Renal Transplant Recipients

In late August 2008, two men with end-stage diabetic nephropathy (a 65-year-old Wisconsin resident [patient A; the index case-patient] and a 41-year-old Iowa resident [patient B]) received renal allografts from the same deceased donor at the University of Wisconsin Hospital and Clinics (UWHC; Madison, WI, USA). Different surgeons in separate operating rooms transplanted the kidneys. Both patients received induction immunosuppressive therapy with basiliximab and maintenance therapy with prednisone, mycophenolate mofetil, and tacrolimus.

During the previous year and peritransplant period, neither patient lived or traveled in babesiosis-endemic regions, which in the Midwest, included parts of Minnesota and Wisconsin but not Iowa (Table), and they did not receive blood transfusions.

Both patients showed seroconversion and development of parasitologically confirmed cases of babesiosis, which were diagnosed ≈8 weeks posttransplantation (Table; Figure 1). At the request of the transplant physicians, both patients were evaluated by the same UWHC infectious disease specialists.

After babesiosis was diagnosed, doses of immunosuppressive medications were decreased and each patient received a 6-week course of oral antimicrobial drug therapy: atovaquone (750 mg, 2×/d for 6 wks) plus azithromycin (1,000 mg, 1×/d for 2 wks, followed by 600 mg, 1×/d for 4 wks). During therapy, symptoms resolved, laboratory parameters returned to reference ranges or values, and *Babesia* parasite DNA became undetectable (Table; Figure 1).

Patient A

On October 2, 2008 (≈5 weeks posttransplantation), during a routine follow-up appointment at the UWHC Transplant Clinic, the wife of patient A mentioned that he had a lack of energy and decreased appetite (onset date not specified). At that clinic visit, his hematocrit was 37%, which approximated his baseline value posttransplantation.

On October 8, he was admitted to the UWHC, as planned, to have his peritoneal dialysis catheter removed the next day. However, at admission, he unexpectedly was found to have a temperature of 39.4°C. His hematocrit values were 33% and 28% on October 8 and 9, respectively. Removal of the catheter was postponed until October 10, and he was discharged after the procedure. Cultures of the

Table. Characteristics of 2 patients who received renal allografts from the same organ donor and became infected with *Babesia microti* parasites, 2008*

Characteristic	Patient A (index case-patient)	Patient B
Type of kidney transplant	Left	Right
Age, y/sex	65/M	41/M
Residence†	Southcentral Wisconsin (urban, nonwooded area of Sauk County)	Iowa (semirural area bordering southwestern Wisconsin)
Cause of end-stage nephropathy	Type 2 diabetes mellitus	Type 1 diabetes mellitus
Pretransplant dialysis	Peritoneal dialysis in Wisconsin	Hemodialysis in Iowa
Other medical history	Diabetic retinopathy; coronary artery disease	Diabetic retinopathy (legally blind); hypertension
Duration of hospitalization for renal transplantation, d‡	6 (late Aug–early Sep)	10 (late Aug–early Sep; patient had moderate delay in graft function)
Clinical manifestations potentially attributable to babesiosis	Fever (39.4°C), sweats, fatigue, anorexia, dark urine	Fever (38°C), fatigue, abdominal pain
Babesia blood-smear examination		
Date of first positive blood smear	Oct 20	Oct 23
Initial parasitemia level, %	8	1
Context for diagnosis	Platelet clumping prompted manual (nonautomated) review of blood smear	Diagnosis of case in patient A prompted evaluation of patient B during a routine clinic visit
Date of last positive blood smear	Oct 24	Oct 23
Date of last *B. microti* PCR-positive blood specimen§	Nov 7	Nov 21
B. microti IFA titer (date)		
Pretransplant serum sample	≤8 (Jul 30)	≤8 (Aug 11)
Posttransplant serum sample	4,096 (Oct 21)	1,024 (Oct 23)
Laboratory values when babesiosis was diagnosed (2, 6, and 16 wks after initiation of therapy)¶		
Hematocrit, %#	21 (21, 45, 49)	35 (41, 37, 44)
Reticulocyte, %	11.7	4.3
Leukocyte count, x 10^9/L**	6.7	5.5
Platelet count, x 10^9/L	157	154
Haptoglobin, mg/dL	<8 (24, 67, 104)	ND (154, 223, 202)
Lactate dehydrogenase, U/L	747 (490, 220, ND)	495 (365, 344, 331)
Creatinine, mg/dL	1.1	1.3
Dates of hospitalization for babesiosis	Oct 20–24	None
Dates of 6-wk course of azithromycin and atovaquone	Oct 20–Dec 1	Oct 23–Dec 4

*IFA, indirect fluorescent antibody; ND, not done.
†Neither patient had lived or traveled in babesiosis-endemic areas in Wisconsin (primarily, the northwestern and northcentral regions) or elsewhere.
‡Preparation of kidneys for transplantation included an in situ flush (initiated 25 min after the donor was declared brain dead and was extubated) with 2 L of University of Wisconsin solution (*15*), each of which was infused in ≤4 min; a flush with 200 mL of this solution after the kidneys were explanted; and continuous circulation with kidney perfusate solution until the kidneys were transplanted.
§Both patients had negative PCR results for followup blood specimens in February 2009.
¶Reference ranges: creatinine, 0.6–1.3 mg/dL; haptoglobin, 30–200 mg/dL; lactate dehydrogenase, 90–200 U/L.
#Hematocrit values posttransplantation were 37% (patient A) and 40% (patient B).
**Differential leukocyte counts were 73% neutrophils, 14% lymphocytes, and 13% monocytes for patient A; and 79% neutrophils, 12% lymphocytes, 8% monocytes, 1% eosinophils, and 1% basophils for patient B.

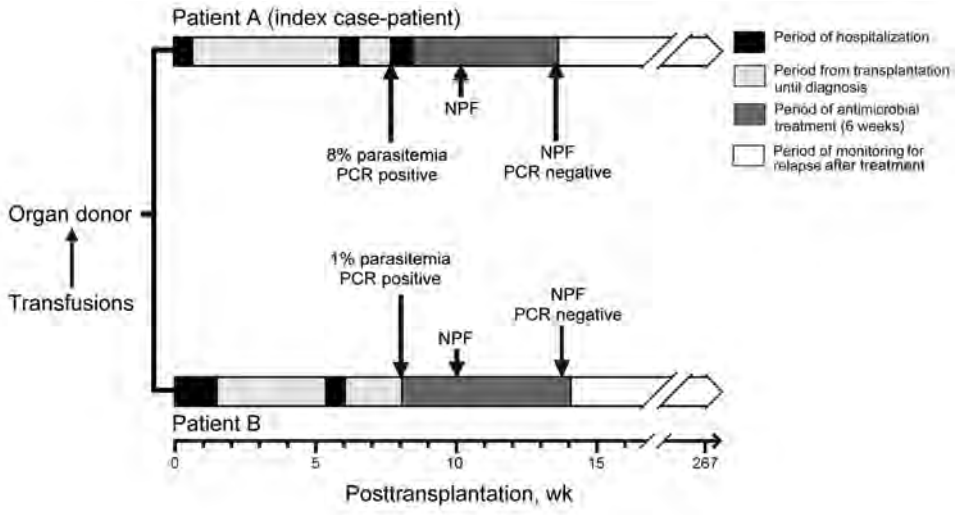

Figure 1. Timelines showing key clinical and laboratory events for 2 renal transplant recipients (patients A and B) infected with *Babesia microti* parasites, Wisconsin, USA, 2008. Trauma, transfusions, death, and organ procurement for the organ donor all occurred on the same day in late August 2008. NPF, no parasites were found by examination of thick and thin blood smears.

catheter tip, blood, and urine specimens were negative for bacterial growth. He was treated empirically with piperacillin/tazobactam during his 2-day hospitalization, followed by a 7-day outpatient course of ciprofloxacin.

On October 16 (day 6 of ciprofloxacin therapy), his wife called the transplant coordinator to report that he had a low-grade temperature (37.5°C) and a 2-day history of drenching sweats. An appointment in the Transplant Clinic was scheduled for October 20 to evaluate his symptoms. During the appointment, he reported a several-day history of darkening urine and progressive fatigue since his previous hospitalization. Per routine for clinic visits, a complete blood count was determined. His hematocrit had decreased to 21% (Table). Because platelet clumping was detected by using an automated hematology analyzer, a blood smear was reviewed manually: intraerythrocytic *Babesia* parasites were visualized at a parasitemia level of 8% (Table; Figure 2). On the same day (October 20), he was admitted to the UWHC, evaluated by the Infectious Disease Service, and began treatment with azithromycin plus atovaquone (Table; Figure 1). Within 48 hours of initiating therapy, his appetite and exercise tolerance increased, and his parasitemia level decreased to <5%.

Patient B

During October 4–9, patient B was hospitalized in Iowa for evaluation of epigastric discomfort, dyspepsia, nausea, and low-grade fever of unclear etiology. His hemoglobin level was 12.1 g/dL. Computed tomography (CT) imaging of his abdomen and pelvis was unremarkable except for enlargement of the pancreatic head (amylase and lipase values were within reference ranges). While hospitalized, he was treated empirically with metronidazole and levofloxacin; a 7-day outpatient course of ciprofloxacin therapy was prescribed.

On October 23, during a routine follow-up appointment in the UWHC Transplant Clinic, he was afebrile but, on prompting, recalled a transient fever (38°C) ≈1 week earlier. In addition, he reported a several-week history of left upper quadrant pain. At examination, he had tenderness to deep palpation of the left upper quadrant, which worsened with deep inspiration. A manual (nonautomated) review of a blood smear was requested explicitly, prompted by diagnosis of the case of babesiosis in patient A 3 days earlier. Intraerythrocytic *Babesia* parasites also were observed on the blood smear for patient B; the parasitemia level was 1%. His hemoglobin level was 11 g/dL, and his hematocrit was 35%. On the same day, he was evaluated in the UWHC Infectious Disease Clinic and began outpatient therapy with atovaquone plus azithromycin. To evaluate his abdominal pain, CT of the abdomen and pelvis was performed on an outpatient basis (November 5); it showed a splenic infarction (Figure 3), which was not detected by CT in early October. During the course of antimicrobial drug therapy, his abdominal pain and constitutional symptoms resolved.

Organ Donor

The organ donor was a 22-year-old man who was a resident of an urban area of Wisconsin to which babesiosis was not endemic. According to his relatives and primary care physician, he had been in good health and did not have any potentially relevant travel or clinical manifestations during the previous year. His only known risk factor for exposure to *Babesia* parasites was receipt of multiple blood transfusions during resuscitation attempts on the day he died from unintentional trauma. Although an autopsy was not performed, a limited number of plasma, serum, and tissue specimens were available for *Babesia* testing. Antibodies against *B. microti* parasites were not detected by retrospective serologic testing of a pretransfusion plasma specimen and 2 posttransfusion serum specimens (IFA titer ≤8). Tissue sections from

both kidneys had negative IHC results. IHC testing of hepatic tissue showed a few rare foci of suspicious staining but no definitive evidence of *Babesia* parasites, and hepatic tissue showed negative results by PCR.

The cornea recipients were contacted, and blood specimens collected ≈3–4 months posttransplantation were tested for evidence of *Babesia* infection. Specimens showed negative results for PCR and IFA analysis, and no parasites were found on blood smears.

Transfusion Investigation

During resuscitation attempts, the organ donor received 20 cellular blood components (19 units of erythrocytes and 1 unit of apheresis platelets) and 13 plasma units. Only 1 of the 33 donors, a 52-year-old man, had evidence of *B. microti* infection. Specimens available for testing were collected 88 and 151 days postdonation and had IFA titers of 256 and 128, respectively; both specimens showed negative results by PCR. The seropositive blood donor was the source of 1 of the organ donor's last erythrocyte transfusions, which was transfused 15 days postdonation.

This blood donor lived in a babesiosis-endemic area of Minnesota (Washington County) and had camped in disease-endemic areas in northern Wisconsin (Ashland County) in May 2008 and in northern Minnesota (Saint Louis County) in July 2008. During the retrospective investigation, he recalled a fever (39.4°C), chills, and diaphoresis, which lasted ≈36 hours, during the first week of June. Although he did not recall any tick bites, his wife reportedly had found a tick on his body (timing and other details not specified). No cellular components from his donation in August were transfused to other patients. After he was found to be seropositive, he was deferred indefinitely from future blood donations. However, he already had donated blood in the interim (in September 2008), and apheresis platelets from the donation had been transfused. A specimen obtained ≈2 months posttransfusion from the platelet recipient was tested in a commercial laboratory and showed negative *B. microti* IFA and PCR results.

Discussion

We investigated parasitologically confirmed cases of babesiosis in 2 recipients of renal allografts from an organ donor whose only known risk factor for exposure to *Babesia* parasites was the receipt of multiple blood transfusions on the day he died. The organ donor and the kidney recipients did not have antibodies against *B. microti* parasites detected by retrospective testing of pretransplantation specimens. However, 1 of the organ donor's blood donors was seropositive when tested postdonation and had risk factors for tick exposure.

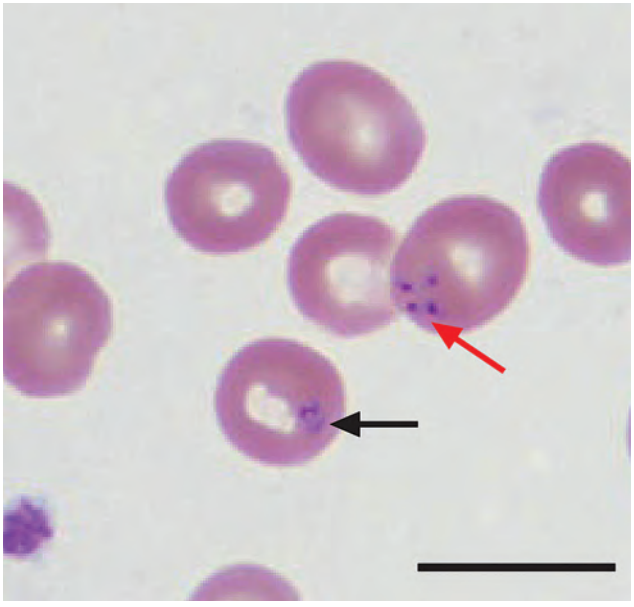

Figure 2. Wright-stained peripheral blood smear from patient A (index case-patient), a renal transplant recipient infected with *Babesia microti* parasites, Wisconsin, USA, 2008. The smear shows intraerythrocytic *Babesia* parasites, a ring form (black arrow), and a Maltese cross or tetrad form (red arrow), which is pathognomonic for babesiosis. Scale bar indicates 10 μm.

The most likely scenario is that the kidney donor served as a conduit of *Babesia* parasites from this blood donor to the kidney recipients (i.e., the blood donor became infected by tickborne transmission, secondary transmission occurred by erythrocyte transfusion, and tertiary transmission occurred by organ transplantation). The possibility that the kidney recipients became infected independently is remote: they did not live, travel, or receive medical care in any known babesiosis-endemic areas in the Midwest or elsewhere; they did not receive any transfusions; and they showed seroconversion posttransplantation, despite being immunosuppressed. Although no subtyping tools are available to establish that the patients were infected with the same *B. microti* strain, they almost assuredly became infected from the same source at approximately the same time.

Previous reports have described organ transplant recipients who became infected with *Babesia* parasites by tickborne- or transfusion-associated transmission in the peritransplant period or thereafter (*10*,*18*–*22*). Transplantation-associated transmission of *B. microti* parasites, which are not known to have an exoerythrocytic tissue phase, has not been described, nor has the occurrence of 3 consecutive routes of transmission (vector, transfusion, and transplantation), which has been reported for West Nile virus (*23*,*24*). The plausibility of transplantation-associated transmission of *B. microti* parasites, in the context of residual parasites in the renal vasculature/fluids after

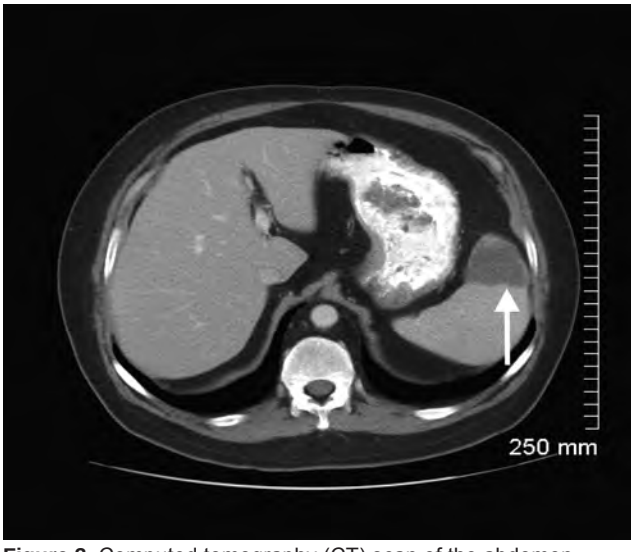

Figure 3. Computed tomography (CT) scan of the abdomen of patient B, a renal transplant recipient infected with *Babesia microti* parasites, Wisconsin, USA, 2008. Taken on November 5, the scan shows a splenic infarction (white arrow) that had not been visualized on a CT scan on October 5. Although the cause of the splenic infarction was not determined, the infarction might have been a complication of babesiosis, as reported for other patients (*16*,*17*).

flushing the organs, is supported in part by data from other contexts (e.g., transfusion-associated cases) that suggest low inocula of the parasite can cause infection (*10*,*25*).

Although we do not have proof that the blood donor was infected when he donated blood or have laboratory evidence that the organ donor briefly harbored the parasite, the negative PCR results for the postdonation specimens from the blood donor and the negative PCR and IHC results for the available posttransfusion specimens from the organ donor are not helpful; only positive results would have been informative. Although other transmission scenarios seem much less probable, the cases of babesiosis in the kidney recipients we report would be noteworthy even if the organ donor recently had acquired the parasite from a tick (i.e., was in the early window period of infection, despite his lack of known risk factors for tickborne transmission).

Diagnosis of babesiosis in the kidney recipients prompted multiagency investigations of the organ donor and his blood donors. However, the cases of babesiosis in the recipients could have been easily missed, which highlights the possibility that other transplantation-associated cases have occurred but were not diagnosed or investigated. For patient A (index case-patient), babesiosis was diagnosed because of the serendipitous finding of parasites on a blood smear that was examined manually because of platelet clumping. His lack of risk factors for tickborne transmission and the possibility of donor-derived infection led to prompt evaluation of patient B. At the time of diagnosis, illness in patient B was milder (lower-level parasitemia, minimal anemia, and transient fever) than that in patient A, even though the 2 patients received similar immunosuppressive regimens.

Babesiosis can be persistent, relapsing, or life threatening in immunocompromised patients (*18*,*19*,*22*,*26*–*28*). Optimal therapy for babesiosis in patients who have received an organ transplant or have impaired immunity for other reasons is not well established and might depend on multiple factors; a uniform recommendation might not be applicable to such a heterogeneous population. In immunocompetent persons, the typical duration of antimicrobial drug therapy for babesiosis is 7–10 days (*6*). We decided to treat both kidney recipients for 6 weeks on the basis of retrospective data for immunosuppressed patients that suggest the likelihood of cure is higher if combination antimicrobial drug therapy is administered for ≥6 weeks, including 2 weeks after *Babesia* parasites are no longer detected on blood smears (*27*). We gave the patients atovaquone plus azithromycin rather than clindamycin plus quinine (the standard of care for severely ill patients [*6*,*29*]) to minimize the likelihood of toxicity during their 6-week treatment courses. In addition, we decreased the doses of their immunosuppressive medications.

Both patients tolerated and responded well to the antimicrobial treatment, without documented relapses. However, clinicians should be aware that clinical resistance reportedly developed in several immunosuppressed patients treated for prolonged periods with atovaquone plus azithromycin (*28*); whether particulars of those patients' treatment regimens (e.g., antimicrobial drug dosing) contributed to development of clinical resistance is not known (*28*,*30*).

The cases of babesiosis we describe not only underscore the plausibility and likelihood of transmission by organ transplantation, but also highlight the emerging role of transfusion-associated babesiosis. For the 3-decade period of 1979 (the year the first known transfusion case occurred) through 2009, a total of 159 transfusion-associated cases of *B. microti* infection were identified in the United States, most (77%) of which occurred during 2000–2009 (*10*). Asymptomatic persons can fulfill all of the criteria for donating blood despite having low-level parasitemia sufficient to cause infection in a transfusion recipient (*10*).

To date, no *Babesia* tests for screening US blood donors have been licensed by the Food and Drug Administration, and no pathogen-reduction technologies for cellular blood components have been approved (*31*–*35*). However, the Blood Products Advisory Committee of the Food and Drug Administration that was convened on May 13, 2015, supported the concepts of year-round *B. microti* serologic testing of all US blood donors and of *B. microti* nucleic acid–based testing of donors in selected states (details remain to be determined) (*36*). Because of donor travels and shipments/distributions of blood

components, transmission by transfusion is not limited to babesiosis-endemic foci (*10*). For example, the seropositive erythrocyte donor we identified had donated blood in a babesiosis-endemic area of Minnesota. This blood was then transported to and transfused in an area of Wisconsin to which babesiosis was not endemic.

As we described, unrecognized tickborne transmission of *Babesia* parasites to the blood donor probably led to transmission by transfusion to the organ donor and subsequent transmission by organ transplantation to both kidney recipients. Clinicians should include babesiosis in the differential diagnosis of unexplained fever and hemolytic anemia after blood transfusion or organ transplantation, even in regions to which babesiosis is not endemic. Suspected cases of iatrogenic transmission should be reported to state and local public health authorities. In addition, cases that might be transfusion or transplantation associated should be reported to the pertinent blood center and organ procurement organization, respectively.

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Immune Responses to Invasive Group B Streptococcal Disease in Adults

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Immunization of nonpregnant adults could help prevent invasive group B *Streptococcus* (GBS) infections, but adult immune responses have not been investigated. We defined capsular polysaccharide (CPS) and pilus island (PI) surface antigen distribution and expression and immune responses to GBS infection in nonpregnant adults. Prospective surveillance from 7 hospitals in Houston, Texas, USA, identified 102 adults with GBS bacteremia; 43% had skin/soft tissue infection, 16% bacteremia without focus, and 12% osteomyelitis. CPS-specific IgG was determined by ELISA and pilus-specific IgG by multiplex immunoassay. CPS types were Ia (24.5%), Ib (12.7%), II (9.8%), III (16.7%), IV (13.7%), and V (12.7%); 9.8% were nontypeable by serologic methods. Pili, expressed by 89%, were most often PI-2a. CPS and pilus-specific IgG increased during convalescence among patients with strains expressing CPS or PI. All GBS expressed CPS or PI; 79% expressed both. Increased antibodies to CPS and PI during recovery suggests that GBS bacteremia in adults is potentially vaccine preventable.

In the United States, group B *Streptococcus* (GBS) has emerged as a frequent cause of invasive infection in nonpregnant adults with underlying medical conditions. The incidence of GBS disease among these patients increased from 3.6 cases/100,000 persons in 1990 to 7.3 cases/100,000 persons in 2007 ($p<0.001$) (*1*). This increase has occurred among adults 18–64 years of age and ≥65 years of age (*2*). The Centers for Disease Control and Prevention Active Bacterial Core surveillance estimated that 28,000 cases of invasive GBS disease occurred in the United States during 2014 and that 25,900 (93%) cases occurred beyond infancy, primarily in nonpregnant adults (*3*). Also, adults account for 90% of the estimated 1,660 annual deaths attributable to GBS infection.

Most cases of invasive GBS disease are caused by 5 capsular types (Ia, Ib, II, III, and V), which together accounted for 88% of cases in nonpregnant adults during 1999–2005 (*2*). The emergence of serotype V in the 1990s was first noted among GBS isolates from nonpregnant adults (*4*,*5*). During 1999–2005, infection with the formerly rare serotype IV increased among nonpregnant adults (*1*). As with invasive pneumococcal disease, the capsule of GBS is a major virulence factor targeted by candidate GBS capsular polysaccharide (CPS)–protein conjugate vaccines; thus, knowledge of prevalence of these antigens is critical for vaccine development. Similarly, another surface component of GBS critical to virulence, the pilus islands (PIs), are limited in number but are found in all strains and are promising as GBS vaccine candidates (*6*–*8*).

Among infants, use of antenatal screening and intrapartum antimicrobial prophylaxis has markedly reduced early-onset (0–6 days of age) but not late-onset (7–89 days of age) GBS disease. Immunization of pregnant women offers the best strategy for prevention of early- and late-onset invasive GBS disease in infants. Immunization of adults also potentially could reduce the GBS disease burden in the United States, but data are needed regarding immune responses during convalescence from invasive disease (*9*). We characterized CPS and PI genotypes and expression among isolates and explored immune responses to these surface antigens during convalescence from invasive infection in a cohort of nonpregnant adults.

Methods

Participants and Setting

We identified cases of GBS bacteremia by conducting prospective, laboratory-based surveillance from March 2012 through October 2014 at 4 Texas Medical Center hospitals (Houston, TX, USA) and 3 suburban affiliates of 1 Texas Medical Center hospital, all of which provided uniform diagnostic evaluations and medical care. Invasive infection was defined as isolation of GBS from blood or cerebrospinal fluid. Potential cases were identified daily at participating hospital laboratories. Nonpregnant adults ≥18 years of age with invasive GBS infection were eligible for enrollment. We excluded those with symptom duration >7 days,

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HIV infection, or polymicrobial bacteremia. An investigator contacted the physician of record for approval before approaching the patient. Study personnel then discussed the study and eligibility with patients and obtained informed consent. The study received approval by the Institutional Review Boards for Human Research at Baylor College of Medicine and participating hospitals' administrative processes.

Specimen Collection

A blood sample was collected from each participant on the day of hospital admission or at enrollment. Demographic and clinical data were recorded from the medical record. Convalescent-phase blood was collected at a mean of 3 (range 2–4) weeks from the date of positive culture. Blood was transported to the investigators' laboratory (Baylor College of Medicine, Houston, TX, USA) where serum was separated, aliquoted, and stored at −80°C until sent to GSK Vaccines (Siena, Italy) for testing.

Bacterial Strains, Media, and Growth Conditions

GBS isolates from each patient were obtained from hospital laboratories. They were then grown in Todd Hewitt Broth (Becton Dickinson, Franklin Lakes, NJ, USA) at 35°C–37°C for serotyping and inoculated directly from plates into trypticase soy broth with 20% glycerol for storage at −80°C.

Serotyping of GBS Isolates

Capsular typing was performed for all isolates. Both the capillary precipitin method (*10*) and the Strep-B-Latex rapid agglutination method (Statens Serum Institut, Copenhagen, Denmark) were used.

Genotyping and Pilus Typing of GBS Isolates

Genomic DNA was prepared from GBS cultures by a standard protocol for gram-positive bacteria by mutanolysin treatment of bacteria and use of a GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, Milan, Italy) according to the manufacturer's instructions. Capsular gene typing was performed for all isolates by multiplex PCR (*11*) modified by including a primer pair specific for detection of serotype IX. Pilus genes were PCR amplified by using primers specifically annealed to conserved genomic regions external to the coding sequences as described (*8*).

Multilocus Sequence Typing

Multilocus sequence typing was performed as described (http://pubmlst.org/sagalactiae/). Clonal complexes (CCs) were assigned by global optimal eBURST analysis of multilocus typing data (*12*).

Fluorescence-Activated Cell Sorter Analysis

Fluorescence-activated cell sorter (FACS) analysis to assess surface expression of CPS and pilus proteins was performed as reported (*13*,*14*). In brief, paraformaldehyde-fixed bacteria were incubated with mouse monoclonal antibodies or immune polyclonal serum specific for CPS type and pilus proteins (*14*). Cells were stained with R-phycoerythrin-conjugated $F(ab)_2$ goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and analyzed with a FACS CANTO II instrument (Becton Dickinson) and FlowJo Software (Tree Star Inc., Ashland, OR, USA).

ELISA

Serum CPS-specific IgG was determined by ELISA. Microtiter plates (96-well) (Nunc MaxiSorp; Sigma Aldrich, St. Louis, MO, USA) were coated overnight at 2°C–8°C with 100 ng of purified CPS Ia, Ib, II, III, IV, or V conjugated to human serum albumin-adipic acid dihydrazide in phosphate-buffered saline (PBS) at pH 7.4. After washing with 0.05% Tween-20 in PBS (PBST), plates were incubated for 90 min with 2% bovine serum albumin in PBST. Serial 2-fold dilutions of test and standard serum in 2% bovine serum albumin in PBST were added, incubated 1 h, and washed. After alkaline phosphatase-conjugated anti-mouse IgG (1:1000 in PBST) was added, plates were incubated for 90 min and then washed. After incubation with p-nitrophenylphosphate (4.0 mg/mL), the reaction was stopped with EDTA 7% (wt/vol) and absorbance was measured at 405 nm. The concentration of specific IgG was determined with a standard titration curve and expressed as micrograms per milliliter (CPS Ia, Ib, III, and V) or ELISA units per milliliter (CPS IV). Standard samples containing 47.2, 35.4, 30.4, 83.5, or 11.6 μg/mL of anti-CPS Ia, Ib, II, III, and V IgG were prepared by pooling serum from women immunized with the specific CPS conjugated to tetanus toxoid (*15*). The CPS IV standard was prepared by pooling unweighted high-titer serum from study participants. The lower limit of quantitation was 0.156 μg/mL for types Ia, Ib, and V; 0.250 μg/mL for types II and III; and 12.5 ELISA units/mL for type IV. A titer corresponding to one half of the lower limit of quantitation was assigned to serum with values below the lower limit of quantitation.

Multiplex Immunoassay

Pilus protein–specific IgG was measured by multiplex immunoassay that used recombinant pilus proteins BP-1, BP-2a, and BP-2b coupled to magnetic beads and expressed as relative Luminex units (RLU) per milliliter. A total of 20 μg of each protein was coupled to carboxyl groups of a specific MagPlex bead set (Luminex Corporation, Austin, TX, USA). Three sets of coupled beads (3,000 beads/region/well) were added to each dilution of the standard curve and serum samples (1:100 and 6 serial 2-fold dilutions), incubated for 90 min in the dark, with shaking at 700 rpm. Beads were washed twice with 200 μL of PBS and incubated with phycoerythrin-conjugated goat anti-human IgG $F(ab')_2$ (Jackson ImmunoResearch Laboratories) at 5

μg/mL for 1 h in the dark, with shaking at 700 rpm. After a second wash, beads were suspended in 100 μL PBS.

Analysis was performed on a FlexMAP3D instrument by using Bio-Plex Manager software version 6.0 (Bio-Rad, Hercules, CA, USA). For each analyte, median fluorescent intensity was converted to RLU per milliliter by interpolation from a 5-parameter logistic standard curve for every bead-region/standard. Standard serum for determination of human IgG specific for GBS pilus proteins was obtained by pooling hyperimmune serum from persons colonized by GBS. A titer of 1 RLU/mL was arbitrarily assigned to the highest concentration point of the calibration curve. The lower limit of quantitation for the pilus proteins was 0.14 RLU/mL for BP-1 and 0.41 RLU/mL for BP-2a and BP-2b.

Statistical Analyses

Clinical features, by patient age group, were compared by using the 2-tailed Fisher exact test. Antibody responses to CPS or pilus proteins were assessed by paired *t*-test on log-transformed data. CPS-specific IgG concentrations were compared for homologous and heterologous (Mann–Whitney U test) GBS serotypes.

Results

Participant Characteristics

Participants comprised 102 nonpregnant adults (race/ethnicity 44% white, 30% black, 25% Hispanic, 1% other) with GBS bacteremia. Mean age was 59.5 years (median 59.7, range 27–91 years). Most (75%) were from Texas Medical Center hospitals. Most (59%) were male. One patient also had meningitis. All patients had ≥1 condition that enhanced risk for invasive GBS disease, including diabetes mellitus (59%), obesity (57%), cardiovascular disease (45%), liver disease (20%), kidney disease (19%), or cancer (10%) (*1*,*2*). The most common expressions of illness were skin/soft tissue infection (43%) bacteremia without focus of infection (16%), osteomyelitis (12%), and endocarditis or endovascular infection (8%). Four patients died, 2 of them before convalescent-phase serum was obtained.

Characteristics of Infecting GBS Isolates

Of 102 isolates, 92 expressed 1 of 6 capsular types. CPS type Ia accounted for 24.5% of isolates, followed by types III (16.7%), IV (13.7%), V (12.7%), Ib (12.7%), and II (9.8%) (Figure 1, panel A). The genotype and serotype for these 92 isolates were concordant. CPS expression was undetectable by latex agglutination and capillary precipitin methods for 10 (9.8%) isolates; 7 were identified by FACS analysis as type Ia (1), II (3), or V (3). The genotypes for the 10 isolates were Ia (1), II (3), and V (6). Genotypes and CCs for all 102 isolates are displayed (Figure 1, panel B); 77% of these isolates were 1 of the 3 lineages (CC1, CC19, or CC23). The hypervirulent CC (CC17) accounted for 35% of type III isolates. Among type IV isolates, CC1 (57%) and CC23 (43%) were exclusively represented.

PI genes were detected in all isolates (Figure 1, panels C and D). Most carried genes for PI-1 and PI-2a (56%) or PI-2a alone (32%). According to flow cytometry, 89% of isolates expressed in vitro ≥1 pilus type on their surface, most often PI-2a alone or with PI-1. PI-2b was expressed only by type IV strains or in combination with PI-1 by type III strains. Genes for PI-1 and PI-2a were present on 8 of 11 isolates not expressing pili. The CPS types for the 11 strains that did not express pili were Ia (1), Ib (2), II (4), IV (2), and V (2). All GBS strains expressed either CPS or pili, and 79% expressed both surface antigens.

Immune Responses

Paired acute- and convalescent-phase serum samples were available from 97 patients, but only 87 were infected with CPS-expressing strains. A significant increase in CPS-specific IgG was observed during the convalescent phase for each of the 6 GBS types causing invasive disease in these 87 patients (Table). However, the concentration of antibodies to CPS in acute- and convalescent-phase serum varied widely (Figure 2). When CPS-specific IgG was expressed as ≥4-fold increases during convalescence, an immune response to their infecting serotype was detected for 50% (Ia), 31% (Ib), 50% (II), 41% (III), 42% (IV), and 46% (V) of patients.

For all patients, CPS-specific IgG concentrations in convalescent-phase serum samples were compared with the infecting GBS serotype and with heterologous GBS serotypes (Figure 3). With the exception of type III, the means and interquartile ranges of CPS-specific IgG were significantly higher ($p<0.001$) for the infecting GBS serotype than for the other 5 serotypes, indicating that the CPS immune response in adults is capsular type specific.

PI-1–specific IgG increased significantly for the 35 patients infected with GBS strains expressing PI-1 when acute-phase serum (geometric mean concentration [GMC] 25.2 RLU/mL [range 0.57–765.0; 95% CI 12.2–52.0]) was compared with convalescent-phase serum (GMC 53.7 RLU/mL [range 0.5–4972.1; 95% CI 24.3–118.7]) ($p = 0.003$). Similarly, PI-2a–specific IgG increased significantly between acute illness (GMC 15.0 RLU/mL [range 0.8–606.5; 95% CI 10–22.5]) and convalescence (GMC 28.7 RLU/mL [range 0.8–1459.9; 95% CI 19.43.2]) for the 66 patients with infection caused by GBS isolates expressing this pilus type ($p<0.001$) (Figure 4). Among the 12 patients infected with GBS strains expressing PI-2b, there was no significant PI-2b–specific IgG response (data not shown). We detected ≥4-fold increases in pilus-specific IgG during convalescence in 20% (PI-1), 16.7% (PI-2a), and 25% (PI-2b) of patients. Among these 20 patients, response to their infecting CPS type was also ≥4-fold for 14 (70%).

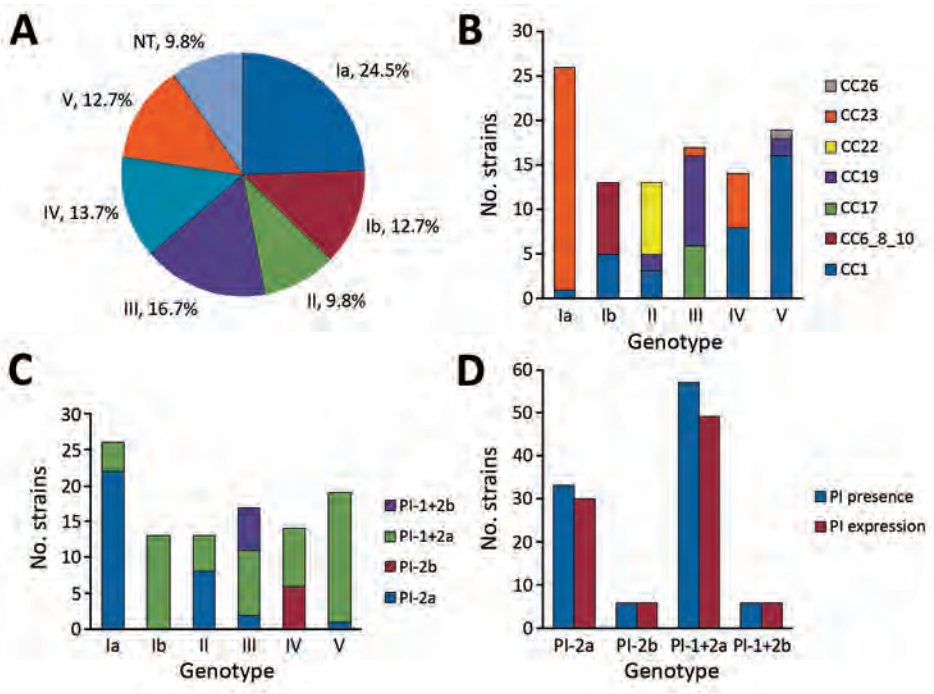

Figure 1. CPS type and PI distribution and expression in 102 group B *Streptococcus* isolates from adults with invasive infection, Houston, Texas, USA. A) CPS distribution of isolates by capillary precipitin method and latex agglutination assay (indicating CPS expression). B) Correlation between CC distribution and CPS genotype by PCR. C) PI and CPS distribution by genotype. D**)** Relationship between pilus genotype distribution and pilus expression in isolates expressing ≥1 pilus type on their surface. CC, clonal complex; CPS, capsular polysaccharide; NT, nontypeable; PI, pilus island.

Discussion

GBS is a common pathogen in nonpregnant adults (*16*). Skoff et al. (*1*) suggest that the increasing prevalence of chronic medical conditions might explain the increasing incidence of GBS disease among adults. Most patients in our cohort had underlying conditions, as has been noted previously (*1*,*17*–*19*). Skin/soft tissue infections were present in 43%, and the presentation was nonfocal for 16%, usually in patients with hepatic disease (*1*,*2*).

Our investigation led to 3 major findings. First, serotype IV GBS was a prominent cause of invasive disease, accounting for 13.7% of cases. Skoff et al. (*1*) noted that type IV cases increased from 0.2% during 1998–1999 to 5.7% during 2005–2006. Characterization of 549 strains from Canada during 2010–2014 indicated that 16.9% of cases with invasive disease were caused by serotype IV (*20*). In our cohort, 6 GBS types were responsible for at least 10% of cases, but only types Ia (24.5%) and III (16.7%) caused more infections than type IV. None of the cases reported here were caused by GBS types VI–IX; Skoff et al. (*1*) identified only 3 of these types among 1,933 isolates from nonpregnant adults. Invasive type IV GBS infections were clinically diverse and included bacteremia without focus (4 patients), skin and soft tissue infections (3), osteomyelitis (3), septic shock (1), endocarditis (1), arthritis (1), and urosepsis (1).

Second, our findings add to limited data regarding GBS virulence factors critical to invasive disease pathogenesis in adults. Most GBS isolates can be assigned to a small number of CCs, including CC1, CC10, CC17, CC19, and CC23 (*21*). Three major lineages (CC1, CC19, and CC23) accounted for 77% of isolates from our cohort. These 3 CCs comprised 64% of isolates from nonpregnant adults in Sweden who had invasive infections during 1988–1997 (*22*). CC19, associated with less invasive type III strains in neonates, accounted for 63% of type III isolates in the patients reported here; the hypervirulent CC17 comprised only 33%, suggesting that less virulent strains may cause invasive disease in adults (*23*,*24*). Among type IV isolates, CC1 and CC23 were exclusively represented, extending the observations of Ferrieri et al (*25*) that type IV isolates from nonpregnant adults with invasive disease were CC1 and that those from invasive infection in infants were CC23. No type IV isolates belonged to the novel epidemic hypervirulent CC17 lineage resulting from the type III to type IV capsular switch (*26*,*27*). Teatero et al. (*20*) assert that emergence of serotype IV in Canada was driven by CC1 sequence type 459 strains, possibly linked to acquisition of resistance to tetracycline, macrolides, and lincosamides.

All strains isolated from the patients reported here expressed CPS, pili, or both surface-associated GBS virulence factors. For a subset of strains, expression was undetectable for CPS (10%) or pilus (11%). The proportion of nontypeable strains (assessed by conventional CPS typing methods) in our cohort was greater than the 6.5% reported by the Centers for Disease Control and Prevention during 2005–2006 (*1*). Also, the proportion of strains for which pilus expression was undetectable was slightly higher than the 6% reported for 289 GBS isolates from the United States and Italy, but these data represented infants and adults with either invasive infection or asymptomatic colonization (*8*).

Table. CPS-specific IgG responses for 87 nonpregnant adults with invasive GBS infection*

GBS type	No. patients†	CPS-specific IgG, GMC (range) [95% CI], μg/mL		p value‡
		Acute-phase sample	Convalescent-phase sample	
Ia	22	26.0 (0.39–609.1) [9.5–71.0]	137.6 (0.35–7,792.2) [50.4–375.6]	<0.001
Ib	13	9.3 (0.08–680.9) [1.6–55.6]	25.2 (0.08–650.5) [4.6–139.5]	0.035
II	10	11.6 (0.13–1,126.8) [1.2–113.6]	47.4 (0.61–2,279.9) [6.1–366.1]	0.032
III	17	1.5 (0.13–153.9) [0.53–4.0]	4.8 (0.13–584.4) [1.1–20.4]	0.044
V	13	3.2 (0.24–596.3) [0.51–19.9]	10.1 (0.43–4,182.0) [1.9–55.0]	0.003
IV§	12	409.5 (17.6–4,823.3) [133.3–1,257.5]	1,060.9 (149.4–9,085.2) [415.7–2,707.6]	0.022

*CPS, capsular polysaccharide; GBS, group B *Streptococcus*; GMC, geometric mean concentration.
†Excludes 10 patients with infection caused by GBS that did not express capsular polysaccharide, 3 for whom convalescent-phase samples were of insufficient quantity for analysis, and 2 who died before collection of convalescent-phase serum.
‡Paired *t*-test on log-transformed data.
§Results are expressed in ELISA units/mL.

Third, as a group, nonpregnant adults demonstrated an immune response to major virulence antigens after invasive GBS infection. CPS-specific IgG to each infecting CPS type and PI-1– or PI-2a–specific IgG almost always increased significantly in convalescent-phase serum. However, the immune response was complex; the range of antibody concentrations in acute- and convalescent-phase serum was broad, a pattern not found in neonates and infants, for whom low concentrations of CPS-specific IgG are uniformly found in acute-phase serum (*28*).

We observed several patterns of immune response. Between acute illness and convalescence, CPS-specific IgG increased by ≥4-fold for 44% of patients. By comparison, CPS-specific IgG increased by ≥4-fold in postimmunization serum from 80%–93% of healthy adults immunized with monovalent GBS conjugate vaccines representing CPS Ia, Ib, II, III, and V (*29*). For other patients, CPS-specific IgG in concentrations at the time of hospital admission were higher than the 0.5–3 μg/mL predicted to be protective against neonatal infection (*30*), suggesting that either the immune response was rapid or that an immune response to CPS is not as critical for nonpregnant adults with invasive infection as it is for neonates. Wessels et al. (*31*) noted that the serum CPS-specific IgG to the infecting GBS isolate in adults with bacteremia was ≥3.5 μg/mL in 7 of 12 serum samples obtained within 2 days of admission. Although acute-phase serum from the patients reported here was almost always collected at the time of admission and those who had symptoms >7 days before admission were excluded from enrollment, for some, a rapid, anamnestic response could have developed before admission. Many patients had diabetes and lower extremity infection or chronic lower extremity disease that could have led to prolonged antigenic stimulation resulting in a high level of CPS-specific IgG that declined during convalescence.

Our study has some limitations. Because we enrolled patients from only 1 city, the diversity of surface antigens relevant to immune response might not be applicable to other regions. Despite obtaining acute-phase serum at admission, the time of bacteremia onset could not be determined, complicating interpretation of immune response results. Our study was not designed to evaluate functional aspects of immune responses, which will be relevant to vaccine design considerations.

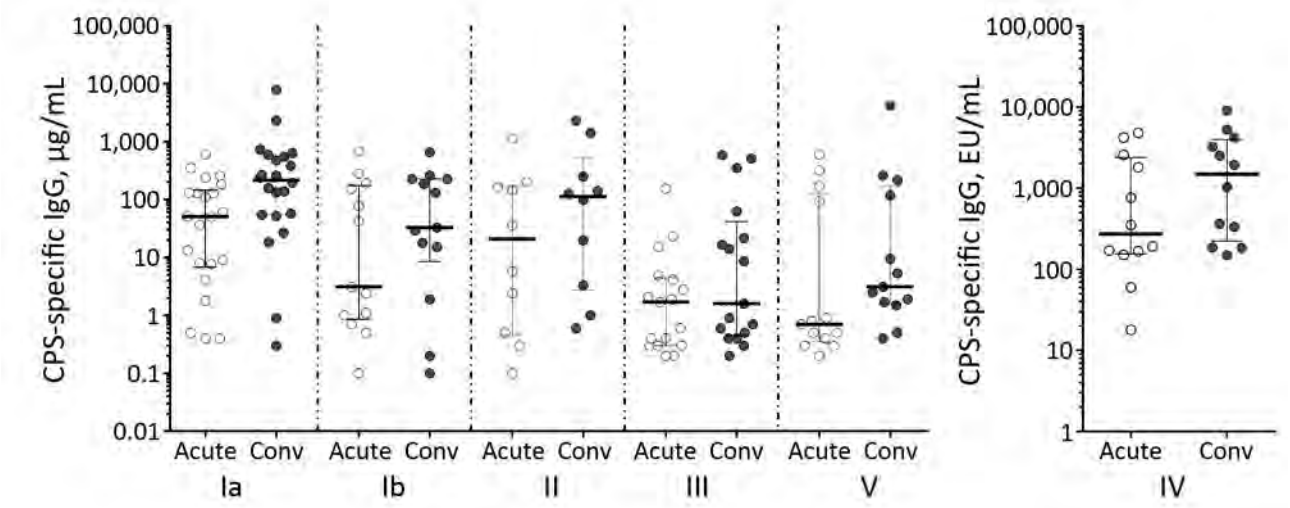

Figure 2. CPS-specific IgG concentrations/titers in acute- versus convalescent-phase serum from patients with group B streptococcal infection, Houston, Texas, USA. Horizontal bars represent median concentrations (± interquartile range) for each patient group. Conv, convalescent; CPS, capsular polysaccharide; EU, ELISA units.

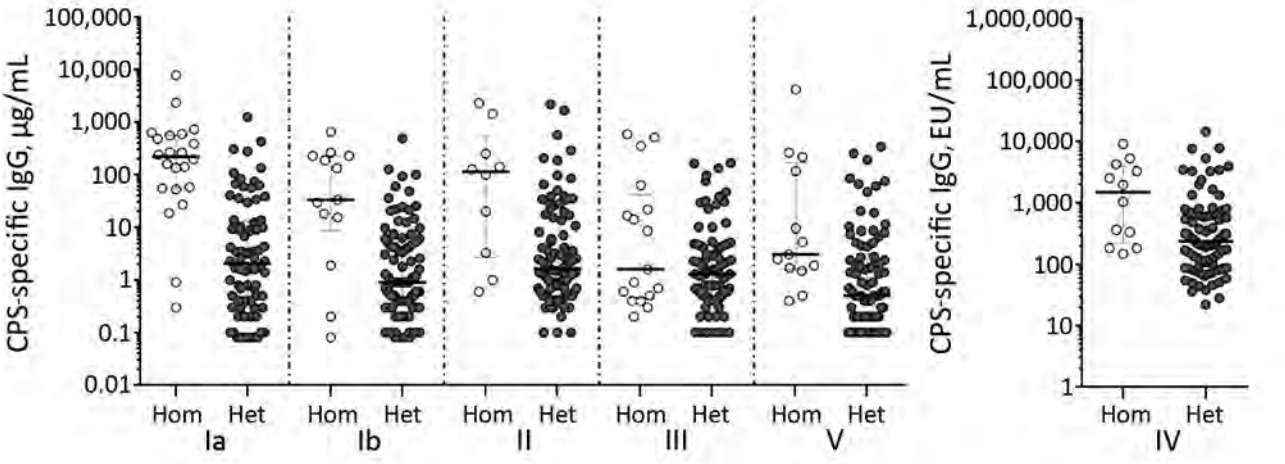

Figure 3. Concentrations of CPS-specific IgG against homologous or heterologous group B streptococcal serotypes in convalescent-phase serum samples from infected patients, Houston, Texas, USA. Horizontal bars indicate median concentrations (± interquartile range) within each group. CPS, capsular polysaccharide; EU, ELISA units; het, heterologous; hom, homologous.

One requirement for a robust protective response is surface availability of antigens for antibody recognition (*7*). In murine models of GBS disease, pilus components induce protective immunity against all tested GBS challenge strains (*8*), and sufficiently high CPS-specific antibodies are associated with disease protection for newborn infants (*30*,*32*). The concept that CPS-protein conjugate vaccines are immunogenic in older adults also is well established (*33*). A 13-valent polysaccharide conjugate vaccine has proven effective at preventing vaccine-type invasive disease caused by *Streptococcus pneumoniae*, another pathogen that results in serious illness in adults with underlying medical conditions and those ≥65 years of age (*34*). Theoretically, a GBS conjugate CPS vaccine also incorporating pilus protein surface antigens could elicit a protective immune response providing protection for those adults at risk for invasive infection.

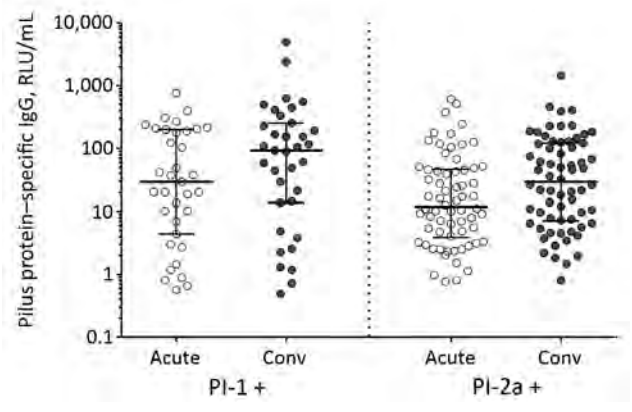

Figure 4. Pilus-specific antibody responses in acute- and convalescent-phase serum from patients infected with group B streptococcal strains expressing pilus type 1 or type 2a, Houston, Texas, USA. Anti-pilus–specific IgG titers were measured by multiplex immunoassay that used recombinant pilus proteins coupled to magnetic beads and expressed in RLU/mL. Horizonal bars represent the median (± interquartile range) within each population. For both comparisons, anti-pilus IgG increased significantly in serum collected during the acute and convalescent phases (p<0.001). p values were calculated by *t*-test on log-transformed data. Conv, convalescent; RLU, relative Luminex units.

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Ambulatory Pediatric Surveillance of Hand, Foot and Mouth Disease as Signal of an Outbreak of Coxsackievirus A6 Infections, France, 2014–2015

Audrey Mirand, François Vié le Sage, Bruno Pereira, Robert Cohen, Corinne Levy, Christine Archimbaud, Hélène Peigue-Lafeuille, Jean-Luc Bailly, Cécile Henquell

The clinical impact of enteroviruses associated with hand, foot and mouth disease (HFMD) is unknown outside Asia, and the prevalence of enterovirus A71 (EV-A71) in particular might be underestimated. To investigate the prevalence of enterovirus serotypes and the clinical presentations associated with HFMD in France, we conducted prospective ambulatory clinic–based surveillance of children during April 2014–March 2015. Throat or buccal swabs were collected from children with HFMD and tested for the enterovirus genome. Physical examinations were recorded on a standardized form. An enterovirus infection was detected in 523 (79.3%) of 659 children tested. Two epidemic waves occurred, dominated by coxsackievirus (CV) A6, which was detected in 53.9% of enterovirus-infected children. CV-A6 was more frequently related to atypical HFMD manifestations (eruptions extended to limbs and face). Early awareness and documentation of HFMD outbreaks can be achieved by syndromic surveillance of HFMD by ambulatory pediatricians and rapid enterovirus testing and genotyping.

Although mostly asymptomatic or self-limited, enterovirus infections comprise a wide spectrum of clinical manifestations in children, which can require medical attention. Periodically, the emergence of an enterovirus serotype is associated with outbreaks of more serious disease resulting in serious illness and even death. Recent examples are the emergence of enterovirus A71 (EV-A71), which was responsible for large hand, foot and mouth disease (HFMD) outbreaks associated with rare but severe rhombencephalitis in Asia, and an EV-D68 epidemic associated with severe respiratory infections (*1*,*2*). Monitoring enterovirus infections and providing laboratory confirmation of the serotypes associated with different clinical presentations are of value for the early detection and awareness of emerging enterovirus infections (*3*,*4*).

EV-A71 is considered to be the most important neurotropic enterovirus in Southeast Asia countries, and EV-A71 vaccines have been developed in China (*5*,*6*). EV-A71 infections, along with other enterovirus serotypes belonging to the species *Enterovirus A* (EV-A) (*7*), are mainly associated with HFMD, which is characterized in children by a brief febrile illness and typical rash, with or without mouth ulcers (*8*). EV-A71 and coxsackievirus (CV) A16 were the most frequent serotypes involved in HFMD outbreaks throughout Asia during 1998–2010 (*1*,*9*). In the past 5 years, however, CV-A6 has emerged as a new important pathogen worldwide (*10*–*19*), and several studies have documented the more severe and extensive dermatologic presentations of CV-A6 HFMD (*16*,*20*–*25*). Surveillance of HFMD could lead to better detection of the upsurge of EV-A71 or another serotype associated with severe or distinct clinical features. In Western countries, surveillance of enterovirus infections is undertaken by virology laboratories and is thus restricted to enterovirus-infected persons admitted to hospitals (*19*,*26*). Children with HFMD or herpangina are usually evaluated and managed in ambulatory settings, and virologic investigations are rarely performed. Consequently, a clear gap exists in the knowledge of the epidemiology and clinical impact of HFMD and herpangina and of the enteroviruses involved in countries outside Asia.

We set up a local surveillance system run by pediatricians in ambulatory care settings that was effective in detecting HFMD outbreaks and the associated enterovirus serotypes (*13*). We have now extended this surveillance to cover the whole of France. The objectives of this study

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were to describe the epidemiology of enterovirus serotypes associated with HFMD and herpangina in France and to compare the clinical characteristics of HFMD and herpangina according to enterovirus serotypes.

Methods

Study Population and Design

The study was a 1-year prospective investigation of children with HFMD or herpangina who were seen by their pediatrician during April 2014–March 2015. The sentinel surveillance was performed by 47 pediatricians selected from among 118 volunteers by stratified sampling in different regions of France; 20 of the 22 French administrative regions were represented (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-0590-Techapp1.pdf). Sentinel pediatricians were requested to collect throat or buccal swab specimens from children with clinically diagnosed HFMD/herpangina. HFMD was defined by the presence of ≥2 of the following signs: buccal or peribuccal ulcers; eruption on palms, soles, buttocks, knees, or elbows; or a generalized eruption. Herpangina was defined by the presence of oral ulcers predominating on the posterior part of the buccal cavity. A standardized case report form collected anonymized information on the patient's demographics (e.g., birth date and sex); clinical signs at presentation, including fever, eruption type, and localization (e.g., palms, soles, buttocks, knees, elbows, lower limbs, upper limbs, generalized, or any other localization), buccal or peribuccal ulcers, gingivostomatitis, herpangina, and digestive/respiratory/ear, nose, and throat/neurologic signs; the onset date of the disease; and the date of sample collection. Environmental data (e.g., number of siblings, attendance at school or a daycare center, and ill contacts) were also recorded. Fever was defined as a rectal temperature >38°C. On the basis of the items checked, typical HFMD was defined as the presence of ≥2 of the following signs, as listed in the HFMD definition of the World Health Organization (*8*): oral ulcerations, eruption on palms, soles, buttocks, knees, or elbows. Clinical signs were considered to be atypical if eruption occurred at anatomic sites not listed in the World Health Organization HFMD definition or if it was generalized.

Written informed consent was obtained from the parents or guardians of all participants, none of whom received a stipend. The study was approved by the Ethics Review Committee of the University Hospital of Clermont-Ferrand, France (reference AU1098), and by the French National Agency for the Safety of Medicines and Health Products (reference 140021-B41).

Sample Collection

Throat or buccal specimens were collected with a flocked swab placed in a universal virus transport system (Copan Italia, s.p.a., Brescia, Italy). After the sampling, swabs were conserved at 2° –8°C and sent weekly to the National Reference Laboratory for Enterovirus and Parechovirus (Clermont-Ferrand, France) for enterovirus testing.

Diagnosis of Enterovirus Infection and Molecular Typing of Strains in Clinical Specimens

Viral RNA was extracted from 200 µL of the universal virus transport medium on the NucliSens easyMAG automated system (bioMérieux, Marcy l'Etoile, France) by the specific B protocol (elution volume 50 µL). Enterovirus diagnosis was performed by real-time reverse transcription PCR (RT-PCR) (Enterovirus R-gene, bioMérieux). Molecular typing (Figure 1, panel A) was first performed by a semi-nested RT-PCR with primers specifically developed for EV-A types (RT-PCR A) that targets the viral protein (VP) 3–VP1 coding region of the enterovirus genome. The first round of the RT-PCR EV-A assays were performed in a final volume of 25 µL with primers HEVAS1405 (5′-GGNTCNTTYATGGCNACNGGNAARATG-3′, location 1,405–1,531 relative to the genome of CV-A6 Gdula strain) and EVAR2C (5′-CGGTGYTTGCTCTTGAACTGCATG-3′, location 4,439–4,416) at a final concentration of 0.5 µmol/L, each by using the One-Step RT-PCR kit (QIAGEN, Courtaboeuf, France). The amplification program was as follows: 1 cycle of 30 min at 50°C; 1 cycle of 15 min at 95°C; 41 cycles of 30 s at 94°C, 50 s at 55°C, and 2 min at 72°C; and a final cycle of 10 min at 72°C. The second round RT-PCR A assays were performed in a final volume of 50 µL by using the Taq polymerase Kit (QIAGEN) and contained 5 µL of the first RT-PCR A amplicons and primers HEVAS1405 and HEVAR2429 (5′-GTNGGRTANCCRTCRTARAACC-3′, location 2,450–2,429) at a final concentration of 0.4 µmol/L. The reaction was run under the following conditions: 1 cycle of 2 min at 94°C; 39 cycles of 15 s at 94°C, 50 s at 54°C, and 50 s at 72°C; and a final cycle of 5 min at 72°C. If results were negative, a semi-nested RT-PCR was performed with primers specific for the species *Enterovirus B* (EV-B), HEVBS1695/EV2C (first round) and HEVS1695/HEVBR132 (second round) (*27*). The amplification programs were the same as those described except for the hybridization step, which was performed at 58°C. Alternatively, genotyping was attempted with nonspecies-specific primers to amplify the partial VP1 gene (*28*). Visible RT-PCR products after gel electrophoresis were purified and subjected to nucleotide sequencing with the same primers used for the amplification for the semi-nested RT-PCR A or, as previously described (*27*,*28*), by using the BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Ville-bon sur Yvette, France). The sequencing was performed on an ABI3500Dx genetic analyzer (Thermo Fisher Scientific). Virus identification was performed by BLAST analysis

(http://www.ncbi.nlm.gov/BLAST) and confirmed by phylogenetic analysis (*13*). The results were prospectively sent to the attending pediatricians, and monthly feedback on the overall findings of the surveillance was provided.

Phylogenetic Analyses

To investigate the spatiotemporal relationships among virus variants, we compared the nucleotide VP1 sequences assigned to the enterovirus serotype CV-A6 with homologous sequences available in public databases. We discarded redundant sequences from the final alignment of 238 sequences of 369 nt (i.e., 159 sequences determined in this study and 79 publicly available sequences). The phylogenetic relationships were inferred using a Bayesian method implemented in the BEAST package version 1.8 (http://beast.bio.ed.ac.uk). The uncorrelated lognormal molecular clock was employed with a flexible Bayesian skyline plot coalescent prior and the general time reversible model of nucleotide substitution. The Markov chain Monte Carlo were run for 200 million generations. We calculated maximum clade credibility trees by using the Tree Annotator program version 1.5.4 in BEAST. Topological support was assessed by calculating the posterior probability (pp) density for each node. All sequences were deposited into the GenBank database (accession nos. LT595894–LT596052). To characterize the EV-A71 strains, we compared partial VP1 sequences with reference sequences for genogroups A–F and subgenogroups B0–B5 and C1–C5. Phylogenetic analysis was performed with the neighbor-joining method and the Tamura-Nei model of sequence evolution implemented in MEGA6 software (http://www.megasoftware.net).

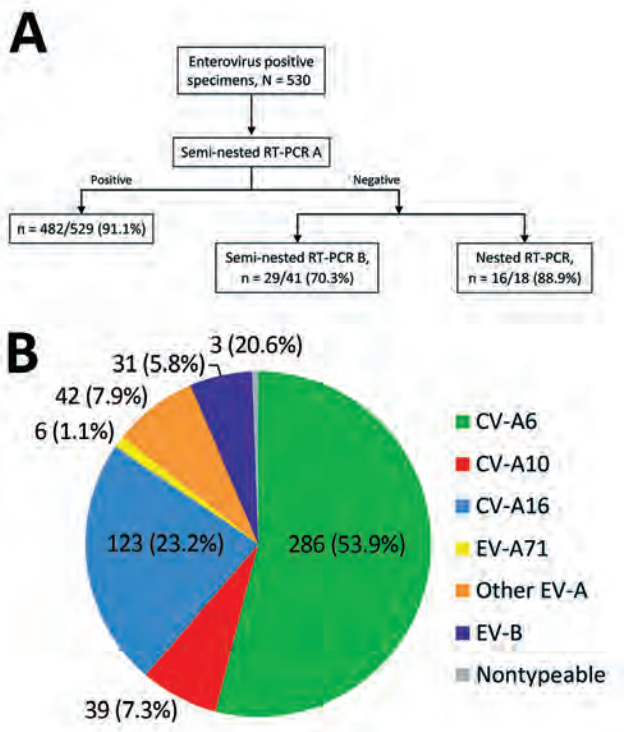

Figure 1. Methodologic approach for enterovirus genotyping and distribution of types associated with hand, foot and mouth disease and herpangina, France, April 2014–March 2015. A) Semi-nested reverse transcription PCR (RT-PCR) A using primers specifically developed for enterovirus types belonging to the EV-A species was first performed for all clinical samples except 1. For this sample, the viral load was low, and the nested RT-PCR described by Nix et al. (*27*) was performed directly. If the semi-nested RT-PCR A was negative, the genotyping was alternatively performed by a semi-nested RT-PCR B with primers specific to the EV-B species (*28*) or a nested RT-PCR (*27*). B) Among other EV-A species, 5 different types were identified: coxsackievirus (CV) A4, n = 18; CV-A8, n = 16; CV-A2 and CV-A5, n = 5 each; and CV-A12, n = 1. Among EV-B species, 12 different types were identified: echovirus (E) 16 (E-16) and E-18 (n = 5 each); E-11 and coxsackievirus B3 (CV-B3; n = 4 each); CV-B1, CV-B2, CV-B4, CV-A9, and E-6 (n = 2 each); and E-3, E-5, and E-25 (n = 1 each). EV-A, *Enterovirus A*; EV-A71, enterovirus A71; EV-B, *Enterovirus B*; RT-PCR, reverse transcription PCR.

Statistical Analyses

We performed statistical analyses with Stata 13 software (StataCorp LP, College Station, TX, USA). The tests were 2-sided, with a type I error set at $\alpha = 0.05$. Patient characteristics were presented as mean (±SD) for continuous data (assumption of normality assessed by the Shapiro–Wilk test) and as the number of patients and associated percentages for categorical parameters. We classified patients according to statistical distribution and epidemiologic relevance into 4 age groups: 1) <1 year old, 2) ≥1 year old, 3) 2 to <3 years old, and 4) ≥3 years old. We compared the independent groups (i.e., age groups and CV-A6 infections [yes/no]) by χ^2 or Fisher exact test for categorical variables and by analysis of variance (ANOVA) or Kruskall-Wallis test for quantitative parameters (assumption of homoscedasticity analyzed by Fisher-Snedecor test). When appropriate (ANOVA or Kruskall-Wallis; $p<0.05$), we performed post hoc tests (Tukey-Kramer after ANOVA and Dunn after Kruskall-Wallis test) for multiple comparisons, particularly for comparisons between classes of age. Multivariate analyses (logistic regression for dichotomous independent variable) were performed to take into account adjustment on covariates fixed according to univariate results and clinical relevance (i.e., age at enrolment and time between onset and consultation).

Results

Of the 659 children enrolled in the study, 523 (79.3%) had an enterovirus infection. Ten patients experienced 2 episodes of HFMD/herpangina during the study period and had a specimen collected; 2 successive enterovirus infections associated with different serotypes occurred in 7 children at intervals of 3 weeks to 5.5 months. For the other 3 patients, only 1 episode was associated with an enterovirus infection.

Overall, 669 specimens were analyzed, of which 530 (79.2%, 95% CI 75.9–82.2) tested positive for enterovirus (Figure 2). Mean patient enterovirus positivity rate for participating pediatricians was 75.1% (range 50.0%–88.9%). Enterovirus-associated HFMD/herpangina showed biannual peaks of activity in early summer (weeks 25–27) and in autumn (week 42) (Figure 3).

An enterovirus serotype was identified for 527/530 (99.4%) of proven infections. The most frequent EV-A serotype was CV-A6 (286/530, 53.9%) followed by CV-A16 (123/530, 23.2%), CV-A10 (39/530, 7.3%), CV-A4 (18, 3.3%), CV-A8 (16, 3.0%), CV-A2 (5, 0.9%), and CV-A5 (5, 0.9%); 1 infection was CV-A12 (Figure 1, panel B). Twelve EV-B serotypes were identified: echovirus 16 (E-16) and E-18 (5 each); E-11 and coxsackievirus B3 (CV-B3) (4 each); CV-B1, CV-B2, CV-B4, CV-A9, and E-6 (2 each); and E-3, E-5, and E-25 (1 each). CV-A6 was predominant during both epidemic waves. Six EV-A71 infections were detected, most associated with typical HFMD (5/6, 83.3%). One patient had generalized eruption. Fever was reported for only 2/6 patients. EV-A71 strains belonged to subgenotypes C2 (n = 5) and C4 (n = 1). The C4 strain was identified in a 3-year-old child from Guangzhou, China, who was on a visit to France (data not shown).

The mean age of enterovirus-infected children was 2.1 years (range 1 month–10.5 years). The highest rate of infections was observed in children 1–2 years of age. Fever was reported in 397/530 (74.9%) of enterovirus-infected children. Cutaneous eruption was observed in 456/530 (86%) children and affected, in decreasing order, the palms, soles, buttocks, and elbows. HFMD was the predominant clinical presentation (342/530, 64.5%). Herpangina was reported in 304/530 (57.4%) of cases and was associated with clinical signs of HFMD in 241/304 (79.2%). Lesions were also frequently observed on the limbs (188/530, 35.4%) and the face (perioral and earlobes, 161/530, 30.4%). Atypical HFMD was observed in 247/530 (46.6%) children (Table 1). The proportions of enterovirus-infected children were not significantly different between children presenting with

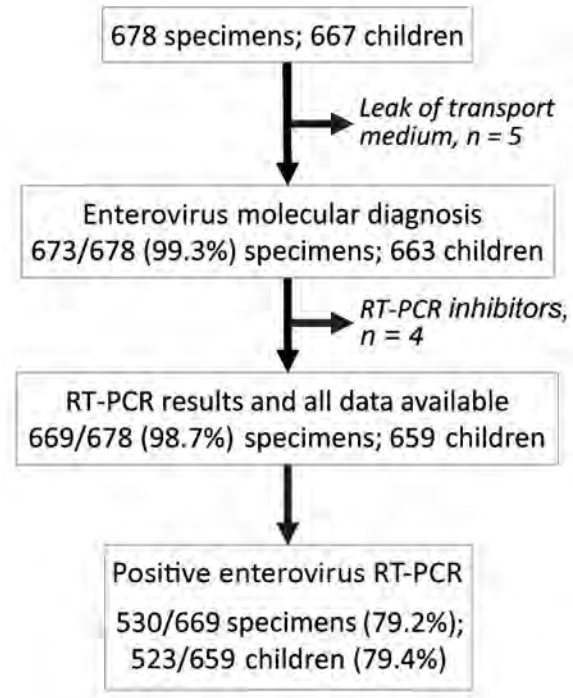

Figure 2. Participant flow diagram of enterovirus testing for the surveillance of hand, foot and mouth disease and herpangina, France, April 2014–March 2015. RT-PCR, reverse transcription PCR.

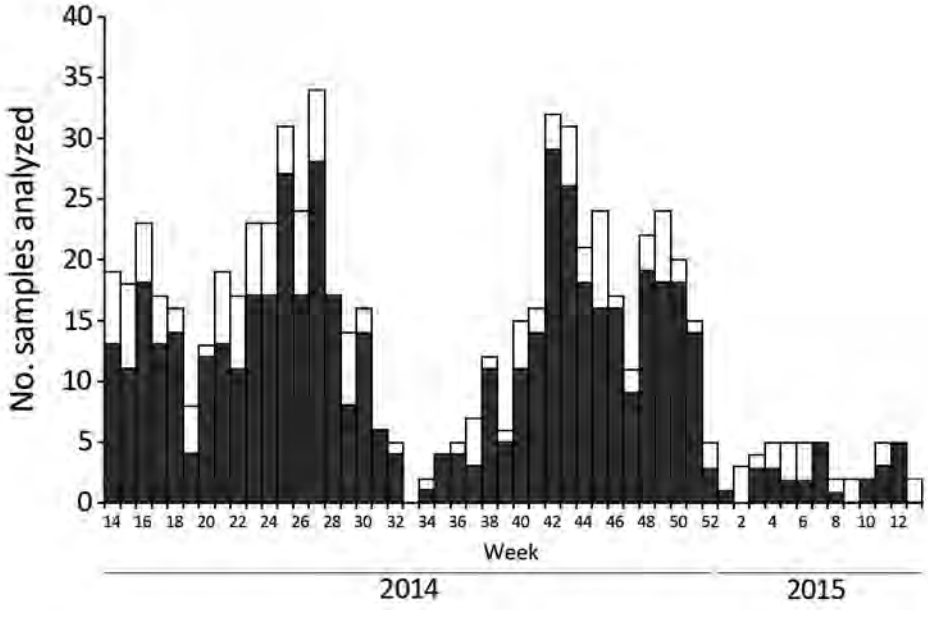

Figure 3. Weekly distribution of enterovirus infections associated with hand, foot and mouth disease and herpangina, France, April 2014–March 2015. Bar sections represent the number of enterovirus-positive (dark gray) and -negative (white) samples analyzed.

Table 1. Demographic and clinical features of patients with CV-A6 infections compared with those with non–CV-A6 infections, France, April 2014–March 2015*

Characteristic	All enterovirus infections, n = 530	CV-A6 infections, n = 286	Non–CV-A6 infections, n = 210	p value†
Age at enrollment, y, mean (SD)	2.1 (1.41)	1.69 (0.93)	2.53 (1.78)	**<0.001**
Male sex, no. (%)	290/523 (55.4)	152/281 (54.1)	123/209 (58.9)	0.29
Time between onset and consultation, d, mean (SD)‡	1.92 (1.35)	2.13 (1.49)	1.63 (1.06)	**<0.001**
Signs and symptoms, no. (%)				
Fever	397 (74.9)	220 (76.9)	147 (70.0)	0.08
Oral ulcerations	224 (42.3)	101 (35.3)	118 (56.2)	**<0.001**§
Gingivostomatitis	79 (14.9)	36 (12.6)	42 (20)	**0.02**
Eruption	456 (86.0)	268 (93.7)	163 (77.6)	**<0.001**§
Vesicular eruption	355 (70.3)	226/278 (81.3)	119/197 (60.4)	**<0.001**§
Nonvesicular eruption	160 (30.2)	94 (32.9)	53 (25.2)	0.07
Localizations of eruption, no. (%)				
Palms	308 (58.1)	190 (66.4)	114 (54.3)	**0.006**§
Soles	279 (52.6)	160 (55.9)	111 (52.9	0.49
Buttocks	251 (47.4)	171 (59.8)	73 (34.8)	**<0.001**§
Elbows / knees	133 (25.1)	90 (31.5)	40 (19.0)	**0.002**§
Lower limbs	170 (32.1)	131 (45.8)	27 (12.9)	**<0.001**§
Upper limbs	119 (22.5)	83 (29.0)	24 (11.4)	**<0.001**§
Generalized eruption	46 (8.7)	27 (9.4)	14 (6.7)	0.27
Trunk	23 (4.3)	13 (4.5)	5 (2.4)	0.20
Face, including perioral ulcerations	161 (30.4)	134 (46.9)	24 (11.4)	**<0.001**§
Diagnosis, no. (%)				
Typical HFMD¶	95 (17.9)	32 (11.2)	62 (29.5)	**<0.001**§
Atypical HFMD	247 (46.6)	181 (63.3)	59 (28.1)	**<0.001**§
Herpangina	304 (57.4)	165 (57.7)	110 (52.4)	0.24
Herpangina alone	63 (11.9)	15 (5.2)	40 (19.0)	**<0.001**§
Other signs, no. (%)				
Digestive signs	61 (11.5)	38 (13.3)	15 (7.1)	**0.03**§
Ear, nose, and throat signs	54 (10.2)	27 (9.4)	26 (12.4)	0.29
Respiratory signs	15 (2.8)	6 (2.1)	7 (3.3)	0.39

*Non–CV-A6 infections only include infections by another type belonging to the enterovirus A species. CV-A6, coxsackievirus A6; HFMD, hand, foot and mouth disease.
†Statistical analyses were performed to compare clinical characteristics of CV-A6 and non–CV-A6 infections. Significant p values (p<0.05) are indicated in bold type.
‡Time from symptom onset and consultation was available for 525 episodes of enterovirus infection.
§Indicates significant differences between CV-A6 infections and non–CV-A6 infections by multivariate analyses.
¶Typical HFMD was defined as the presence of ≥2 of the following signs: oral ulcerations, eruption on palms, soles, buttocks, knees, or elbows, excluding any other localization. Atypical HFMD was defined by the presence of ≥2 of those signs plus the involvement of another anatomic site.

typical HFMD (95/116, 81.9%) or atypical HFMD (247/281, 87.9%).

"Eczema coxsackium or herpeticum" was reported in 8 children, of whom 7 had a CV-A6 infection and 1 a CV-A10 infection. An eruption mimicking a Gianotti-Crosti syndrome caused by different enterovirus serotypes (CV-A6 [n = 3]; CV-A10 [n = 1]; or CV-A16 [n = 1]) was reported in 5 of these 8 children. Exposure to ill contacts was reported for 155/469 (33%) enterovirus-infected children with available environmental data, a proportion significantly higher than that among non–enterovirus-infected children (25/127, 19.7%; p = 0.004).

Patients with CV-A6 HFMD/herpangina were significantly younger than patients with other EV-A serotypes (p<0.001) (Table 1). The clinical features of CV-A6-associated HFMD were also significantly different. Atypical HFMD was more frequently reported in CV-A6-infected children (181/286, 63.3%) than in children infected with other enterovirus serotypes: CV-A16 (42/123, 34.1%), CV-A10 (14/39, 35.8%), CV-A4 and E-16 (2 cases each), and CV-A12, E-6, E-11, E18, or CV-B4 (1 case each). Typical HFMD or herpangina alone were significantly more frequent in children infected by the other EV-A serotypes (p<0.001) (Table 1). The most frequent serotypes associated with typical HFMD were CV-A16 (54/95, 56.8%) and CV-A6 (32/95, 33.7%), followed by EV-A71 (n = 4), CV-A8 and CV-A10 (n = 2 each), and E-5 (n = 1). The most frequent serotypes associated with herpangina alone were CV-A6 (14/63, 22.2%), CV-A4 (11/63, 17.4%), CV-A8 (10/63, 15.9%), and CV-A10 (8/63, 12.7%). The differences between CV-A6 infections and non–CV-A6 infections remained statistically significant by multivariate analyses.

Atypical HFMD was less frequent in patients <1 year of age than in children 1–2 and ≥3 years of age. Herpangina alone was more frequent in children <1 year of age than in older children (Table 2).

The CV-A6 strains sampled in France in 2014 and 2015 were grouped in 6 co-circulating lineages supported by high posterior probability values (Figure 4). The nucleotide identities within lineages ranged from 95.3% to 98.9% (98.3%–99.5% amino acid identities). Between

Table 2. Demographic and clinical features associated with CV-6 infections in 4 age groups of patients, France, April 2014–March 2015*

Characteristic	<1 y, n = 63	1–2 y, n = 146	2–3 y, n = 50	≥3 y, n = 26	p value†
Male sex, no. (%)	35/62 (56.4)	80/146 (55.5)	26/49 (53.1)	10/25 (40	0.52
Time between onset and consultation, d, mean (SD)‡	1.84 (1.31)	2.27 (1.43)	1.87 (1.48)	2.46 (2.02)	0.09
Signs and symptoms, no. (%)					
Fever	54 (85.7)	105 (71.92)	38 (76)	22 (84.6)	0.13
Oral ulcerations	17 (27)	53 (36.3)	19 (38)	12 (46.1)	0.32
Perioral ulcerations	18 (28.57)	67 (45.9)	25 (50)	15 (57.7)	**0.003**
Eruption	53 (84.1)	143 (97.9)	45 (90)	26 (100)	**0.001**
Vesicular eruption	43 (71.7)	120 (83.9)	41 (83.7)	21 (84)	0.21
Nonvesicular eruption	20 (31.7)	55(37.7)	11 (22)	7 (26.9)	0.20
Localizations of eruption, no. (%)					
Palms	41 (65.1)	96 (65.75)	33 (66)	20 (76.9)	0.71
Soles	34 (54)	86 (58.9)	24 (48)	16 (61.5)	0.53
Buttocks	29 (46)	100 (68.5)	30 (60)	12 (46.1)	**0.009**
Elbows or knees	15 (23.8)	49 (33.6)	16 (32)	10 (38.5)	0.46
Lower limbs	20 (31.8)	75 (51.4)	26 (52)	10 (38.5)	**0.04**
Upper limbs	8 (12.7)	55 (37.7)	17 (34)	3 (11.5)	**<0.001**
Generalized eruption	9 (14.3)	14 (9.6)	3 (6)	1 (3.9)	0.41
Trunk	ND	ND	ND	ND	ND
Face, including perioral ulcerations	21 (33.3)	70 (48)	27 (54)	15 (57.7)	0.07
Diagnosis, no. (%)					
Typical HFMD§	10 (15.9)	17 (11.6)	3 (6)	2 (7.7)	0.38
Atypical HFMD	30 (47.6)¶	100 (68.5)¶	32 (64)	19 (73.1)¶	**0.02**
Herpangina	45 (71.4)	76 (52.1)	29 (58)	15 (57.7)	0.08
Herpangina alone	9 (14.3)	2 (1.4)	4 (8)	0	**0.001**
Other signs, no. (%)					
Digestive signs	7 (11.1)	16 (11)	10 (20)	5 (19.2)	0.28
Ear, nose, and throat signs	4 (6.4)	18 (12.3)	3 (6)	2 (7.7)	0.41
Respiratory signs	ND	ND	ND	ND	ND

*For 1 patient, age was not known. CV-A6, coxsackievirus A6; HFMD, hand, foot and mouth disease; ND, not determined because of low sample size (<15 patients total).
†Significant p values (p<0.05) are indicated in bold type.
‡Time from symptom onset and consultation was available for 525 episodes of enterovirus infection.
§Typical HFMD was defined as the presence of ≥2 of the following signs: oral ulcerations, eruption on palms, soles, buttocks, knees, or elbows, excluding any other localization. Atypical HFMD was defined by the presence of ≥2 of those signs plus the involvement of another anatomic site.
¶Indicates significant differences between age groups.

lineages, the nucleotide identities ranged from 91.9% to 96.3% (97.5%–98.4% amino acid identities), the highest divergence being observed between lineages 1 and 2 and the lowest between 5 and 6. In lineage 1, the virus strains collected in France were temporally distantly related to viruses collected 1–3 years earlier in the United Kingdom. In the other 5 lineages, the CV-A6 virus strains sampled in France displayed close temporal relationships to viruses recovered in other countries in Europe since 2010. In the lineages 2 and 6, close genetic and temporal relationships were also estimated between virus strains recovered in France and countries in Asia. The 2014 CV-A6 viruses in lineage 3 were genetically related to those recovered in 2010 in France.

Discussion

This prospective ambulatory clinic–based surveillance of HFMD/herpangina in children revealed the global effect of these diseases in France. Data were collected from a standardized report of clinical signs, which provided a comprehensive description of the clinical characteristics of these syndromes associated with different enterovirus serotypes. During April 2014–March 2015, CV-A6 infections were associated with HFMD in 74.4% cases and herpangina in 57.7% of cases. These proportions were inverted during the 2010 HFMD outbreak in central France: 50% for HFMD cases and 70% for herpangina (*13*). In addition, the dermatologic presentation of CV-A6 HFMD cases was more frequently unusual (63.3%), with eruptions extending beyond the typical sites of HFMD (i.e., soles, palms, buttocks, and knees or elbows) than in HFMD cases caused by other enterovirus serotypes. Our findings suggest that the clinical presentation of CV-A6 infections in France shifted to atypical HFMD during 2010–2014, as observed in China during 2008–2013 (*18*). In our study, the co-circulation of 6 virus lineages in 2014 is consistent with the hypothesis of multiple introductions of genetically distinct CV-A6 strains. In addition, a genetic analysis of complete CV-A6 genomes showed that strains collected during the 2012–2013 outbreak in Shanghai, China, were recombinant compared with strains collected before 2009 and were more frequently associated with generalized rash (*29*).

Comparative analyses of whole virus genomes should be expanded on large sequence data derived from prospective epidemiologic studies to investigate whether the

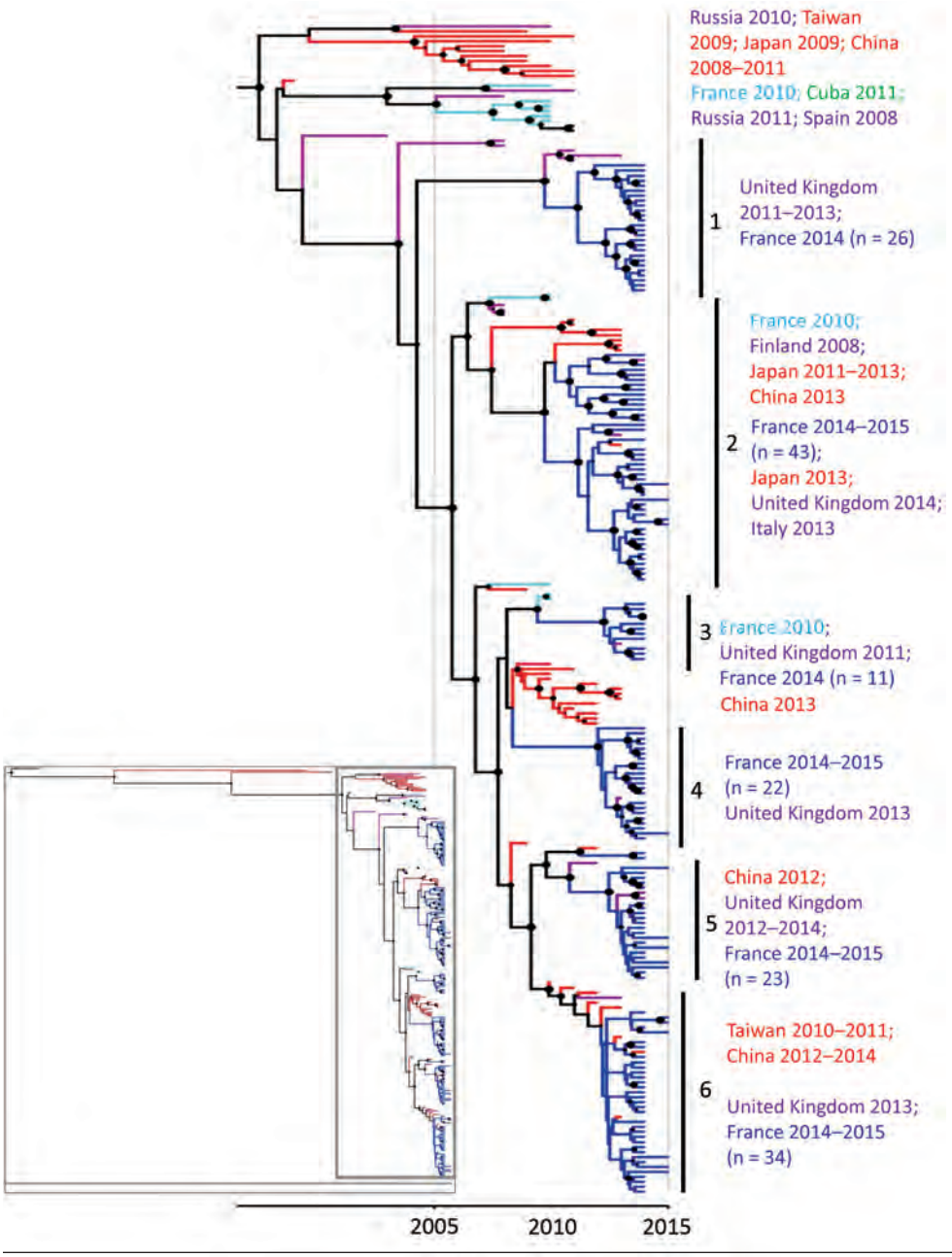

Figure 4. Phylogenetic tree based on partial viral protein (VP1) coding sequences of coxsackievirus (CV) A6, France, April 2014–March 2015. The maximum credibility tree is inferred with the partial VP1 sequence (369 nt, position 2,441–2,808 relative to the Gdula CV-A6 prototype strain). The phylogenetic relationships were inferred with a Bayesian method by using a relaxed molecular clock model. The tree was reconstructed using Figtree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). For clarity, the sequence names are not included in the tree. Circle sizes are proportional to posterior probability. Each tip branch represents a sampled virus sequence. The continents/countries where the virus strains were sampled are indicated by different colors: Europe, purple; France 2010, light blue; France 2014–2015, dark blue; the Americas, green; Asia, red. The inset shows the complete tree, with the box indicating the portion enlarged for clarity.

changes in the clinical features of CV-A6 infections reported here are determined by viral factors. The relationship between CV-A6 and atypical HFMD has been reported in earlier studies that described frequent unusual morphology or extent of cutaneous findings (*15*,*20*–*25*,*30*), such as "eczema coxsackium," Gianotti-Crosti-like eruption, and purpuric eruption (*21*). However, these studies were either retrospective, focused on severe or atypical HFMD, or performed in dermatology pediatric centers, which might have biased the clinical spectrum of CV-A6 HFMD cases toward more severe or atypical presentations. Although we cannot exclude the possibility that pediatricians were more prone to include children with unusual presentations of HFMD, our study confirms that CV-A6 is more frequently associated with atypical HFMD even in an ambulatory setting.

Of note, atypical HFMD was reported in 66/244 (27%) of non–CV-A6-associated HFMD cases. This result might be attributable to the definition of atypical HFMD we used, which was, in contrast to that of typical HFMD (*8*), the involvement of a nontypical anatomic site for HFMD. The lack of a consensus definition of atypical HFMD and the fact that the collected clinical data vary between studies hamper rigorous comparisons between them. Further investigations based on prospective ambulatory clinic–based

surveillance of HFMD are needed to determine whether our observation is attributable to a specific increase in the circulation of CV-A6 or to a global change in the transmission of enterovirus strains. The documentation of unusual presentations of HFMD by enterovirus genotyping is useful for detecting the emergence of a new serotype with distinct clinical features.

The clinical courses of typical and atypical HFMD seemed similar in our cohort because no complications were reported. The extensive or unusual nature of the cutaneous manifestations of CV-A6 HFMD does not seem to increase the risk for severe systemic illness (*21*). Although to a lesser extent than with EV-A71, severe CV-A6 HFMD cases, defined by the presence of neurologic signs (e.g., meningitis, encephalitis, acute flaccid paralysis, and seizures) or cardiopulmonary signs, have been reported, with a frequency ranging from 3.6 % to 18.2% during the recent CV-A6 outbreaks in China (*18*,*31*–*34*). Meningitis rather than encephalitis was more frequently associated with severe CV-A6 HFMD (*32*,*33*). However, clinicians should be aware of the potential neurotropism of all EVs. As exemplified by EV-A71 outbreaks (*35*) and more recently EV-D68 outbreaks (*2*), the high rate of circulation of CV-A6, either symptomatic or asymptomatic, can lead to the more frequent observation of this serotype in association with neurologic signs.

As is the case with many common and self-limited illnesses, the children in our study might not have attended all medical consultations, thus rendering the surveillance incomplete. The comprehensive recruitment of all children with HFMD/herpangina is time-consuming and not feasible in the routine practice of ambulatory pediatrics, which might have resulted in a lower inclusion rate. We do not have recorded long-term follow-up or the specific CV-A6–associated clinical entities described by Mathes et al. (*13*), and we were not able to assess the occurrence of onychomadesis or desquamation of the extremities, which have been frequently associated with CV-A6 outbreaks (*9*–*11*,*15*,*20*,*32*).

This study contributes to a more comprehensive view of the epidemiology of HFMD/herpangina in France and the clinical spectrum of HFMD/herpangina associated with enterovirus, in particular with CV-A6. The often unusual presentation of HFMD can be challenging for clinicians, and this study might therefore help improve the differential diagnosis of HFMD by primary care physicians and the detection of future HFMD outbreaks. Syndromic surveillance of HFMD/herpangina by pediatricians in ambulatory setting with prospective and standardized collection of clinical data in combination with enterovirus testing and genotyping are useful for monitoring the epidemiology of enterovirus infections, for the timely detection of peaks of highest activity, and for determining the enterovirus serotypes involved, leading to better detection of outbreaks associated with EV-A71 or any other serotype associated with severe or distinct clinical features.

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Increased Hospitalizations for Neuropathies as Indicators of Zika Virus Infection, according to Health Information System Data, Brazil

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Evidence is increasing that Zika virus can cause extensive damage to the central nervous system, affecting both fetuses and adults. We sought to identify traces of possible clinical manifestations of nervous system diseases among the registers of hospital admissions recorded in the Brazilian Unified Health System. Time series of several diagnoses from the International Classification of Diseases, 10th Revision, were analyzed by using control diagrams, during January 2008–February 2016. Beginning in mid-2014, we observed an unprecedented and significant rise in the hospitalization rate for congenital malformations of the nervous system, Guillain-Barré syndrome, encephalitis, myelitis, and encephalomyelitis. These conditions are compatible with viral infection and inflammation-associated manifestations and may have been due to the entrance of Zika virus into Brazil. These findings show the necessity of adequately diagnosing and treating suspected cases of Zika virus infection and also that health surveillance systems can be improved by using routine data.

The recent spread of Zika virus across the globe has worried citizens and public health authorities. In late February 2016, the World Health Organization (WHO) declared an international state of emergency because of the advance of microcephaly associated with the Zika virus (*1*). Previously considered a minor infection with mild symptoms and a low mortality rate, infection with Zika virus was seen as less severe than that caused by other arboviruses transmitted by mosquitoes of the genus *Aedes*, such as dengue, yellow fever, and chikungunya viruses.

However, recent studies have shown that the infection has a high potential for causing damage to the central nervous system (CNS), leading to certain congenital malformations (*2*) and neuropathies, such as Guillain-Barré syndrome (GBS) (*3*–*6*). The neurotropic nature of the virus and its ability to cross the blood-brain and placental barriers (*7*) were demonstrated in laboratory experiments, and damage to developing brains has been shown by imaging of fetuses and newborns (*8*). Other neuropathies may be associated with the Zika virus and could have passed unnoticed through health information systems.

Some studies suggest that sporting events in 2013 and 2014 may have led to the introduction of Zika virus into Brazil (*9*). Further spread to other regions might have occurred by 2014. The first warnings of the epidemic in Brazil came on April 20, 2015 (*10*), with a report about the large number of cases of rash of unknown origin, which began at the end of December 2014 in various regions of the state of Pernambuco, followed by an alert issued by the state of Rio Grande do Norte (*11*). In May 2015, the spread of Zika virus among the local populations was laboratory confirmed, first in the Pernambuco and Bahia, then in other states of the center-west and southeast regions (*12*). The distribution of Zika virus in Brazil during 2014 and 2015 is summarized in Figure 1, according to case reports and epidemiologic data produced by the Federal Ministry of Health and state secretaries of health (*13*).

One strategy for acquiring morbidity data is to analyze hospital admission records. The Hospital Information System (SIH, in Portuguese) is primarily financial and administrative in nature, but it can be used as a data source for epidemiologic studies and surveillance because of advantageous characteristics, such as national coverage, relative consistency and standardization in terms of data generation, and rapid production and dissemination of data. These features facilitate its incorporation into epidemiologic alert systems, especially those for infectious diseases.

An analysis of hospital admissions data can provide a baseline that enables identification of trends in the number of admissions for neuropathies that affect the nervous system, as well as inflection points that depart abruptly from these trends. Long-term analyses of diseases potentially associated with Zika virus continue to be nonexistent in Brazil and have become urgently needed to bring

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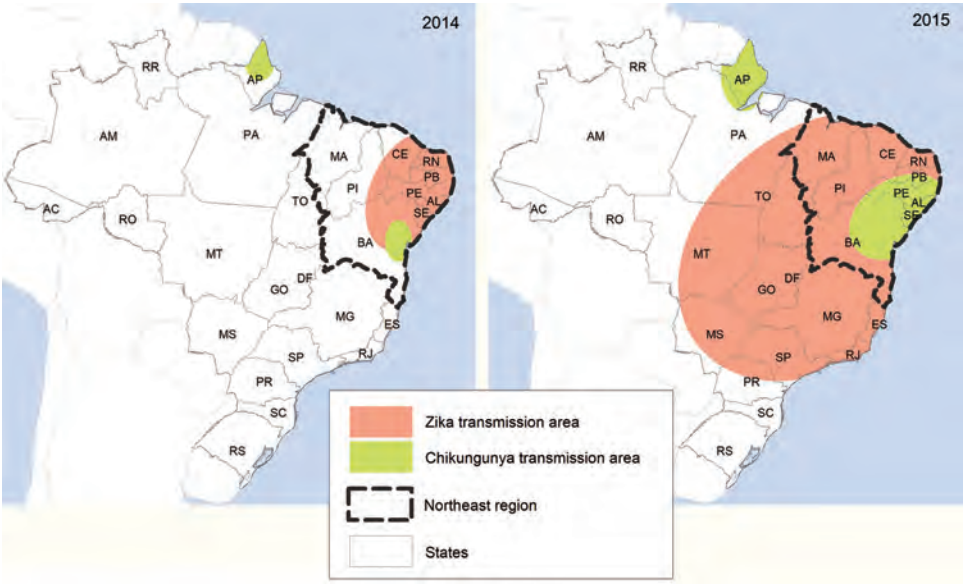

Figure 1. Approximate distribution of areas of local spread of Zika virus in Brazil, 2014 and 2015. Adapted from (*13*). State abbreviations: AC, Acre; AL, Alagoas; AP, Amapá; AM, Amazonas; BA, Bahia; CE, Ceará; GO, Goiás; DF, Distrito Federal; ES, Espírito Santo; MA, Maranhão; MT, Mato Grosso; MS, Mato Grosso do Sul; MG, Minas Gerais' PA, Pará; PB, Paraíba; PR, Paraná; PE, Pernambuco; PI, Piauí; RJ, Rio de Janeiro; RN, Rio Grande do Norte; RS, Rio Grande do Sul; RO, Rondônia; RR, Roraima; SP, São Paulo; SC, Santa Catarina; SE, Sergipe; TO, Tocantins.

into focus the scope of the problem, as well as delineate monitoring strategies for the epidemic in Brazil and other affected countries.

The main objective of this study was to analyze temporal patterns in hospitalizations in separate Brazilian regions during January 2008–February 2016, for neuropathies, which could have been associated with Zika virus infection in separate Brazilian regions. This period includes the time before the epidemic and the onset of the Zika epidemic in Brazil.

Methods

This is a descriptive study of a time series from a population dataset. We sought to evaluate historical trends in neurologic conditions potentially related to infection with Zika virus, based on the principal diagnosis assigned at discharge as the main reason for hospitalization. Data were made available by the Informatics Department of the National Health System through the Hospital Information System during January 1, 2008–February 29, 2016, according to each patient's state of residence.

We selected the health problems analyzed in this study on the basis of monitoring protocols put in place to track reports of microcephaly related to infection with Zika virus (*14*) and track neurologic conditions for which the patient had a history of previous viral infection (*15*). We considered all hospital admissions for the following: 1) congenital malformations of the nervous system (International Classification of Diseases, 10th Revision [ICD-10] Q00-Q07) in children <1 year of age, 2) GBS (ICD-10 G610; 3) other potential and unspecific clinical manifestations such as encephalitis, myelitis, and encephalomyelitis (ICD-10 G040-G049); 4) abortion and related problems (ICD-10 O03-O07); in particular, 5) spontaneous abortion (ICD-10 O03). In Brazil, abortion is permitted in cases of risk to the mother and fetus, as well as in cases of sexual violence. Other cases of induced abortion are attended to in hospitals when the woman initiates an abortion, when an incomplete abortion leads to complications, or in cases of hemorrhage or infection (*16*).

Time series were assembled by using aggregate data tabulated by month and region. Because the Northeast region was, until the end of 2015, the center of the Zika outbreak and of the surge in microcephaly cases, we analyzed this region and compared the results with those from the other Brazilian regions in aggregate. We calculated crude hospital admission rates for each month of the study using population estimates provided by the Brazilian Geography and Statistics Institute (Brazil). With respect to congenital malformations of the nervous system in children <1 year of age, we calculated the rate of hospitalizations using the number of live births per month as the denominator. In the case of abortion, we obtained the rate of hospitalizations using the number of live births per month as the denominator with a lag of 7 months, which reduced the effect of seasonality so common in data for births as well as for abortions.

The total number of hospitalizations can vary greatly by state due to variations in staffing at the local level and disparities in access to services for utilizers of the public health system (*17*). To control for the effect of the capacity of health services to provide hospital beds, we used the same techniques and parameters for the analysis of the general causes of hospitalizations, which include admissions for malformations among newborns (ICD-10, Chapter XVII: Congenital Malformations, Deformities and Chromosomal Anomalies) and all admissions for nervous system diseases (ICD-10, Chapter VI).

We compared the monthly hospitalization rates of the Northeast region with rates in non-Northeast Brazilian regions, added to a 95% CIs (1.96-fold SDs from the mean) as upper limits for detection of peaks in the time series. These data were plotted as control charts, which allowed for evaluation of long-term trends, seasonality, and anomalies in the series by visual inspection. We tested the difference between observed and expected number of hospitalization—assuming the historical baseline as a control parameter—by calculating the rate ratio (IRR) and CI using Poisson regression.

Results

The rate of hospitalizations for congenital malformations of the nervous system (ICD-10 Q00-Q07) presented a stable mean value of 40/100,000 live births in the Northeast region until September 2015. As of November 2015, however, we observed an increase in this rate, which, in the last months of the series, reached a mean value of 170 hospitalizations for congenital malformations of the nervous system per 100,000 live births, an increase ≈4 times higher than historical rates (IRR 4.2; 95% CI 3.8–4.6). In February 2016, all regions exhibited rates of ≈100 hospitalizations for congenital malformations of the nervous system per 100,000 live births, a 2-fold increase over the national historical baseline. From November 2015 through February 2016, a total of 1,027 hospitalizations for congenital malformations of the nervous system were recorded nationwide; 448 of these occurred in the Northeast region. This region is responsible for ≈830,000 (28%) of the overall annual live births in Brazil.

In the Northeast region, the hospitalization rate for GBS (ICD-10 G610) was 0.05/100,000 residents until May 2015, when an outbreak occurred, which peaked in July 2015. From June 2015 through February 2016, the hospitalization rate was 0.11/100,000 residents, an increase by a factor of 2.7 (95% CI 2.5–3.0). During July through October 2015, 377 GBS hospitalizations were recorded in the Northeast region, an excess of ≈240 hospitalizations in the region.

The hospitalization rate for CNS inflammatory diseases, represented by encephalitis, myelitis, and encephalomyelitis (ICD-10 G040-G049), increased in the Northeast region, principally as of September 2014, from a baseline of 0.05 hospitalizations/100 residents to 0.11 hospitalizations/100,000 residents (IRR 2.0; 95% CI 1.9–2.2). In the rest of the country, a stable base line of 0.05 hospitalizations/100,000 residents was observed. From September 2014 through February 2016, hospitalizations for encephalitis, myelitis, and encephalomyelitis in the Northeast region reached 1,115, an excess of ≈570 hospitalizations.

Figure 2 shows the evolution during 2008–2016 of the rate of congenital malformations of the nervous system in children <1 year of age (per 100,000 live births) and of the rates of hospitalization (per 100,000 residents) for GBS and encephalitis, myelitis, and encephalomyelitis in the Northeast region compared with rates in the rest of the country. Figure 2, panel A, shows the abrupt increase in hospitalization rates for congenital malformations of the nervous system among children <1 year of age as of October 2015 in the Northeast region. Meanwhile, the rest of the country exhibited a slight increase in these same rates. Some peaks of hospitalizations can be seen in August 2014 and February 2015, when the rate observed in the Northeast region surpasses the CI for the rate in the rest of the country.

With respect to GBS (Figure 2, panel B), hospitalizations peaked sharply during June–August 2015 in the Northeast region. In a more detailed analysis, we identified the first peak of GBS in Pernambuco State during May 2015, followed by the states of Ceará, Bahia, and Alagoas in the subsequent months. This trajectory is consistent with the early reports of Zika and rash epidemic epidemiologic alerts (*11*,*12*).

A historical series of the rates of hospitalization for inflammatory diseases of the CNS, represented by encephalitis, myelitis, and encephalomyelitis, shows a pattern change in recent years (Figure 2, panel C). Until 2013, the rates of the series in the Northeast region varied within the range of the rest of Brazil. By the end of 2014, the Northeast region showed an increase in relation to the rest of the country, with values above the CIs for the series for the rest of Brazil. In mid-2015, the number of hospitalizations peaks again during August–October. This pattern suggests a more accentuated annual cycle, showing peaks during the second semester of the 2014 and 2015.

We did not observe changes for abortions and related problems in the times series, including for spontaneous abortions (results not shown). We also did not observe changes in the total number of hospitalizations or in hospitalizations for nervous system problems (ICD-10, Chapter VI) in the regions during the study period. This pattern may demonstrate the stability of the health system in terms of specialty beds, especially for neurology.

Discussion

In this study, we analyzed time trends for hospitalizations for some neuropathies. We found increases in the number of hospitalizations for congenital malformations of the nervous system, GBS, and some inflammatory diseases of the CNS. These complications began to cause more hospitalizations, with strong fluctuations over the course of the study period, beginning even before the first warning in November 2015 about the possible effects of infection with Zika virus on microcephaly and other malformations. These shifts from the previous baseline were not, therefore, influenced by new procedures and norms that have been

incorporated into the health system over the last months. As such, the complications that we present here can be thought of as early signals of neurologic complications that were unperceived through health surveillance systems, but that may be related to the entrance and circulation of the Zika virus in Brazil, especially in the Northeast region.

Our results show that the rates of hospitalization in the Northeast region for some neuropathies were subject to abrupt changes beginning in mid-2014, when it is believed that Zika virus was introduced to the country (*9*) (and later produced outbreaks in 2015). Conversely, the onset of hospitalizations for congenital malformations of the nervous system observed in November 2015 may be a result of the first public health warnings about Zika-related microcephaly, issued in the same month. Taking into consideration the fact that CNS malformations originate, typically, in the first 3 months of gestation (*18*), infection may have occurred in mid-2015, which coincides with the observed peaks of GBS and CNS inflammatory diseases. The hospitalization rates have slightly increased nationally during the first months of 2016, probably in reaction to warnings about microcephaly and the ensuing necessity to keep neonates hospitalized to investigate potential cases.

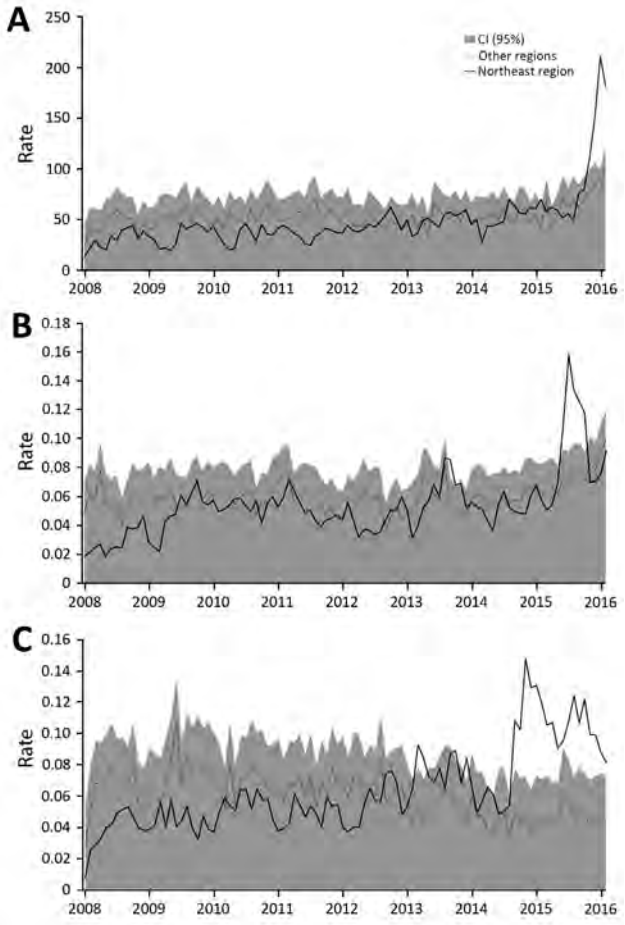

Figure 2. Hospitalizations in the Northeast region of Brazil, indicated by codes from the International Classification of Diseases, 10th Revision, January 2008 through February 2016. A) Congenital malformations of the nervous system (ICD-10; Q00-Q07) per 100,000 live births; B) Guillain-Barré syndrome (ICD-10; G610) per 100,000 residents; and C) encephalitis, myelitis and encephalomyelitis (ICD-10; G040-G049) per 100,000 Northeast region residents, compared with hospitalizations for these conditions in the rest of the country. Source: Hospital Information System (SIH); www.datasus.gov.br.

With respect to problems related to abortion, including spontaneous abortion, hospitalizations rates remained stable over the study period. We expected an increase in the number of abortions and related problems, especially spontaneous abortions, which can occur in cases of serious congenital abnormalities (*19*). Equally, public health warnings about the surge in microcephaly cases may have provoked an increase in the number of induced abortions. In Brazil, legislation permits abortion in cases of risk to the mother and fetus, or in cases of sexual violence, but studies have shown that health information systems substantially underestimate abortion, and also document bureaucratic delays of health systems in performing legal abortions (*16*). The possibility of an increase in the number abortions and related complications in the coming months should not be discounted.

The concentration of neuropathies in the Northeast states remains a mystery for researchers and health surveillance services. First, we can assume that the virus entered Brazil mainly in this region (*12*,*20*) and that, with the spread of the epidemic to other regions, the number of cases of neuropathies will increase in the rest of the country. On the other hand, the theory persists that other risk factors, such as chronic diseases (*21*) and co-infections (*22*,*23*), might interact with and potentiate neurologic manifestations in persons infected with Zika virus. The region had one of the worst droughts in its history during 2010–2013, resulting in contamination of drinking water, food insecurity (*24*), and outbreaks of diarrhea across the region (*25*), which could have affected the immunity status of the population. In addition, concomitant outbreaks of Zika virus infection and dengue, as was observed in French Polynesia (*26*) and in northeast region of Brazil, could potentiate the neurologic effects of infection with Zika virus. Chikungunya virus was also circulating in the region during 2014 and 2015, although in more restricted areas (Figure 1) and with less magnitude, and it can also produce severe neurologic complications such as GBS (*27*).

Another issue is the higher rate of hospitalizations during the winter months (June–September), when in general the incidence of vector-transmitted diseases, such as dengue, is lower because of milder temperatures (*28*). In this case, we can presume a latency period between infection with the Zika virus and hospitalization for neurologic complications. A study carried out in Salvador, a chief

city in the Northeast region, demonstrated a lag of 5–9 weeks (mean 2 months) between the Zika virus epidemic and cases of GBS (*29*). Another hypothesis is that transmission of the virus persists beyond seasonal limits, due to other means of transmission, such as through sexual contact (*30*).

Our study has certain limitations. First, a detection bias could exist (i.e., the alerts about the Zika epidemic could have led to greater rigor on the part of health services to diagnose and register the neuropathies and congenital abnormalities we have examined, which would result in overestimation of hospitalization rates). However, because the first alerts about microcephaly related to Zika virus infections were issued in November 2015, and only in February 2016 did WHO declare a public health emergency, the previous months' records may have not been affected by health surveillance guidelines, with the exception of microcephaly.

A second limitation concerns the fact that the health information system for hospitalizations registers events and not individual patients. As such, rehospitalizations can be contained within that figure, leading to overestimation of hospitalization rates. These 2 limitations likely do not substantially affect the results of this study because they would have had to occur uniformly across the nation in the period studied.

This study can contribute to a greater understanding of the Zika epidemic in Brazil and its additional effect on certain neuropathies. The data sources and methods we used in this study can be used for monitoring and warning systems in cases of emergency. Implementation would require obtaining and statistically analyzing time series data, to establish a baseline of adverse events and identify peaks that could represent outbreaks of disease.

To monitor the effects of Zika virus, public health researchers must consider its various clinical manifestations that may be registered in different ICD codes, even in dispersed chapters, such as viral encephalitis (ICD-10 A83) and provisional code (ICD-10 U06), as recommended by WHO. The hospital routine may account for the diversity of codes used to describe complications derived from Zika virus. The admission of a patient to the hospital is first justified by a preliminary ICD code. Throughout the period of hospitalization, new examinations and procedures are carried out, which can alter this classification. At the end of hospitalization period, a code for the principal diagnosis is assigned to the outpatient, based on procedures performed and their costs, which is not necessarily related to the disease etiology. A wide range of ICD codes should be taken into account in this situation, with anticipation of all possible reasons for hospital admission potentially related to Zika complications.

New detection tools for outbreaks and means of communicating warnings should be pursued to identify real trends and, at the same time, minimize false alarms and panic that could be provoked in populations potentially affected by the Zika epidemic. The history of neuropathies caused by viruses is about to change, not only because of the effects that Zika virus has been found to have on the CNS, but also because of the effort of gathering evidence that will help construct this history.

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Global *Escherichia coli* Sequence Type 131 Clade with $bla_{CTX\text{-}M\text{-}27}$ Gene

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The *Escherichia coli* sequence type (ST) 131 C2/*H*30Rx clade with the $bla_{CTX\text{-}M\text{-}15}$ gene had been most responsible for the global dissemination of extended-spectrum β-lactamase (ESBL)–producing *E. coli*. ST131 C1/*H*30R with $bla_{CTX\text{-}M\text{-}27}$ emerged among ESBL-producing *E. coli* in Japan during the late 2000s. To investigate the possible expansion of a single clade, we performed whole-genome sequencing for 43 Japan and 10 global ST131 isolates with $bla_{CTX\text{-}M\text{-}27}$ (n = 16), $bla_{CTX\text{-}M\text{-}14}$ (n = 16), $bla_{CTX\text{-}M\text{-}15}$ (n = 13), and others (n = 8). We also included 8 ST131 genomes available in public databases. Core genome-based analysis of 61 isolates showed that ST131 with $bla_{CTX\text{-}M\text{-}27}$ from 5 countries formed a distinct cluster within the C1/*H*30R clade, named C1-M27 clade. Accessory genome analysis identified a unique prophage-like region, supporting C1-M27 as a distinct clade. Our findings indicate that the increase of ESBL-producing *E. coli* in Japan is due mainly to emergence of the C1-M27 clade.

The global increase in resistance to the third-generation cephalosporins and fluoroquinolones among extraintestinal pathogenic *Escherichia coli* (ExPEC) is a public health concern because of the importance of these drugs in treating serious infections (*1*). The extended-spectrum β-lactamases (ESBLs), especially CTX-M types, contribute to third-generation cephalosporin resistance among ExPEC, and specific mutations in quinolone resistance–determining regions in *gyrA* and *parC* mainly contribute to fluoroquinolone resistance (*2*). The increase in resistance among ExPEC has resulted mainly from the recent expansion of a pandemic clonal group known as *E. coli* sequence type (ST) 131, which is usually multidrug resistant and is associated with CTX-M-15, the most prevalent β-lactamase among ESBL-producing ExPEC (*2*). ST131 harbors more virulence factors than other antimicrobial-resistant ExPEC and can cause severe infections (*2*,*3*).

Recent studies using whole-genome sequencing (WGS) analysis revealed that ST131 comprises different lineages or clades (*4*,*5*). Price et al. found a dominant fluoroquinolone-resistant lineage (named *H*30R) in North America that contains the *fimH* 30 allele and was associated with characteristic quinolone resistance–determining region mutations (*2*,*4*). ST131 with the $bla_{CTX\text{-}M\text{-}15}$ gene formed a distinct cluster within the *H*30R lineage, referred to as the *H*30Rx clade (*4*). Petty et al. confirmed these findings using a collection of strains from 6 countries (*5*). In their study, *H*30R and *H*30Rx clades correspond to clade C and clade C2 (subset of clade C), respectively. The other clade C subset, clade C1, included ST131 isolates with different CTX-Ms than $bla_{CTX\text{-}M\text{-}15}$.

Globally, the CTX-M-15–producing C2/*H*30Rx clade is mostly responsible for the pandemic of ExPEC with ESBLs (*2*), but in Japan, ExPEC with $bla_{CTX\text{-}M\text{-}15}$ is rare despite the predominance of ST131 among ESBL-producing isolates (*6*). Before 2005, ST131 C1/*H*30R negative for Rx containing $bla_{CTX\text{-}M\text{-}14}$ predominated among Japanese ST131 (*6*). In 2006, ST131 C1/*H*30R with $bla_{CTX\text{-}M\text{-}27}$ was detected in Japan, and the numbers of this lineage escalated since 2010 and are responsible for the substantial increase of ESBL-producing ExPEC in Japan (*6*). Moreover, $bla_{CTX\text{-}M\text{-}27}$ is confined to ST131, whereas other CTX-Ms, such as $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$, are equally present among ST131 and non-ST131 *E. coli* isolates (*3*).

$bla_{CTX\text{-}M\text{-}27}$ is an infrequent global $bla_{CTX\text{-}M}$ allele that differs by only 1 nt from $bla_{CTX\text{-}M\text{-}14}$, which results in 1 aa change at position 240 (*1*,*6*). ST131 with CTX-M-27 had previously been reported from other countries, such as Korea (isolation year 2008), China (2013–2014), Australia (2009–2010), Nepal (2013–2014), Cambodia (2004–2005), Israel (2008–2009), Czech Republic (2008–2011), Switzerland (2011), Spain (2012), France (2012), Portugal (2013–2014), Netherlands (2011), Canada (2005), and United States (2013) (*2*, *5*–*15*). Because of the rapid

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increase of CTX-M-27–producing ST131 in Japan since 2010 (*6*), we designed a study to characterize these isolates using WGS techniques.

Materials and Methods

Bacterial Isolates

We selected 43 nonduplicate ST131 clinical isolates collected from 2 multicenter surveillance programs in Japan for WGS to represent 3 major ESBL-producing ST131 (CTX-M-27–producing *H*30R, 13 isolates; CTX-M-14–producing *H*30R, 9 isolates; CTX-M-15–producing *H*30Rx, 11 isolates) and other ST131 (CTX-M-14+CTX-M-15–producing *H*30Rx, 2 isolates; CTX-M-14–producing *H*30Rx, 1 isolate; CTX-M-14–producing *H*22, 1 isolate; CTX-M-2–producing *H*22, 1 isolate; TEM-producing *H*30, 2 isolates; non–ESBL-producing *H*30R, 3 isolates) in Japan (*6*) (Table). One of the surveillance programs collected ESBL-producing *E. coli* isolates during 2001–2010 at 10 acute-care hospitals in the Kyoto and Shiga prefectures of Japan (*6*); the other program collected all *E. coli* isolates during December 2014 at 10 acute-care hospitals in the 5 prefectures in central Japan. ST131 isolates were identified by PCR specific for *mdh* and *gyrB* alleles, O25b or O16 *rfb* variants, *fimH* allele, and *H*30Rx status (*6*). The selection process of the Japanese ST131 ensured equal representation by geographic location, specimen type, and date of isolation.

In addition to isolates from Japan, we obtained 10 CTX-M–producing ST131 isolates from global collections that previously had been characterized by multilocus sequence typing (MLST) (Table; online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/11/16-0519-Techapp1.pdf). We selected all of the CTX-M-27 producers, 1 CTX-M-14 producer per country, and 2 CTX-M-15 producers. We also sought public databases for ST131 *H*30 and included sequence data for 8 isolates from countries other than Japan: CTX-M-27 producers (3 raw reads, 2 draft genomes); CTX-M-14 producer (1 raw read); and CTX-M-15–producing C2/*H*30Rx (2 complete genomes) (Table; online Technical Appendix Table 1) (*5*,*9*,*16*–*18*).

WGS

We used the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA) to prepare libraries for sequencing. Samples were multiplexed and sequenced on an Illumina MiSeq for 600 cycles (300-bp paired-end) or NextSeq500 for 300 cycles (151-bp paired-end). The ST131 genomes were sequenced at an average depth of 44.03 (SD ± 14.70) and an average coverage of 97.73% (SD ± 0.93%) using the 5,109,767-bp EC958 chromosome as previously described (*16*).

Core Genome Analysis

We used a core genome single-nucleotide polymorphism (SNP)–based approach to create a phylogenetic tree. We identified SNPs using raw read mapping followed by duplicate read removal, realignment, quality score recalibration, and variant filtering (online Technical Appendix). Reads from 53 isolates sequenced in this study and 4 isolates (S100EC, S107EC, S108EC, and S135EC) (*5*) were aligned against a reference genome of EC958, and SNPs were called. The remaining 4 draft or complete genomes underwent whole-genome alignment against EC958 to make EC958-like pseudo-chromosomes that contained only SNPs. The SNP-only core genome was identified as the blocks of >500 bp common to all 61 study isolates to ensure that each block represented a common segment from good alignment in each isolate and that the block had enough length to enable identification (*5*). A maximum-likelihood tree was built using RAxML (*19*). A recombination-free tree was also build by excluding recombination sites identified using a Bayesian analysis software BRATNextGen (*20*).

Comparative Genomic Analysis

To define presence of genes and their alleles, we used SRST2 with trimmed reads or BLAST+ (executables [http://blast.ncbi.nlm.nih.gov/]) with assembled draft genomes and following databases or typing schemes: ResFinder antimicrobial resistance gene database, VFDB and VirulenceFinder virulence gene databases, serotypeFinder O:H typing database, PlasmidFinder plasmid replicon database, MLST (http://mlst.ucc.ie/mlst/dbs/Ecoli), plasmid MLST, *fimH* typing, *gyrA*/*parC* typing, ST131 virotyping, and detection of *H*30Rx-specific *ybbW* SNPs, plasmid addiction systems, and bla_{CTX-M} genetic environment (online Technical Appendix). We used pangenome analysis to identify clade specific segments among draft or complete genomes. BRIG was used to visualize similarity of genomes to ST131 genomic islands (*16*) and to the ST131 reference plasmid pEC958 (*21*).

Statistical Analysis and Sequence Data Accession Numbers

We compared categorical variables using Fisher exact test. A p value <0.05 was considered statistically significant. We conducted our statistical analysis using Stata, version 13.1 (StataCorp, College Station, TX, USA). The sequences were deposited in the DDBJ Sequence Read Archive database (accession no. DRA004266 and DRA004267).

Results

Bacterial Isolates

The study comprised 60 clinical and 1 environmental ST131 isolates (Table; online Technical Appendix Table

Table. ST131 isolates included in study of EBSL-producing *Escherichia coli*, Japan*

	Country/prefecture of isolation (no. isolates; year)			
	*H*30			
	*H*30R			
Type of ESBL	*H*30R, n = 39	*H*30Rx, n = 18	*H*30, n = 2	*H*22, n = 2
CTX-M-27, n = 21	Japan/Kyoto, Shiga, Aichi (13; 2004–2014), Australia (3; 2009, 2010),† United States (2; 2013, 2014),‡ Canada (1; 2008), Thailand (1; 2013), Vietnam (1; 2011)			
CTX-M-14, n = 17	Japan/Kyoto, Shiga, Hyogo (9; 2002–2014), Canada (2; 2005, 2009),† France (1; 2008), New Zealand (1; 2010), South Africa (1; 2008), United States (1; 2008)	Japan/Kyoto (1; 2009)		Japan/Kyoto (1; 2007)
CTX-M-15, n = 15		Japan/Kyoto, Shiga, Osaka (11; 2006–2014), Canada (2; 2009), UK (1; 2005),§ United States (1; 2008)¶		
CTX-M-14 and CTX-M-15, n = 2		Japan/Kyoto (2; 2010, 2014)		
CTX-M-2, n = 1				Japan/Kyoto (1; 2004)
TEM, n = 2			Japan/Kyoto (2; 2005, 2009)	
Negative, n = 3	Japan/Shiga, Hyogo, Osaka (3; 2014)			

*ESBL, extended-spectrum β-lactamase; ST, sequence type.
†Raw reads were downloaded from European Nucleotide Archive (accession no. ERA118286) for 3 isolates from Australia (S100EC, S107EC, S108EC) and 1 isolate from Canada (S135EC).
‡MRSN17749 draft genome (GenBank assembly accession no. GCA_000770275.1) and lEH71520 draft genome (GenBank assembly accession no. GCA_000681435.1).
§EC958 complete genome (GenBank assembly accession no. GCA_000285655.3).
¶JJ1886 complete genome (GenBank assembly accession no. GCA_000493755.1).

1). We confirmed the types of β-lactamase genes, ST131 status, *fimH* allele numbers, and *H*30Rx status using draft genomes.

Core Genome SNP-based Phylogenetic Tree

We identified a 4,086,650-bp core genome that included 5,280 SNPs by mapping and alignment of the 61 study isolates to EC958 (Figure 1). The ciprofloxacin-resistant isolates with *gyrA* 1AB and *parC* 1aAB alleles formed the C/*H*30R cluster that comprised the C2/*H*30Rx and C1/*H*30R clades. The C2/*H*30Rx clade included isolates with $bla_{\text{CTX-M-15}}$ (n = 15) and $bla_{\text{CTX-M-14}}$ (n = 1) and isolates with both $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ (n = 2) (Figure 1). The C1/*H*30R clade included isolates with $bla_{\text{CTX-M-27}}$ (n = 21) and $bla_{\text{CTX-M-14}}$ (n = 14) and isolates without ESBLs (n = 3) (Figure 1). Within the C1/*H*30R clade, 19 of 21 CTX-M-27–producing isolates clustered into a distinct group, named the C1-M27 clade (Figure 1). *E. coli* ST131 C1-M27 comprised isolates from Japan (n = 13; isolation years 2004–2014), Australia (n = 2; 2009–2010), United States (n = 2; 2013–2014), Canada (n = 1, 2008), and Thailand (n = 1, 2013).

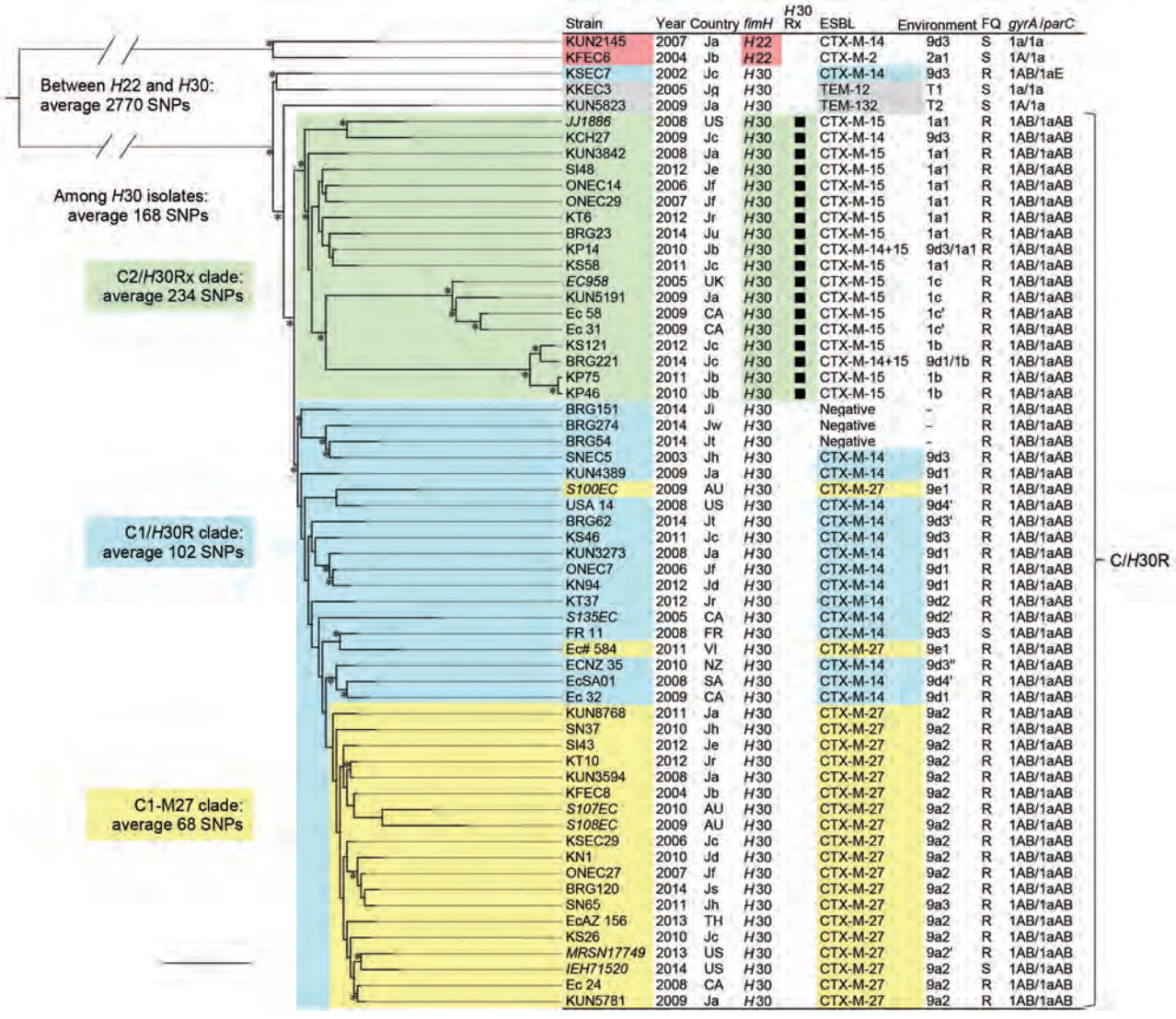
Analysis of the core genome showed that 79 segments (i.e., 304,782 bp, including 3,453 SNPs) were associated with recombination sites (online Technical Appendix Figure 1). This finding suggests that recombinant segments contained 65% of SNPs with subsequent higher frequency of SNPs compared with nonrecombinant regions (average 11 vs. 0.48 SNPs/kb, respectively). The phylogenetic tree created without recombination sites showed the same results as the phylogenetic tree obtained with recombination sites (online Technical Appendix Figure 2). In addition to the core genome–based phylogeny with or without recombination sites, the C1-M27 clade was defined by a unique accessory genome of the M27PP1.

The C1-M27 Clade–Specific Region

The pangenome analysis of genomes from all the isolates identified an 11,894-bp region named M27PP1 that was specific to all the isolates from the C1-M27 clade. Further analysis using the BLAST database and Sanger sequencing for gap filling showed that this region was identical to a prophage-like genomic island (GenBank accession no. CP006632) in *E. coli* PCN033 that belonged to phylogenetic group D and was isolated from a pig in China. The BLAST database also identified 2 similar sequences (i.e., 99.9% homology): A CMY-2 containing plasmid pEQ011 (GenBank accession no. NC_023315) in an *E. coli* isolate from a horse in Ireland (*22*) and a multidrug-resistant plasmid pSD853_88 (GenBank accession no. JF267652) found in a bovine *Salmonella enterica* isolate in the United States. M27PP1 was inserted into chromosome creating a 7-bp direct repeat region (Figure 2). PCN033 had the same flanking structure as the M27PP1,

whereas the 2 plasmids (pEQ011, pSD853_89) contained only a 44-bp similar segment at 5′ side and other parts of these plasmids were not found in the C1-M27 clade isolates.

Two *E. coli* ST131 C1-M27 isolates (i.e., KUN5781 and Ec 24) had an additional insertion region of 19,352 bp, named M27PP2, situated upstream of M27PP1. M27PP2 was accommodated within the same 7-bp direct repeat region (Figure 2). M27PP2 included a 15,555-bp region that showed 88.9% homology to a prophage-like sequence in the chromosome of the γ proteobacterium HdN1 (GenBank



Strain	Year	Country	*fimH*	H30 Rx	ESBL	Environment	FQ	*gyrA*/*parC*
KUN2145	2007	Ja	*H22*		CTX-M-14	9d3	S	1a/1a
KFEC6	2004	Jb	*H22*		CTX-M-2	2a1	S	1A/1a
KSEC7	2002	Jc	*H30*		CTX-M-14	9d3	R	1AB/1aE
KKEC3	2005	Jg	*H30*		TEM-12	T1	S	1a/1a
KUN5823	2009	Ja	*H30*		TEM-132	T2	S	1A/1a
JJ1886	2008	US	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
KCH27	2009	Jc	*H30*	■	CTX-M-14	9d3	R	1AB/1aAB
KUN3842	2008	Ja	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
SI48	2012	Je	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
ONEC14	2006	Jf	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
ONEC29	2007	Jf	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
KT6	2012	Jr	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
BRG23	2014	Ju	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
KP14	2010	Jb	*H30*	■	CTX-M-14+15	9d3/1a1	R	1AB/1aAB
KS58	2011	Jc	*H30*	■	CTX-M-15	1a1	R	1AB/1aAB
EC958	2005	UK	*H30*	■	CTX-M-15	1c	R	1AB/1aAB
KUN5191	2009	Ja	*H30*	■	CTX-M-15	1c	R	1AB/1aAB
Ec 58	2009	CA	*H30*	■	CTX-M-15	1c'	R	1AB/1aAB
Ec 31	2009	CA	*H30*	■	CTX-M-15	1c'	R	1AB/1aAB
KS121	2012	Jc	*H30*	■	CTX-M-15	1b	R	1AB/1aAB
BRG221	2014	Jc	*H30*	■	CTX-M-14+15	9d1/1b	R	1AB/1aAB
KP75	2011	Jb	*H30*	■	CTX-M-15	1b	R	1AB/1aAB
KP46	2010	Jb	*H30*	■	CTX-M-15	1b	R	1AB/1aAB
BRG151	2014	Ji	*H30*		Negative	-	R	1AB/1aAB
BRG274	2014	Jw	*H30*		Negative	-	R	1AB/1aAB
BRG54	2014	Jt	*H30*		Negative	-	R	1AB/1aAB
SNEC5	2003	Jh	*H30*		CTX-M-14	9d3	R	1AB/1aAB
KUN4389	2009	Ja	*H30*		CTX-M-14	9d1	R	1AB/1aAB
S100EC	2009	AU	*H30*		CTX-M-27	9e1	R	1AB/1aAB
USA 14	2008	US	*H30*		CTX-M-14	9d4'	R	1AB/1aAB
BRG62	2014	Jt	*H30*		CTX-M-14	9d3'	R	1AB/1aAB
KS46	2011	Jc	*H30*		CTX-M-14	9d3	R	1AB/1aAB
KUN3273	2008	Ja	*H30*		CTX-M-14	9d1	R	1AB/1aAB
ONEC7	2006	Jf	*H30*		CTX-M-14	9d1	R	1AB/1aAB
KN94	2012	Jd	*H30*		CTX-M-14	9d1	R	1AB/1aAB
KT37	2012	Jr	*H30*		CTX-M-14	9d2	R	1AB/1aAB
S135EC	2005	CA	*H30*		CTX-M-14	9d2'	R	1AB/1aAB
FR 11	2008	FR	*H30*		CTX-M-14	9d3	S	1AB/1aAB
Ec# 584	2011	VI	*H30*		CTX-M-27	9e1	R	1AB/1aAB
ECNZ 35	2010	NZ	*H30*		CTX-M-14	9d3''	R	1AB/1aAB
EcSA01	2008	SA	*H30*		CTX-M-14	9d4'	R	1AB/1aAB
Ec 32	2009	CA	*H30*		CTX-M-14	9d1	R	1AB/1aAB
KUN8768	2011	Ja	*H30*		CTX-M-27	9a2	R	1AB/1aAB
SN37	2010	Jh	*H30*		CTX-M-27	9a2	R	1AB/1aAB
SI43	2012	Je	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KT10	2012	Jr	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KUN3594	2008	Ja	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KFEC8	2004	Jb	*H30*		CTX-M-27	9a2	R	1AB/1aAB
S107EC	2010	AU	*H30*		CTX-M-27	9a2	R	1AB/1aAB
S108EC	2009	AU	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KSEC29	2006	Jc	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KN1	2010	Jd	*H30*		CTX-M-27	9a2	R	1AB/1aAB
ONEC27	2007	Jf	*H30*		CTX-M-27	9a2	R	1AB/1aAB
BRG120	2014	Js	*H30*		CTX-M-27	9a2	R	1AB/1aAB
SN65	2011	Jh	*H30*		CTX-M-27	9a3	R	1AB/1aAB
EcAZ 156	2013	TH	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KS26	2010	Jc	*H30*		CTX-M-27	9a2	R	1AB/1aAB
MRSN17749	2013	US	*H30*		CTX-M-27	9a2'	R	1AB/1aAB
IEH71520	2014	US	*H30*		CTX-M-27	9a2	S	1AB/1aAB
Ec 24	2008	CA	*H30*		CTX-M-27	9a2	R	1AB/1aAB
KUN5781	2009	Ja	*H30*		CTX-M-27	9a2	R	1AB/1aAB

Figure 1. Core genome single-nucleotide polymorphism (SNP)–based phylogenetic tree of *Escherichia coli* sequence type (ST) 131 isolates. This maximum-likelihood phylogram is based on a 4,086,650-bp core genome and a total of 5,280 SNPs. The tree is rooted by using the outgroup *H*22 isolates, and asterisks indicate bootstrap support >90% from 100 replicates. Strains that had previously been sequenced are in italics. The Country columns indicate places of isolation: Ja to Jw, Japan (a to w indicates hospitals); AU, Australia; CA Canada; FR, France; NZ, New Zealand; SA, South Africa; TH, Thailand; UK, United Kingdom; US, United States; VI, Vietnam. Environment column shows a type of genetic environment of ESBL genes (online Technical Appendix Table 2, http://wwwnc.cdc.gov/EID/article/22/11/16-0519-Techapp1.pdf). FQ columns indicate ciprofloxacin susceptibilities (S, susceptible; R, resistant). KSEC7 had a *parC* 1aE allele including G250A (S80K) mutation in addition to a 1a allele. The ciprofloxacin-resistant C/*H*30R cluster comprised the C2/*H*30Rx and C1/*H*30R clades. All of the *H*30Rx isolates belonged to the C2/*H*30Rx clade. The C1/*H*30R clade included CTX-M-14–producing *H*30R, non–ESBL-producing *H*30R, and CTX-M-27–producing *H*30R isolates. CTX-M-27–producing isolates belonged to the C1-M27 clade within the C1/*H*30R clade except 2 isolates (S100EC and EC# 584). The bootstrap value for the root of the C1-M27 clade was 64%. An average of 68 SNPs was found among the C1-M27 clade, whereas an average of 158 SNPs was found between the C1-M27 clade and 2 non–C1-M27 clade isolates with $bla_{CTX-M-27}$. Scale bar indicates 100 SNPs.

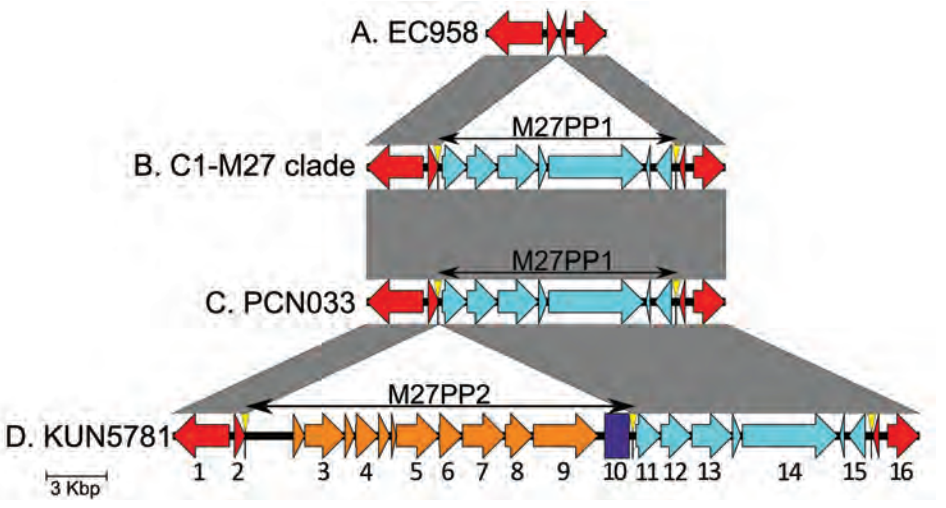


Figure 2. Genetic environments of the C1-M27 clade–specific region of *Escherichia coli*. All isolates other than the C1-M27 clade isolates had the type A structure in their chromosome (red arrows; gene locus tags shown in the bottom are annotated according to EC958). The C1-M27 clade isolates except 2 isolates (KUN5781 and Ec 24) had the type B structure. A 11,894-bp region (M27PP1; predicted genes shown in light blue arrows) is inserted into the type A structure creating the 7-bp direct repeat (CCGTTCT; yellow triangle). The inserted sequence M27PP1 is identical to a prophage-like genomic island in *E. coli* PCN033 chromosome (GenBank accession no. CP006632), which had the similar flanking structure (structure C, 98.8% similarity). M27PP1 included phage-like integrase and recombinase. The identical M27PP1 sequence was found in all of the C1-M27 isolates with the use of additional Sanger sequencing. Only the draft genome of IEH71520 had 98.7% coverage to the M27PP1 sequence because of contig discontinuity. KUN5781 and Ec 24 had the type D structure, of which an additional 19,352-bp region (M27PP2) is inserted into the type B structure by creating the same 7-bp direct repeat (yellow triangle). The M27PP2 includes a total of 15,555-bp region (genes shown in orange arrows), which was 88.9% similar to a prophage-like region in γ proteobacterium HdN1 chromosome (GenBank accession no. FP929140) and a following 1,221-bp region is 99.8% similar to IS*Sen4* (purple box). Code to gene locus tags: 1, 958RS23365; 2, 958RS23370; 3, HDN1F03950; 4, HDN1F03970; 5, HDN1F04000; 6, HDN1F04010; 7, HDN1F04020; 8, HDN1F04030; 9, HDN1F04040; 10, ISSen4; 11, 033RS22420; 12, 033RS22425; 13, 033RS22430; 14, 033RS22440; 15, 033RS22450; 16, 958RS23380.

accession no. FP929140) and 99.8% homology to the insertion element IS*Sen4*.

Genomic Comparison of the ST131 Genomic Islands and Virulence Genes

The sequences of the study isolates were similar to the ST131 genomic islands in EC958 and JJ1886 (a CTX-M-15–producing C2/*H*30Rx strain obtained in the United States from a patient with fatal urosepsis) (Figure 3) (*17*). The C1-M27 clade isolates lacked the prophage 1 region present in EC958 (Figure 3). This prophage 1 region, specific for ST131, was present among the non–C1-M27 ST131 isolates in this study, except for BRG23 and EcSA01. The presence of ExPEC-associated virulence genes is shown in online Technical Appendix Figure 3. The *senB* enterotoxin gene was more common in C1/*H*30R (than in C2/*H*30Rx). No significant differences existed in the distribution of virulence genes between *E. coli* ST131 C1-M27 and other isolates.

Plasmid Replicons, Addiction Systems, and Antimicrobial Drug Resistance Genes

We compared the study isolates with pEC958, the plasmid present in EC958 that carries $bla_{\text{CTX-M-15}}$ (online Technical Appendix Figure 4). The C1-M27 clade lacked the first part of the transfer region (*tra*) present in pEC958. Some regions common to both C2/*H*30Rx and C1/*H*30R clades were present in pEC958. The C1/*H*30R clade producing CTX-M-27 or CTX-M-14 (including C1-M27) contained mostly F1:A2:B20 replicons, whereas the C2/*H*30Rx clade producing CTX-M-15 contained mainly F2:A1:B- replicons (online Technical Appendix Figure 5). The C1-M27 clade was negative for Tn*2* containing $bla_{\text{TEM-1}}$. Two C1-M27 isolates from Thailand and the United States were also positive for $bla_{\text{NDM-1}}$ (online Technical Appendix Figure 5).

Discussion

A previously unreported clade named C1-M27 within C1/*H*30R clade is responsible for the epidemic of ESBL-producing ExPEC in Japan and has already been disseminated to 5 countries on 3 continents. ST131 containing $bla_{\text{CTX-M-27}}$ responsible for human infections has been reported from various continents (*2*) and is especially common among ESBL-producing ExPEC in certain countries, such as Israel, the Czech Republic, and Switzerland (*2*,*13*,*14*). CTX-M-27–producing ST131 also is present among nonclinical and nonhuman *E. coli* isolates, including fecal specimens of healthy children attending day care centers in France; fecal specimens of healthy adults in China, Portugal, and the Netherlands; samples from sick dogs and cats in Japan; samples from water birds from central Europe and Swiss rivers and lakes; and samples of well water from China (*2*,*10*,*11*,*15*,*23*–*25*). The most common ESBL among *E. coli* ST131 in nonhuman samples is CTX-M-27 (*2*,*23*–*25*). ST131 with $bla_{\text{CTXM-15}}$ is rare among animal and environmental *E. coli* isolates (*26*). Our analysis of IEH71520,

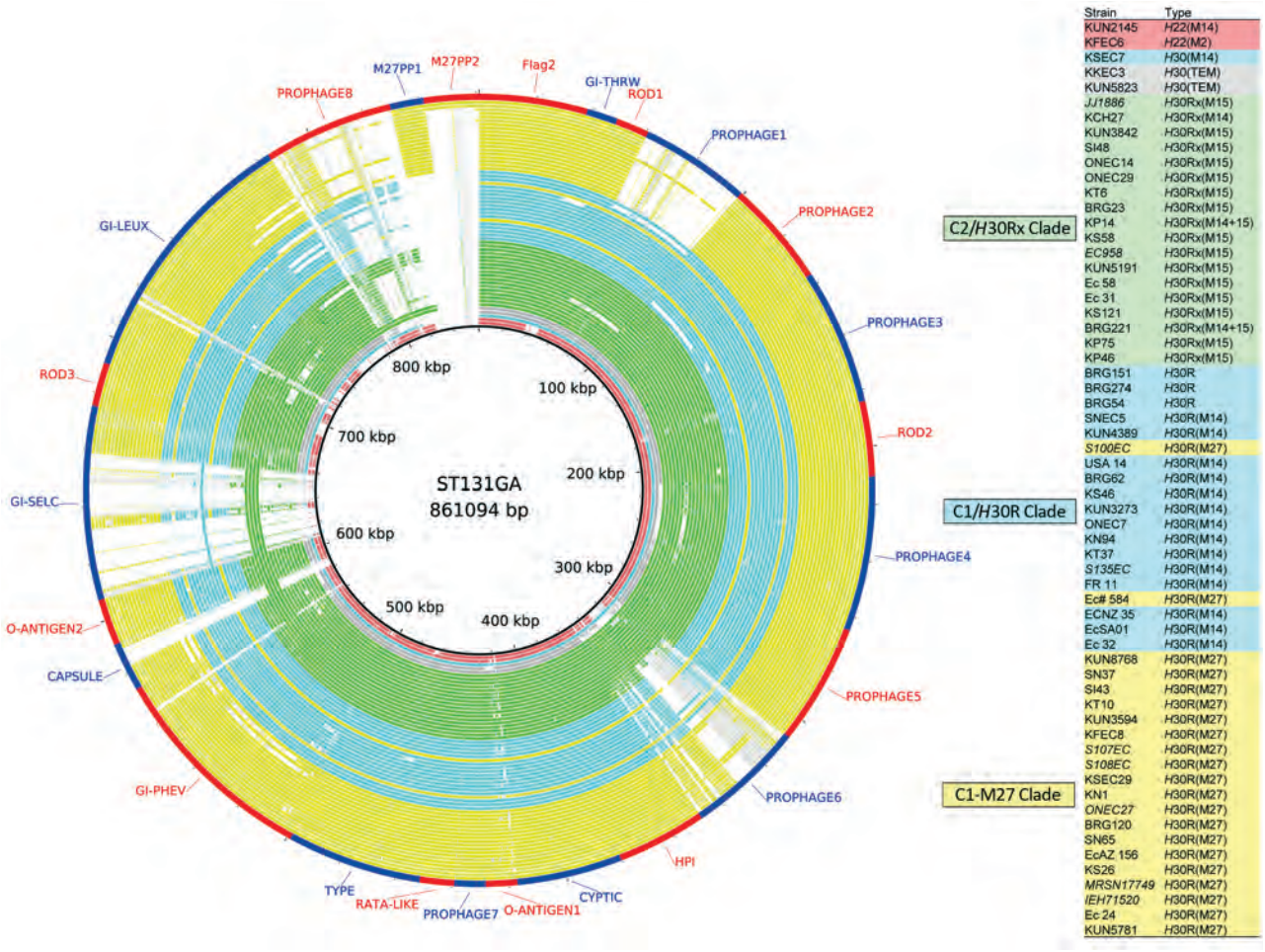


Figure 3. Genome similarities to the *Escherichia coli* sequence type (ST) 131 genomic islands and the C1-M27 clade–specific region. Rings drawn by BRIG show the presence of these regions. Colored segments indicate >90% similarity and gray segments indicate >70% similarity by BLAST comparison between the regions of interest and each genome. Extended-spectrum β-lactamase types are indicated in parentheses of Type column. The regions from Flag2 to GI-*lueX* were found in EC958, the prophage 8 region was found in JJ1886, and the M27PP1 and M27PP2 were found as the C1-M27 clade–specific regions in this study. Prophage 6, capsule, GI-*selC*, and prophage 8 regions were present in some C2/*H*30Rx isolates but were absent in C1/*H*30R isolates.

an *E. coli* isolate from vacuum cleaner dust in the United States (*15*), showed that this ST131 isolate belong to the C1-M27 clade. The C1-M27 clade is likely to be present among animal and environmental ST131, and such isolates might act as a hidden reservoir for the introduction of ST131 containing $bla_{CTX-M-27}$ into human medicine.

E. coli ST131 C1-M27 had an additional, unique prophage-like region (M27PP1) within its chromosome, lacked the prophage 1 genomic island previously identified in ST131 C2/*H*30Rx, and were negative for the transposon Tn*2* containing bla_{TEM-1} (Figure 3; online Technical Appendix Figure 5). The direct flanking repeat sequences surrounding M27PP1 suggest that this region was introduced into *E. coli* ST131 C1/*H*30R with $bla_{CTX-M-27}$ by a recombination event that was then followed by the clonal expansion of the C1-M27 clade.

Recent studies focusing on evolutionary history of ST131 suggested that C1/*H*30R and C2/*H*30Rx clades emerged ≈30 years ago, after their acquisition of *gyrA*-1AB and *parC*-1aAB alleles from C0/*H*30 (non-R) clade (*27*,*28*). The phylogeny and smaller numbers of SNPs in the C1-M27 clade (Figure 1) suggest that this clade was recently diverged from the C1/*H*30R. In the time-scaled phylogeny presented by Stoesser et al. (*27*), a cluster that included 6 CTX-M-27–producing isolates from Cambodia, Thailand, and Laos in 2007–2011 was present within the C1/*H*30R clade. This cluster, supposed to be the C1-M27 clade, diverged in the early 2000s, supporting our hypothesis.

CTX-M-27–producing ST131 that belongs to the *H*41 lineage previously had been described from Japan (*6*) and China (*15*). The characterization of the Japanese ST131

*H*41 isolates showed different genetic structures flanking the $bla_{\text{CTX-M-27}}$ from those structures present in *E. coli* ST131 *H*30R (*6*). The flanking regions previously characterized in ST131 *H*41 were identical to the flanking regions in ST131 non–C1-M27 from this study. It seems there are 2 types of structures flanking the $bla_{\text{CTX-M-27}}$ among *E. coli* ST131; 1 type is confined to clade C1-M27 (i.e., 208 bp of ΔIS*Ecp1* upstream and ΔIS*903D* downstream), whereas another structure (i.e., 388 bp of ΔIS*Ecp1* upstream and full IS*903D* downstream) is distributed among non–C1-M27 isolates, including ST131 *H*41 (*6*). Therefore, ST131 *H*41, through horizontal transfer of $bla_{\text{CTX-M-27}}$, is unlikely to have played a substantial role in the emergence of the C1-M27 clade.

Two ST131 isolates with $bla_{\text{CTX-M-27}}$ from Australia and Vietnam did not belong to the C1-M27 clade (Figure 1). These 2 isolates differ from the C1-M27 clade in that their core genomes had more SNPs (158 vs. 68), contained the prophage 1 ST131-specific region, and lacked the M27PP1 and M27PP2 elements. Moreover, the genetic environment surrounding the $bla_{\text{CTX-M-27}}$ differed from *E. coli* ST131 C1-M27 (as described previously). The isolate from Vietnam lacked *mph*(A)-*mrx-mphR*, *tetR-tet*(A), *sul2-strA-strB*, and In*54* resistance genes, compared with the C1-M27 clade (online Technical Appendix Figure 5). These differences indicate that some ST131 isolates might acquire $bla_{\text{CTX-M-27}}$ independently from the C1-M27 clade.

Our study has several limitations. Most isolates originated from Japan. However, we were able to include ST131 C1-M27 isolates from 5 countries on 3 continents and C1/*H*30R isolates producing CTX-M-14 or CTX-M-15 from 6 countries on 4 continents. Another limitation was that we were able to obtain only 1 environmental ST131 isolate with $bla_{\text{CTX-M-27}}$ (IEH71520). Future studies that include environmental isolates will provide additional insights into molecular epidemiology and evolutionary history of the C1-M27 clade. We could not analyze plasmid contents of $bla_{\text{CTX-M-27}}$ because $bla_{\text{CTX-M-27}}$–containing contigs were too short. The sequencing of plasmids that contain $bla_{\text{CTX-M-27}}$ obtained from various ST131 clades (including the C1-M27 clade) should also be undertaken.

In conclusion, we showed that the recent increase in ESBL-producing *E. coli* from Japan resulted from emergence of a ST131 C1/*H*30R subclade with $bla_{\text{CTX-M-27}}$. This clade, named C1-M27, had unique genomic characteristics and was present in ST131 from Thailand, Australia, Canada, and the United States. Our findings suggest that the C1-M27 clade is contributing to the global success of ST131. *E. coli* ST131 C1-M27 poses a major new public health threat because of its global distribution and association with the very dominant C/*H*30 lineage. We urgently need rapid cost-effective detection methods for *E. coli* ST131 C1-M27 and well-designed epidemiologic and molecular studies to understand the dynamics of transmission, risk factors, and reservoirs for ST131 C1-M27. These efforts will provide insight into the emergence and spread of this multidrug-resistant clade that will lead to information essential for preventing the spread of ST131.

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Multidrug-Resistant *Corynebacterium striatum* Associated with Increased Use of Parenteral Antimicrobial Drugs

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Release date: October 12, 2016; Expiration date: October 12, 2017

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

- Analyze characteristics of infections with corynebacteria
- Distinguish anatomic sites of clinically relevant infection with *Corynebacterium striatum*
- Evaluate patterns of antimicrobial resistance of *C. striatum*
- Compare clinical outcomes of infections with *C. striatum* vs. coagulase-negative staphylococci

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Corynebacterium striatum is an emerging multidrug-resistant bacteria. We retrospectively identified 179 isolates in a clinical database. Clinical relevance, in vitro susceptibility, and length of parenteral antimicrobial drug use were obtained from patient records. For patients with hardware- or device-associated infections, those with *C. striatum* infections were matched with patients infected with coagulase-negative staphylococci for case–control analysis. A total of 87 (71%) of 121 isolates were resistant to all oral antimicrobial drugs tested, including penicillin, tetracycline, clindamycin, erythromycin, and ciprofloxacin. When isolated from hardware or devices, *C. striatum* was pathogenic in 38

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(87%) of 44 cases. Patients with hardware-associated *C. striatum* infections received parenteral antimicrobial drugs longer than patients with hardware-associated coagulase-negative staphylococci infections (mean ± SD 69 ± 5 days vs. 25 ± 4 days; p<0.001). *C. striatum* commonly shows resistance to antimicrobial drugs with oral bioavailability and is associated with increased use of parenteral antimicrobial drugs.

Corynebacteria are a normal component of the microbiota of human skin and mucous membranes. At the University of Washington Medical Center (Seattle, WA, USA), *Corynebacterium striatum* has historically been the second most commonly isolated *Corynebacterium* species, after *C. jeikium* (*1*). Although *C. striatum* is a frequent colonizer (*2*), it might also be implicated as a true pathogen when isolated in multiple samples from sterile sites or from indwelling hardware and devices (*2*–*9*). Determination of whether an isolate represents infection, colonization, or contamination is based upon clinical judgment.

Although early reports indicated that *C. striatum* isolates were frequently susceptible to many antimicrobial drugs, including β-lactams, tetracycline, and fluoroquinolones (*8*–*11*), more recent studies have demonstrated increasing multidrug resistance (*12*–*17*). To clarify the spectrum of disease associated with *C. striatum*, we retrospectively extracted clinical information for immunocompetent patients with *C. striatum* isolates to determine clinical relevance, antimicrobial drug susceptibilities, and length of parenteral therapy. For patients with device-associated infections, we compared the length of parenteral therapy for *C. striatum* with that for coagulase-negative staphylococci, other low-virulence organisms that commonly colonize the skin.

Methods

Patients

We used an algorithm-based query of the De-Identified Clinical Data Repository maintained by the Institute for Translational Health Sciences of the University of Washington (Seattle, WA, USA) to identify 213 patients from the university medical center infected with *C. striatum* isolated from a clinical sample during 2005–2014. Adult patients (>18 years of age) were included if *C. striatum* was isolated from a specimen submitted for bacterial culture and identified to the species level. Because we were interested in whether *C. striatum* would be considered pathogenic in an immunocompetent population, patients with active immunosuppression were excluded from our analysis. We excluded 34 patients with immunosuppressant use at the time of culture with microbiological growth on the basis of pharmacy records (defined as documentation of use of corticosteroids, methotrexate, infliximab, adalimumab, tacrolimus, cyclosporine, mycophenolate mofetil, or rituximab), or with a diagnosis of active malignancy or HIV/AIDS according to codes from the International Classification of Diseases, 9th Revision. Our algorithm-based method was confirmed by using a manual chart review.

Bacterial Identification and In Vitro Drug Susceptibility Testing

Corynebacteria were identified to the species level if isolated in pure culture or if deemed to be clinically meaningful if present in a polymicrobial culture. Identification of corynebacteria was initially performed during 2005–2012 by using the RapID CB Plus Kit (Thermo Fisher Scientific, Waltham, MA, USA). This kit correctly identifies 95% of *Corynebacterium* isolates to the species level (*18*). During 2012–2014, corynebacteria were identified by using matrix- assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MALDI Biotyper and Biotyper software versions 3.0 and 3.1; Bruker Daltonics, Billerica, MA, USA) and a cutoff score of 2.0. Use of MALDI-TOF mass spectrometry with the Bruker system database correctly identifies corynebacteria to the genus level for >99% of isolates and correctly identified *C. striatum* in 100% (51/51) of clinical isolates tested (*19*).

Phenotypic susceptibility testing was performed by using the E-test (bioMérieux, Marcy l'Étoile, France) and Remel blood Mueller Hinton agar (Thermo Fisher Scientific) and incubation for 24–48 h at 35°C in ambient air, according to breakpoints of the Clinical and Laboratory Standards Institute (*20*). Because breakpoints have recently changed (*21*), we tested whether shifting the breakpoint would alter our results. Susceptibilities were available for penicillin, ciprofloxacin, clindamycin, erythromycin, and tetracycline. For patients from whom >1 isolate of *C. striatum* were obtained, we considered only the first isolate in our analysis.

Clinical Data Extraction

We obtained the following variables from chart review by manual extraction: patient location when the culture was obtained (i.e., inpatient versus outpatient), whether the culture grew *C. striatum* in pure culture or was polymicrobial, whether the treating physician considered the isolate to be clinically relevant or a contaminant, length of parenteral therapy administered, and whether adverse events were documented. If the treating physician did not comment on the isolate, the isolate was categorized as clinically irrelevant. Adverse events were defined as clinical event necessitating a change in antimicrobial agent and were graded according to the Common Terminology for Adverse Events (*22*). Serious adverse events were considered grade 3 or 4. The outpatient parenteral antimicrobial therapy practices at the University of Washington Medical Center monitor for adverse events by weekly measurement of a complete

blood count and monitoring of renal function. We used these weekly measurements as a proxy to verify ongoing administration of outpatient parenteral therapy in combination with review of documentation in clinical notes.

Matched Case–Control Analysis

In a subset of patients with hardware-associated osteomyelitis or infections of implanted cardiac devices, we performed a matched case–control analysis to examine length of parenteral therapy in *C. striatum* cases compared with that for persons infected with coagulase-negative staphylococci (controls). Persons infected with coagulase-negative staphylococci were chosen as controls because isolates are frequently found in clinical samples (enabling appropriate matching), colonize the skin, and generally have low virulence, similar to *C. striatum*. Cases were matched to controls on the basis of age (± 5 years), site of infection, and presence or absence of hardware associated with the infection site.

Statistical Analysis

Statistical analysis was performed by using Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). Data are reported as mean ± SE. Parametric data were compared by using the *t*-test, and nonparametric data were compared by using χ^2 or Mann-Whitney U tests. A p value <0.05 was considered significant.

Results

We identified 179 immunocompetent adult patients infected with *C. striatum* isolated from clinical specimens. A substantial proportion (48%, 86/179) of these isolates were believed to be from clinically relevant infections. For comparison, in our laboratory system during a similar period, ≈42,000 isolates of *Staphylococcus aureus* and 4,800 isolates of coagulase-negative staphylococci were recovered with in vitro susceptibilities determined.

Consistent with previous reports that *C. striatum* is a colonizer of the skin and mucous membranes, isolates were frequently reported in sputum and skin samples (65/179) (Figure 1). Interpretation of clinical relevance varied markedly by sample site. Isolates from deep surgical specimens, such as bone and surgical hardware, were generally considered to be clinically relevant (88%–95%), whereas isolates from urine, sputum, or skin were rarely considered to be clinically relevant (10%–15%) (Figure 1). Isolates from bronchoalveolar lavage samples were indicated by clinicians to be clinically relevant in 45% (9/20) of cases, and all of these patients were empirically treated with vancomycin for healthcare-associated pneumonia for a mean ± SE duration of 11 ± 1.9 days. No clinical failures for vancomycin therapy were documented.

Eighty percent (143/179) of isolates were found in an inpatient setting. Susceptibility testing was performed for 121/179 isolates, and 72% (87/121) were resistant to all oral antimicrobial drugs tested (Figure 2). The percentage of drug-resistant isolates obtained from the inpatient setting did not differ from that found in the outpatient setting (p = 0.27).

We determined in vitro susceptibilities for several antimicrobial drugs (Table). In general, MIC distributions were bimodal, whereby most drugs had low MICs or high MICs, except for vancomycin, which was universally active in vitro. The breakpoint of the Clinical and Laboratory Standards Institute for penicillin changed in 2015 (*21*) from 1 mg/L to 0.125 mg/L. This change increased the rate of isolates considered penicillin resistant from 85% to 98%, but did not affect the number of isolates without an oral drug option because all isolates with a penicillin MIC <1.0 mg/L were susceptible to tetracycline. Daptomycin was rarely formally tested in our laboratory during this period (n = 6), including the 2 patients we previously described (*14*). Initial MICs for daptomycin were 0.032–0.125 mg/L, but it was rarely used by clinicians in our cohort. Similarly, linezolid was rarely tested (n = 10), was uniformly active in vitro, but was not used clinically.

All patients with infections deemed to be clinically relevant were initially given vancomycin, except 1 patient with a documented allergy to vancomycin who received daptomycin. As expected, the length of time parenteral antimicrobial drugs were administered varied widely by anatomic site of infection and presence of a foreign body. Prosthetic joint infections were treated with parenteral therapy for 54 ± 7 days, and other hardware- or device-associated infections were treated for 65 ± 10 days. One patient with a ventricular assist device–associated infection who received vancomycin for 650 days was excluded from this analysis (outlier).

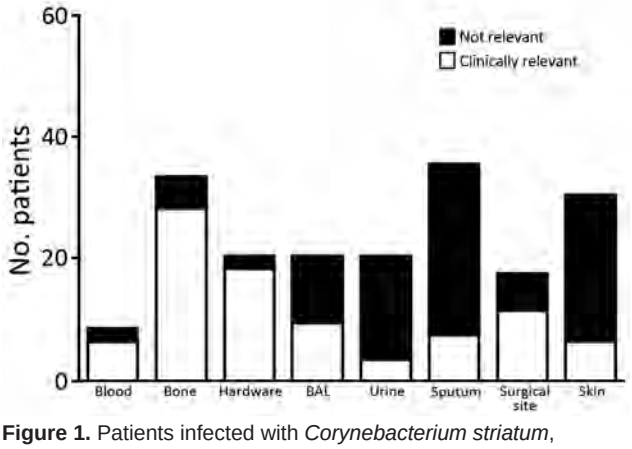

Figure 1. Patients infected with *Corynebacterium striatum*, by specimens isolated from a particular anatomic site, at the University of Washington Medical Center, Seattle, Washington, USA, 2005–2014. Hardware, surgical specimen obtained from a location anatomically in connection with a foreign device; BAL, bronchoalveolar lavage; urine, specimen isolated from a urine sample (we were unable to determine presence or absence of a catheter); surgical site, deep surgical specimen; skin, wound swab from a nonsurgical superficial specimen.

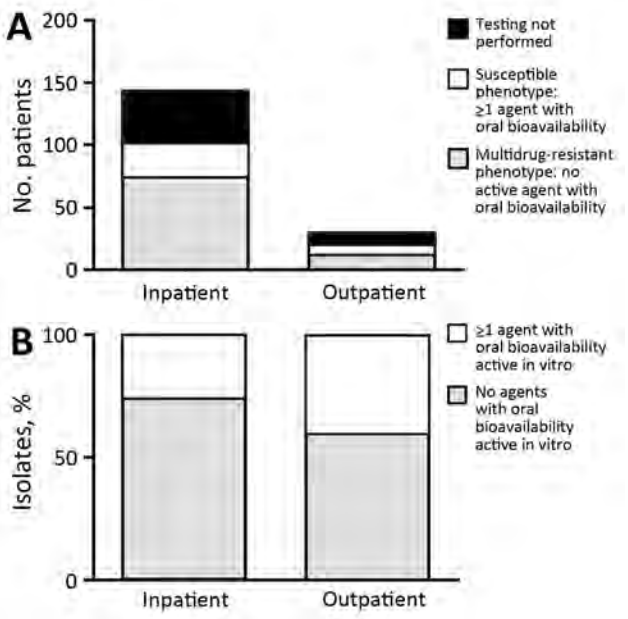

Figure 2. Numbers (A) and percentages (B) of *Corynebacterium striatum* isolates from patients at the University of Washington Medical Center, Seattle, Washington, USA, 2005–2014, with a multidrug-resistant phenotype for all antimicrobial drugs tested (penicillin, ciprofloxacin, clindamycin, erythromycin, and tetracycline). Inpatient or outpatient indicates clinical setting in which cultures were performed.

In a subset restricted to the 38 patients with hardware (including prosthetic joint) or device-associated infections deemed clinically meaningful, we successfully matched 27 patients with control patients infected with coagulase-negative staphylococci. Eleven patients could not be matched for site of infection or age. When we compared control patients infected with coagulase-negative staphylococci with patients infected with *C. striatum*, we found that those infected with *C. striatum* had a longer course of parenteral antimicrobial drugs (69 ± 5 days vs. 25 ± 4 days; p<0.001) (Figure 3). *C. striatum* isolates were more likely to be monomicrobial (14/27, 52%) than coagulase-negative staphylococci (5/27, 19%) (p = 0.02).

Five serious adverse events were associated with parenteral antimicrobial drugs in the *C. striatum* group and only 1 serious adverse event in the coagulase-negative staphylococci group (p = 0.19). The serious adverse events (all associated with vancomycin) were 1 drug reaction with eosinophilia and systemic symptoms syndrome, 2 acute kidney injuries with creatinine levels >3 times baseline values, and 3 absolute neutrophil counts <1,000 cells/mm^3.

Discussion

Because *C. striatum* can be a component of the skin microbiota, determination of whether microbiologic growth in a clinical sample represents an infection depends on clinical judgment. Previous investigations have described *C. striatum* primarily as a nosocomial pathogen, frequently in the setting of underlying malignancy or organ transplantation (*15*,*23*,*24*). Our report documents that clinicians encountering *C. striatum* in clinical samples of immunocompetent patients frequently consider the isolate to be a true pathogen.

Prior studies of small groups of patients have indicated that isolation of *C. striatum* from bone or a medical device is typically considered by the treating physician to be relevant (*24–26*), and our study confirms these findings. Furthermore, our study provides support for the pathogenic role of *C. striatum* in hardware-associated infections because we found that these infections with *C. striatum* were more likely to be monomicrobial than infections with coagulase-negative staphylococci. In contrast to some reports in which isolation from a respiratory sample was frequently determined to be clinically relevant (*15*,*27*,*28*), less than half of the respiratory isolates in our study were considered to reflect lower respiratory tract infections.

We documented that a multidrug-resistant phenotype of *C. striatum* directly affects clinical care. Osteomyelitis and hardware-associated infections are difficult to treat, often requiring a prolonged course of antimicrobial drugs. In some situations, guidelines recommend a limited duration of parenteral therapy followed by a longer period of oral therapy (*29*). A lack of well-tolerated oral treatment options active against *C. striatum* would be expected to lead to a longer duration of use of parenteral antimicrobial drugs for patients with these infections, and our matched case–control study confirmed this expectation.

The longer that parenteral antimicrobial therapy is necessary, the greater the likelihood of adverse events associated with intravenous access. These events include a rate of line events (mostly thrombosis) ranging from 5 to 17 episodes/100 devices and infection rates of 0.5 to 5 infections/100 lines (*30*). Although we documented more adverse events associated with treatment in the *C. striatum* group than in the coagulase-negative staphylococci group, this difference did not achieve a priori statistical significance, and we did not capture line thrombosis events. Furthermore, parenteral therapy is associated with substantially increased costs, even when comparing an inexpensive parenteral antimicrobial drug (vancomycin) with an expensive oral antimicrobial drug (linezolid) (*31*). In addition, parenteral options for *C. striatum* will be increasingly limited because our group and others have reported clinical failures caused by rapid development of high-level daptomycin resistance (*14*,*16*,*25*). Because daptomycin resistance can emerge rapidly, it is reasonable to assume that increased daptomycin use could also cause resistance to this drug.

One oral treatment option for multidrug-resistant *C. striatum* infections is linezolid. Although testing for

Table. Drug resistance patterns for *Corynebacterium striatum* isolates from patients at the University of Washington Medical Center, Seattle, Washington, USA, 2005–2014*

Characteristic	Penicillin, n = 121	Tetracycline, n = 119	Clindamycin, n = 103	Erythromycin, n = 72	Ciprofloxacin, n = 119	Vancomycin, n = 120
Mean MIC	18	34	209	66	51	0.6
Median MIC	8	32	256	16	32	0.5
MIC range	0.125–256	0.125–256	0.25–256	0.25–256	0.125–256	0.125–1
MIC_{50}	8	32	256	16	32	0.5
MIC_{90}	32	64	256	256	32	1

*Resistance is defined as per Clinical and Laboratory Standards Institute Guidelines (*20*). Values are in milligrams/liter. MIC_{50}, concentration required to inhibit 50% of bacteria; MIC_{90}, concentration required to inhibit 90% of bacteria.

C. striatum linezolid susceptibility is rarely performed in our clinical microbiology laboratory, to our knowledge, linezolid resistance has never been reported for corynebacteria. Nevertheless, linezolid is poorly tolerated during the long courses of treatment required for hardware- or device-associated infections and has shown a rate of adverse events leading to treatment discontinuation ranging from 34% to 80% (*32*,*33*). None of our patients in our study were treated with linezolid for a prolonged time, which most likely reflects reluctance of physicians to use an agent with such a high rate of toxicity.

We demonstrated that multidrug resistance was common even in isolates that were not considered to be clinically meaningful in an outpatient setting. We hypothesize that these resistant strains of *C. striatum* are probably circulating in the community rather than emerging under nosocomial pressure, but further studies would be needed to establish the ecologic niche of drug-resistant *C. striatum* strains.

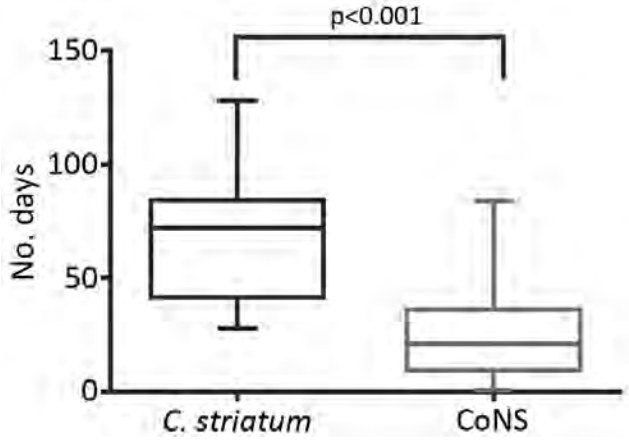

Figure 3. Length of use of parenteral intravenous antimicrobial drugs in matched case–control analysis of *Corynebacterium striatum* isolates and isolates of coagulase-negative staphylococci in patients with hardware-associated infections, University of Washington Medical Center, Seattle, Washington, USA, 2005–2014. Horizontal lines within boxes indicate median values, whiskers indicate minimum and maximum values, and boxes indicate 25th and 75th percentiles. Mean durations of parenteral antimicrobial drug use for patients infected with *C. striatum* and those infected with coagulase-negative staphylococci were compared by using the Mann-Whitney U test. CoNS, coagulase-negative staphylococci.

Strengths of our study include a systems-wide approach to *C. striatum* infections in immunocompetent hosts and the large number of *C. striatum* case reports we reviewed. Detailed clinical information, including the treating physician's interpretation of the sample, was linked to microbiological isolates. *C. striatum* will probably be recognized in more clinical settings because use of MALDI-TOF mass spectrometry enables *C. striatum* to be rapidly identified to the species level with a high degree of confidence without molecular techniques (*19*).

Limitations of our study include its retrospective nature and use of data from 1 health system, which restricted potential generalizability of the results. Given that our study was a retrospective study conducted over a 10-year period, we also cannot state whether the isolates are clonally related or reflect a diverse group with divergent mechanisms of drug resistance. In addition, we relied on the treating physician's interpretation to determine the clinical relevance of an isolate. We have no other way of determining whether the isolate was causing disease, but we believe that this limitation is indicative of general clinical practice. Determining causality would require a different series of mechanistic investigations.

Our study demonstrates that *C. striatum* is an emerging multidrug-resistant pathogen. Our results highlight the need to identify corynebacteria to the species level, which is now readily performed by using MALDI-TOF mass spectrometry, and perform susceptibility testing for any isolate that is believed to be clinically meaningful. The frequent resistance of *C. striatum* to all easily tolerated oral antimicrobial drugs supports the need for development of new agents with good oral bioavailability and acceptable long-term safety profiles that are active against gram-positive organisms.

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Risk Factors for Middle East Respiratory Syndrome Coronavirus Infection among Healthcare Personnel

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Healthcare settings can amplify transmission of Middle East respiratory syndrome coronavirus (MERS-CoV), but knowledge gaps about the epidemiology of transmission remain. We conducted a retrospective cohort study among healthcare personnel in hospital units that treated MERS-CoV patients. Participants were interviewed about exposures to MERS-CoV patients, use of personal protective equipment, and signs and symptoms of illness after exposure. Infection status was determined by the presence of antibodies against MERS-CoV. To assess risk factors, we compared infected and uninfected participants. Healthcare personnel caring for MERS-CoV patients were at high risk for infection, but infection most often resulted in a relatively mild illness that might be unrecognized. In the healthcare personnel cohort reported here, infections occurred exclusively among those who had close contact with MERS-CoV patients.

Middle East respiratory syndrome coronavirus (MERS-CoV), first identified in 2012, has emerged as a cause of severe acute respiratory illness in humans. As of May 1, 2016, a total of 1,728 laboratory-confirmed cases, including 624 deaths, have been reported globally (*1*). All reported cases have been directly or indirectly linked to countries in or near the Arabian Peninsula, including a recent outbreak in South Korea resulting from a single imported case in a person with history of travel to the Middle East (*2*,*3*). Increasing evidence suggests that dromedary camels are a natural host for MERS-CoV and that camel-to-human transmission can occur, initiating short chains of human-to-human transmission (*4*–*7*). Numerous questions about the epidemiology of MERS-CoV remain unanswered.

Healthcare settings are important amplifiers of transmission (*6*,*8*,*9*). A 2014 case series of 255 MERS-CoV infections in Saudi Arabia found that 31% of cases occurred among healthcare personnel (HCP), and among case-patients who were not HCP, 87.5% had recent healthcare exposure (*9*). Current MERS-CoV infection control recommendations are based on experience with other viruses rather than on a complete understanding of the epidemiology of MERS-CoV transmission (*10*,*11*).

The World Health Organization recently issued an urgent call for studies to better understand risk factors for infection and transmission (*12*). Published case series of healthcare-associated MERS-CoV infections have major limitations, including lack of control groups and lack of serologic confirmation of infection status, leaving wide knowledge gaps, such as mode of and risk factors for transmission in healthcare settings, attack rate among HCP, and spectrum of illness for MERS-CoV infection (*13*). To address these gaps, we retrospectively studied MERS-CoV infection among a cohort of HCP in a hospital in Saudi Arabia.

Methods

The study was conducted at King Faisal Specialist Hospital and Research Center (Jeddah, Saudi Arabia) during May–June 2014. This multispecialty hospital has 360 beds, including an 18-bed medical intensive care unit (MICU) and a 38-bed emergency department (ED). Seventeen patients with confirmed MERS-CoV infection were in the hospital during March 24–May 3, 2014. The hospital had no cases of MERS-CoV before March 24, 2014. All patients with suspected or confirmed MERS-CoV infection were placed in private rooms equipped

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with negative pressure ventilation. Patients in whom MERS-CoV infection was not suspected initially were transferred to negative-pressure rooms as soon as the diagnosis was suspected or confirmed. During the outbreak, all HCP who had contact with MERS-CoV cases were screened for symptoms and underwent testing for MERS-CoV RNA by real-time reverse transcription PCR (rRT-PCR) of nasopharyngeal swab specimens.

We assessed risk factors for a case, defined as a MERS-CoV antibody–positive serum sample from an HCP, among 3 cohorts of HCP. Two cohorts, 1 each from the ED and MICU, comprised all HCP who worked in those hospital units during March 24–May 14, 2014, the period during which those units treated patients known to have MERS-CoV infection. In addition, we included a cohort of all HCP who worked in a unit (neurology) that was not known to house any MERS-CoV patients during the study period.

Every healthcare worker in each cohort was recruited to enroll. Participants provided a serum sample and were interviewed by trained study personnel using a standardized questionnaire. Although HCP were from different cultural, language, and educational backgrounds, all spoke English fluently. All questionnaires were conducted in English. Interviews were conducted during May 28–July 10, 2014. In addition to age, sex, occupation, and co-morbidities, we collected information on signs, symptoms, and treatment from March 31, 2014, through the day of interview. Contacts with MERS-CoV patients were described, including patient care activities, duration of contact, and exposure to body fluids. Information about infection control training and use of personal protective equipment (PPE) during encounters with MERS-CoV patients was collected. We assessed exposures outside the hospital, including household exposures to persons with MERS-CoV, contact with animals, and travel.

Serum samples were screened for antibodies against MERS-CoV (Hu/Jordan-N3/2012) nucleocapsid (N) protein by ELISA. The recombinant MERS-CoV N indirect ELISA was developed by using a modified version of the HKU5.2 N ELISA previously described (*13*) Serum was considered positive when the optical density values were ≥0.36 (mean absorbance 405 nm of serum from US blood donors + 3 SD) with an assay specificity of 98.1% (544/555). Samples that were positive by ELISA were confirmed by immunofluorescence assay, microneutralization assay, or both (*14*). A positive serologic test result required confirmation by immunofluorescence assay or microneutralization assay. HCP whose serum sample tested positive for MERS-CoV antibodies were considered to have evidence of MERS-CoV infection (case-HCP); seronegative persons were considered uninfected.

We analyzed data using SAS version 9.3 (SAS Institute, Cary, NC, USA). As appropriate, we compared dichotomous variables using χ^2 and Fisher exact tests. Cochran-Armitage tests for trend were used for ordinal variables. We performed multivariate logistic regression with backward stepwise elimination for exposures with univariate $p \leq 0.2$. Variables with $p \leq 0.1$ were retained in the final generalized linear model using a logit link to estimate risk.

We obtained written informed consent from all participants. The Institutional Review Board of the King Faisal Specialist Hospital and Research Centre approved the study.

Results

Of 363 HCP eligible for the MICU (178 HCP), ED (137 HCP), and neurology unit (48 HCP) cohorts, 292 (80.4%) HCP were enrolled: 131 (73.5%) from the MICU, 127 (92.7%) from the ED, and 34 (70.8%) from the neurology unit. Of the 292 enrolled persons, 9 were excluded because serum specimens were unavailable.

For study participants who worked in units that treated MERS-CoV patients, the attack rate was 8.0% (20/250) and varied by hospital unit: MICU, 11.7% (15/128); ED, 4.1% (5/122). (The attack rate in the neurology unit, where no known MERS-CoV patients were treated, was 0% [0/33].) Attack rates in the MICU and ED also varied by occupation; radiology technicians had the highest attack rate (29.4% [5/17]), followed by nurses (9.4% [13/138]), respiratory therapists (3.2% [1/31]), and physicians (2.4% [1/41]). No clerical staff (7 participants) or patient transporters (14 participants) were seropositive. Most participants (64.4% [161/250]) were female; attack rate did not differ by sex (male 7.9%, female 8.1%; $p = 0.95$). The mean age of seropositive HCP was 40 years (range 29–59 years) and of seronegative HCP 37 years (range 18–66 years).

The most common manifestations of illness among case-HCP were muscle pain, fever, headache, and dry cough (Table 1). These signs and symptoms, along with shortness of breath, occurred significantly more often among seropositive than among seronegative HCP. Seropositive HCP were also more likely to report gastrointestinal symptoms ($p<0.001$). Of the 20 case-HCP, 3 (15%) were asymptomatic, 12 (60%) had mild illness (symptomatic illness not requiring hospital admission), 2 (10%) had moderate illness (required hospital admission but not mechanical ventilation), and 3 (15%) had severe illness (required mechanical ventilation). All case-HCP survived, and all had been previously tested for MERS-CoV by rRT-PCR of nasopharyngeal swab specimens, but only 5 (25%) rRT-PCRs were positive.

Nineteen (95%) of 20 case-HCP reported having been in the same room as or within 2 meters of a patient known to be infected with MERS-CoV. The 1 seropositive HCP who had no MERS-CoV patient contact reported being in an automobile with a symptomatic person subsequently

Table 1. MERS-CoV symptoms reported by healthcare personnel, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia, March–July 2014*

Symptom	Seropositive, no./No.† (%)	Seronegative, no./No.† (%)	p value
Muscle pain	13/20 (65.0)	66/260 (25.4)	0.0001
Fever	12/19 (63.2)	42/258 (16.3)	<0.0001
Dry cough	11/20 (55.0)	80/262 (30.5)	0.02
Headache	11/20 (55.0)	80/262 (30.5)	0.02
Diarrhea	7/20 (35.0)	21/262 (8.0)	0.0001
Nausea	7/20 (35.0)	18/262 (6.9)	<0.0001
Shortness of breath	7/20 (35.0)	32/261 (12.3)	0.005
Runny nose	6/19 (31.6)	92/263 (35.0)	0.76
Chills	6/20 (30.0)	23/261 (8.8)	0.003
Sore throat	5/20 (25.0)	118/263 (44.9)	0.08
Vomiting	4/20 (20.0)	10/262 (3.8)	0.01
Productive cough	3/18 (16.7)	39/263 (14.8)	0.74
Rash	1/20 (5.0)	4/259 (1.5)	0.26
None	3/20(15.0)	94/263 (35.7)	0.019

*MERS-CoV, Middle East respiratory syndrome coronavirus.
†Denominator is the number of healthcare personnel who responded to the question.

confirmed to have MERS-CoV infection. We therefore limited our analysis of risk factors, including PPE use, to any study participant who reported direct contact (i.e., within 2 meters) with MERS-CoV patients in the hospital (Table 2, http://wwwnc.cdc.gov/EID/article/22/11/16-0920-T2.htm). Total time spent in a MERS-CoV patient's room or handling the patient's bedding, equipment, or fluids did not significantly differ between seropositive and seronegative HCP (p = 0.93), nor did the number of MERS-CoV patients cared for during the study period (median 3.0 and 5.0 patients for seropositive and seronegative HCP, respectively; p = 0.75). We found no association between animal contact and infection.

We assessed HCP's self-reported use of PPE during care of MERS-CoV patients, stratified by type of equipment and type of patient interaction (Table 3). HCP who reported always covering their nose and mouth with either a medical mask or N95 respirator had lower risk for infection than did HCP reporting not always or never doing so, although this association was statistically significant only among HCP present in the room where aerosol-generating procedures were conducted. HCP who reported always using a medical mask for direct patient contact were ≈3 times more likely to have MERS-CoV infection than were HCP who reported not always or never using a medical mask (98% of whom reported always or sometimes using an N95 respirator), a trend that was not statistically significant (p = 0.10). Conversely, those who reported always using N95 respirators for direct patient contact were less likely to be seropositive, a trend that approached statistical significance (p = 0.07).

Because medical mask and N95 respirator use were strongly and inversely correlated, we built separate multivariate models, one that assessed risk for medical mask use (model 1) and another that assessed risk for N95 respirator use (model 2). In both models, having participated in infection control training that included information about MERS-CoV prevention was associated with a significant and strong protective effect, and there was a strong but statistically insignificant trend toward increased risk among smokers. In model 1, HCP who reported always using a medical mask for direct MERS-CoV patient care were significantly more likely to be seropositive than those who reported not always or never wearing a medical mask (almost all of whom sometimes or always wore an N95 respirator) (relative risk [RR] 2.73, 95% CI 0.99–7.54). This model also included past or current smoking (RR 2.54, 95% CI 0.93–6.96) and participation in MERS-CoV infection control training (RR 0.28, 95% CI 0.10–0.80). In model 2, N95 respirator use was associated with a strong protective trend; HCP who always used an N95 respirator for direct MERS-CoV patient care were 56% less likely to be seropositive than were those who reported not always or never using an N95 respirator (almost all of whom sometimes or always wore a medical mask) (RR 0.44, 95% CI 0.15–1.24). This model also included past or current smoking (RR 2.51, 95% CI 0.92–6.87) and participation in MERS-CoV infection control training (RR 0.33, 95% CI 0.12–0.90).

Discussion

We report this seroepidemiologic study to quantify the risk for MERS-CoV infection among HCP. The findings have important implications for infection control practice. Our results suggest that the attack rate of MERS-CoV infection among healthcare workers is substantially higher than that in previous reports that used nonserologic methods of detection (*15–17*). The spectrum of illness appears to be broader than previously described; infection caused a relatively mild illness in most cases. Infections occurred almost exclusively among HCP having close contact with a MERS-CoV patient.

Most HCP in this cohort reported always covering their nose and mouth with a medical mask or N95 respirator when caring for a MERS-CoV patient, which appeared to protect against infection among HCP participating in aerosol-generating procedures. When we stratified by type of mask, we observed an increased risk for MERS-CoV infection among HCP who reported always using medical masks and, conversely, a lower risk among those who reported always using N95 respirators. Taken together, these results raise the hypothesis that short-range aerosol transmission might have factored in transmission. Previous studies suggest that some respiratory viruses (e.g., influenza, severe acute respiratory syndrome coronavirus, rhinovirus) that are transmitted primarily by droplets and/or contact might simultaneously be spread through aerosol under certain conditions and perhaps by certain patients (*18–22*). Aerosol transmission in close proximity to the patient might not

Table 3. PPE used by healthcare personnel during care of MERS-CoV patients, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia, March–July 2014*

PPE used, contact type	Always wore PPE,† no. seropositive/total‡ (%)	Sometimes/never wore PPE,§ no. seropositive/total‡ (%)	RR (95% CI)	p value
Gloves	18/197 (9.1)	0/21 (0)	NA	NA
Gown	11/139 (7.9)	7/79 (8.9)	0.89 (0.36–2.21)	0.81
Eye protection				
Direct contact	1/47 (2.1)	17/165 (10.3)	0.21 (0.03–1.51)	0.13
Aerosol-generating procedure	3/62 (4.8)	11/100 (11.0)	0.44 (0.13–1.51)	0.25
Covering of nose and mouth with medical mask or N95 respirator¶				
Direct patient contact	11/151 (7.3)	7/66 (10.6)	0.69 (0.28–1.69)	0.43
Aerosol-generating procedures	8/133 (6.0)	6/32 (18.8)	0.32 (0.12–0.86)	0.03
Medical mask				
Direct patient contact	9/69 (13.0)	9/142# (6.3)	2.06 (0.86–4.95)	0.10
Aerosol-generating procedures	5/81 (6.2)	8/76 (10.5)	0.59 (0.20–1.71)	0.39
N95 respirator				
Direct patient contact	6/116 (5.2)	12/101** (11.9)	0.44 (0.17–1.12)	0.07
Aerosol-generating procedures	5/90 (5.6)	9/73 (12.3)	0.45 (0.16–1.29)	0.16

*MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not applicable; PPE, personal protective equipment; RR, relative risk.
†Reported always wearing PPE indicated in table row when caring for MERS-CoV patients.
‡Total number of healthcare personnel who responded to the question about PPE.
§Reported not always or never wearing PPE indicated in table row when caring for MERS-CoV patients.
¶Reported use of medical mask and N95 respirator were not mutually exclusive categories; therefore the number of healthcare personnel reporting always wearing either an N95 respirator or always wearing a medical mask does not sum to the "covering of nose and mouth with medical mask or N95 respirator" category.
#Of the 142 who reported not always or never wearing a medical mask, 139 (98%) reported always or not always wearing an N95 respirator (55% always, 45% not always).
**Of the 101 who sometimes or never wore an N95 respirator, 96 (95%) reported always or not always wearing a medical mask (35% always, 65% not always).

necessarily be accompanied by long-range transmission because the risk for such transmission might be affected by the infectious dose, the amount of aerosolized particles generated at the source, and the rate of biologic decay of the agent (*22*). We found no evidence of long-range aerosol transmission. Until additional information about the mode of MERS-CoV transmission is available, it seems prudent to take precautions against aerosol spread in healthcare settings when feasible to do so.

The combined attack rate for HCP who worked in units known to house patients with MERS-CoV infection (8%) was substantially higher than that in previous studies, which described attack rates for HCP of ≤1% (*15*–*17*). These prior studies did not use serologic methods to detect infection but rather relied on rRT-PCR of nasopharyngeal swabs. All 20 seropositive HCP in our study were screened with nasopharyngeal swabs, and only 5 (25%) of these tests showed evidence of MERS-CoV by rRT-PCR. Therefore, screening for viral shedding using nasopharyngeal swabs might be an insensitive method for detecting infection, perhaps because of variability in timing of samples in relationship to exposure, and studies relying solely on this method of case detection might underestimate attack rates.

Our study suggests that almost all MERS-CoV infection among HCP occurs among those having close contact with patients known to be infected with MERS-CoV. We observed the highest attack rates among radiology technicians, followed by nurses. We hypothesize that radiology technicians most likely were exposed while obtaining portable chest radiographs, a procedure that requires close contact (e.g., positioning the patient for cassette placement) with patients who might be likely to have worsening respiratory status and be highly contagious. We identified no seropositive HCP who worked in the unit not known to house any MERS-CoV patients, suggesting that the background rate of MERS-CoV infection among HCP was low in the absence of known exposure to infected patients and that the virus was not circulating widely among staff.

HCP who had undergone infection control training specific to MERS-CoV had a lower risk for infection. This finding underscores the critical need for adequate infection control training, especially in settings with ongoing transmission of epidemiologically important pathogens.

We observed a broad spectrum of illness among HCP, and in most cases illness was relatively mild. Most illnesses were characterized by myalgia, fever, headache, and dry cough. Gastrointestinal symptoms were present in 50% of infected HCP; and 3 (15%) reported no symptoms. Most seropositive HCP with symptoms sought care, but only a small minority were recognized as having MERS-CoV infection. All 20 infected HCP survived, and only 5 required hospitalization. The spectrum of illness we observed was broader than that described in previous case series of MERS-CoV infection (*15*,*23*,*24*), which probably were biased toward identifying patients with more severe illness because testing for MERS-CoV infection has largely been triggered by case definitions requiring evidence of pneumonia (*10*). The observation that most MERS-CoV infections among HCP are likely to be relatively mild and unrecognized has potentially important implications for

infection control practice. Although little is known about risk for transmission from persons with mild MERS-CoV-infection, HCP with unrecognized MERS-CoV infection might be a reservoir for transmission to hospitalized patients who are more susceptible to severe illness because of underlying illnesses. Transmission from persons with unrecognized MERS-CoV infections might have contributed to the major role healthcare-associated transmission has played in the epidemiology of MERS-CoV (*6*,*8*,*9*). Thus, control of transmission in healthcare settings might depend on maintaining a low threshold for suspicion of MERS-CoV infection among exposed HCP and other persons with a relatively mild viral syndrome.

Our study did not identify strong associations with underlying chronic illnesses, most likely because the prevalence of such conditions was low (<10%) in this population. HCPs with a history of smoking had a risk for infection almost 3 times that of nonsmokers. We found no association between MERS-CoV infection and sex. Most case series to date have demonstrated a male predominance among case-patients (*15*,*23*,*24*), but our study suggests this association might be explained by social and behavioral factors that increase exposure to MERS-CoV, rather than a sex-specific difference in biological susceptibility.

Our study has several strengths. We compared MERS-CoV infected and uninfected HCP to determine risk factors for acquiring infection during patient care. The use of serologic testing to determine infection status enabled unbiased case ascertainment, an examination of the full spectrum of disease, and a comparison of the risks associated with a wide range of specific patient care activities.

Our study also has limitations. First, questionnaires were administered several weeks after possible exposures, and therefore the potential exists for recall bias. Recall bias can limit assessment of important variables, such as frequency of exposure and duration of contact during specific procedure. However, HCP and interviewers were unaware of their serologic status at the time of interview; their answers would not have been influenced by knowledge of these results. Moreover, symptoms of illness were unlikely to have introduced systematic bias to responses because most uninfected and infected groups reported illness. Second, we used only 1 serum sample for serologic testing. Because of the retrospective nature of our study, baseline serologic tests were not conducted, and therefore the potential exists for false-positive results. However, seroprevalence of MERS-CoV antibodies in Saudi Arabia is low (0.15%), making misclassification bias unlikely (*25*). Third, infected asymptomatic HCP could serve as a potential source of infection to other HCP. Given the retrospective nature of our study, we were not able to characterize these potential exposures. Fourth, as is common with early studies of emerging infectious diseases, sufficiently powering studies can be difficult. Whether negative findings were true null findings or due to small sample sizes is unclear.

In conclusion, we report results of a seroepidemiologic study to quantify risk for MERS-CoV infection among HCP. The attack rate appears to be substantially higher than that in prior reports that used nonserologic methods of detection. Infection in this population most often results in mild illness that might be overlooked; programs to identify and exclude ill HCP who have been exposed to patients with MERS-CoV might help eliminate this reservoir for transmission. Our findings also suggest N95 respirators might be more protective against MERS-CoV infection while in close contact with an infected patient and highlight the possible role of short-range aerosol transmission of MERS-CoV in healthcare settings. Education about standard and MERS-CoV infection control practices appears to be protective, suggesting that adherence to basic practices can effectively prevent MERS-CoV infection among HCP.

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Epidemiology of La Crosse Virus Emergence, Appalachia Region, United States

Sharon Bewick, Folashade Agusto, Justin M. Calabrese, Ephantus J. Muturi, William F. Fagan

La Crosse encephalitis is a viral disease that has emerged in new locations across the Appalachian region of the United States. Conventional wisdom suggests that ongoing emergence of La Crosse virus (LACV) could stem from the invasive Asian tiger (*Aedes albopictus*) mosquito. Efforts to prove this, however, are complicated by the numerous transmission routes and species interactions involved in LACV dynamics. To analyze LACV transmission by Asian tiger mosquitoes, we constructed epidemiologic models. These models accurately predict empirical infection rates. They do not, however, support the hypothesis that Asian tiger mosquitoes are responsible for the recent emergence of LACV at new foci. Consequently, we conclude that other factors, including different invasive mosquitoes, changes in climate variables, or changes in wildlife densities, should be considered as alternative explanations for recent increases in La Crosse encephalitis.

In recent years, several vectorborne diseases have reemerged either at new locations or to new levels in locations where they have historically ranged. Commonly cited factors for reemergence include evolution of novel vector or pathogen strains (*1*), increased human mobility or disease spread by infected travelers, decreased herd immunity (*2*), landscape change (*3*), climate change (*4*), and invasion of new regions by competent disease vectors (*5*). Although disease translocations across continents are almost always a result of human transport, pathogens that exhibit novel regional spread, increased transmission in preexisting locations, or both are more difficult to explain. Such is the case with La Crosse encephalitis, a mosquitoborne viral disease currently emerging in the US Appalachian region (Appalachia, comprising Tennessee, North Carolina, Virginia, and West Virginia). With 30–180 cases of severe LACV disease reported annually (*6*) and an estimated total disease annual incidence as high as 300,000 cases, LACV is rapidly becoming a leading cause of encephalitis in the United States (*7*,*8*). For patients with severe cases, LACV disease has lifelong neurologic consequences (*6*) and carries an estimated fatality rate of 0.5%–1.9% (*6*,*9*).

Previously, most LACV disease cases were associated with forested areas in the midwestern United States (*10*), where LACV was maintained through a cycle involving the eastern tree-hole mosquito (*Ochlerotatus triseriatus*), hereafter called the tree-hole mosquito, and mammals of 3 species: eastern chipmunks (*Tamias striatus*), gray squirrels (*Sciurus carolinensis*), and fox squirrels (*Sciurus niger*) (*10*,*11*). However, since the mid-1990s, Appalachia has emerged as a new focus for the disease (*8*,*12*–*14*). One potential explanation is the introduction of the invasive Asian tiger mosquito (*Aedes albopictus*), hereafter called the tiger mosquito (*15*). This suggestion is based on the laboratory-demonstrated competence of the tiger mosquito (*16*,*17*), isolation of LACV from field-collected tiger mosquito pools (*18*), observation of LACV-positive tiger mosquitoes at sites of LACV infections of humans (*19*), and the coincidental link between tiger mosquito invasion and the emergence of LACV in the Appalachian region (*12*). Unfortunately, although these observations demonstrate the potential for the tiger mosquito to influence LACV dynamics, the contribution of this mosquito to observed increases in LACV transmission remains unclear. One obstacle to identifying the role of the tiger mosquito in LACV emergence is our limited understanding of the interaction between invasive species and native disease cycles and how this interaction affects disease transmission, both within natural reservoirs and to human hosts. Epidemiologic modeling is a powerful tool, useful for understanding the outcomes of different transmission pathways in other disease systems. To our knowledge, however, no dynamic models for LACV disease have been developed, even for regions where the tree-hole mosquito is the only disease vector. We therefore developed a series of compartmental models (Figure 1) for LACV. Using these models, we then explored LACV dynamics in systems with (native) and without (invaded) tiger mosquitoes to assess the likelihood that the tiger mosquito is responsible for the emergence of LACV in Appalachia.

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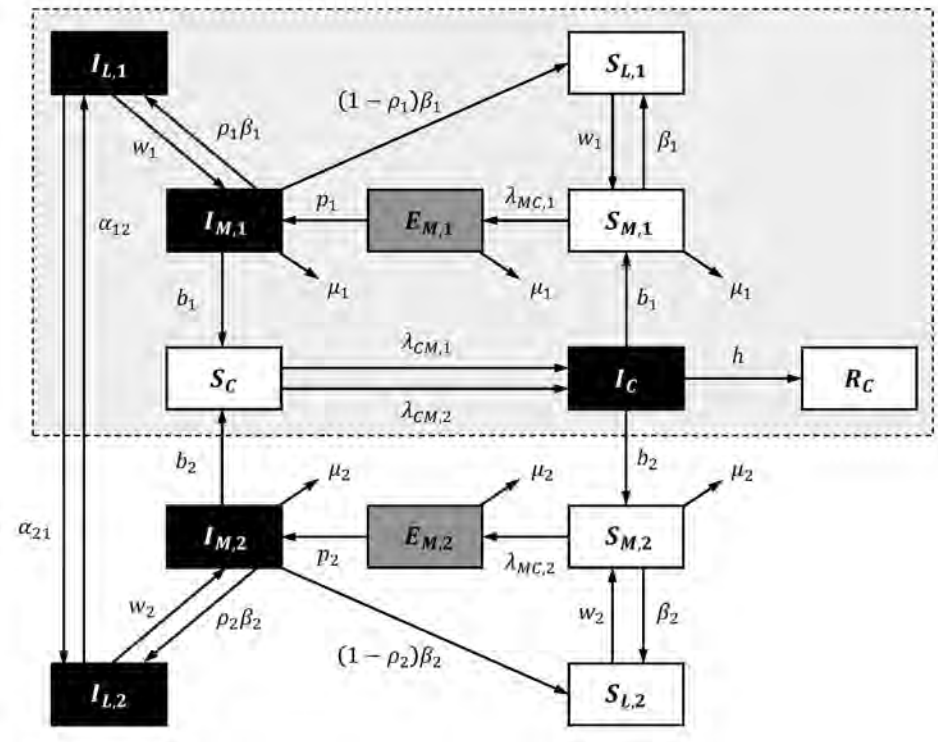

Figure 1. Schematic illustrating transitions/interactions in the compartmental model for La Crosse virus disease. Subscripted 1, 2, and C denote parameters and state variables for the eastern tree-hole mosquito, Asian tiger mosquito, and host populations, respectively. State variables and parameters are described elsewhere (see Basic Model and Table A1, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). Black boxes indicate infected classes; gray boxes, exposed classes; and white boxes, susceptible/recovered classes. Dashed box with gray shading demarks the subset of transitions/interactions that define the native system before tiger mosquito invasion.

Methods

Model

We built 3 models: 1) the tree-hole model, in which the tree-hole mosquito is the only LACV vector; 2) the tiger model, in which the tiger mosquito is the only LACV vector; and 3) the tree-hole/tiger model, in which mosquitoes of both species simultaneously serve as LACV vectors. In the third model, mosquitoes of either species may be driven extinct through competitive exclusion; thus, although both vectors are potentially present, it is possible that only 1 persists. For all models, we assumed that the vertebrate host was the eastern chipmunk. The basic dynamic system (Figure 1), including all relevant assumptions and system parameterizations, is fully described elsewhere (see Basic Model, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).

Basic Reproduction Number, R_0

To determine whether sustained LACV transmission is predicted, we considered the basic reproduction number, R_0, for each of the 3 models (see Basic Reproduction Numbers, R_0, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). For $R_0>1$, LACV transmission can be sustained. For $R_0<1$, LACV will go extinct.

Transmission Pathways

The tree-hole/tiger model, which represents current LACV spread throughout much of Appalachia and the Midwest, accounts for 2 vector species and, thus, 4 transmission pathways: 1) horizontal transmission between tree-hole mosquitoes and chipmunks, 2) vertical (transovarial) transmission by tree-hole mosquitoes, 3) horizontal transmission between tiger mosquitoes and chipmunks, and 4) vertical transmission by tiger mosquitoes. To determine the relative contributions of the different transmission pathways to R_0, we used elasticity analyses (*20*). Large elasticities indicate transmission routes that contribute most to disease maintenance and spread (see Elasticity Analysis of Transmission Pathways, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).

LAC Dynamics

R_0 analyses reflect equilibrium conditions, which are good approximations of full system behavior when seasonality is weak or when the system reaches equilibrium rapidly within a single season. Because the relevance of seasonality for LACV is unknown, we also considered fully dynamic multiseason extensions to each of our models (see LAC Dynamics, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). Using dynamic simulations, we estimated the fraction of scenarios (i.e., parameter combinations) resulting in sustained LACV transmission above a critical threshold. This is the numerical equivalent of R_0 but may differ as a result of seasonality. For dynamic simulations in which LACV persists, we also quantified season-long host seroprevalence rates, peak rates of mosquito

infection, and the timing of peak transmission to humans. Last, we considered the potential for the tiger mosquito to act as a bridge vector, linking LACV transmission in wildlife reservoirs to infections in human populations.

Latin Hypercube Sampling

Measurements of system parameters vary, for example, as a result of geographic differences in abiotic variables, differences in local mosquito or chipmunk populations, differences in circulating virus strains, or measurement error. To capture model predictions over empirically determined parameter ranges, we used Latin hypercube sampling, followed by sensitivity analyses with partial rank correlation coefficients (PRCCs) (see Latin Hypercube Sampling and PRCC, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html) (*21*).

Results

Basic Reproduction Number, R_0

We found that sustained LACV transmission can occur according to most (60%) tree-hole model scenarios but only a small fraction (3%) of tiger model scenarios (Figure 2) (see Latin Hypercube Sampling and PRCC, and Tables A2, A3, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). This finding is surprising because the average tiger mosquito population has approximately twice as many biting females per hectare as does the average tree-hole mosquito population (see Table A2 at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html), which reflects the higher larval carrying capacity and faster larval maturation rate of tiger mosquitoes than those of tree-hole mosquitoes. Clearly, the numerical abundance of tiger mosquitoes does not compensate for the lower rates of horizontal and vertical LACV transmission and the lower rates of their biting on key host species (see Basic Model, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).

In the 2-vector system, our results for the tree-hole/tiger model indicated a similar outcome—that the invasion of tiger mosquitoes into tree-hole mosquito populations should reduce the fraction of scenarios (from 60% to 37%) in which LACV transmission is viable. Thus, instead of causing the emergence of new LACV foci, tiger mosquitoes should instead drive LACV out of regions where previously it could persist. This result is again a function of the poor intrinsic capability of tiger mosquitoes to serve as LACV vectors. It also depends on asymmetric competition between tiger and tree-hole mosquitoes (see Latin Hypercube Sampling and PRCC and Table A3, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). For example, whereas 14% of parameter combinations yielded tiger mosquitoes competitively excluding tree-hole mosquitoes, for only 0.03% of parameter combinations was the converse true. Moreover, even when tiger and tree-hole mosquitoes were predicted to coexist, the tree-hole mosquito population declined by an average of 63% through interspecific competition. By contrast, interspecific competition reduced the tiger mosquito population by an average of only 16%. Not surprisingly, then, when both mosquito species were present, most (average 78%) were tiger mosquitoes. Because the tiger mosquito is the less competent of the 2 LACV vectors, its invasion actually reduces the likelihood of LACV transmission.

Transmission Pathways

Elasticity analysis of the 4 virus transmission pathways in the tree-hole/tiger model indicated that, in most scenarios, the pathway that contributes most to disease spread is

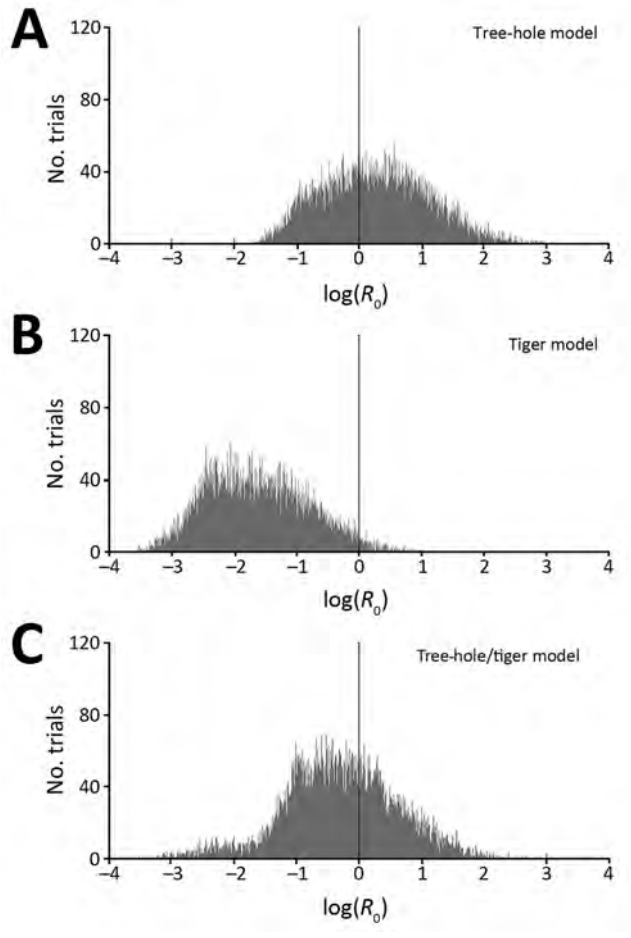

Figure 2. Histograms of basic reproduction numbers (R_0) for La Crosse virus, based on Latin hypercube sampling analyses with 10,000 randomly selected parameter sets (ranges shown at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). A) Tree-hole model, B) tiger model, and C) tree-hole and tiger model. In each panel, the black vertical line at $\log(R_0) = 0$ corresponds to the general breakpoint between growing and shrinking infection rates and thus represents the threshold for La Crosse virus disease persistence.

horizontal transmission by tree-hole mosquitoes (Figure 3). Vertical transmission by tree-hole mosquitoes can also contribute but usually only when the role of tiger mosquitoes is minimal. For scenarios in which tiger mosquitoes contribute to spread, the main pathway is horizontal transmission either by tiger mosquitoes alone or in combination with horizontal transmission by tree-hole mosquitoes. By contrast, vertical transmission by tiger mosquitoes rarely affects disease dynamics; when it does, it is only in systems in which horizontal transmission by tiger mosquitoes is already the major mode of disease spread.

LACV Dynamics

Predictions from dynamic models were similar to predictions for R_0 (LACV transmission in 46% of scenarios) and matched many expectations from LACV systems (Table; see also LAC Dynamics at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). First, in the native system (the tree-hole model) and the invaded system (the tree-hole/tiger model), predicted host seroprevalence rates were remarkably high, approaching 100% toward the end of the season (mean [median] end-of-season host seroprevalence rates of 89% [99%] in the tree-hole model and 84% [97%] in the tree-hole/tiger model). These rates are consistent with findings from Wisconsin where, at least in high-quality mosquito habitats, multiple surveys have demonstrated that antibody prevalence rates among chipmunks can be well over 50%, often nearing 100% late in the season (*15*,*22*). At the same time, our model predicted very low numbers of LACV-positive mosquitoes, even in the native system (mean [median] yearly averages of 2.0% [1.6%] for the tree-hole model). Again, this finding is highly consistent with observed minimum field infection rates that range from 0.26 to 12.5 (*14*,*23*–*26*). Of note, predicted rates of infection among overwintering eggs were even lower than rates of infection among adult populations (mean [median] end-of-season infection rates of 0.63% [0.49%] for the tree-hole model.) This finding reflects the fact that transovarial transmission is <100% and that overwintering eggs are laid later in the season, sometimes after peak LACV transmission has subsided. Again, predicted rates of egg infection strongly agree with field data indicating that 0.29%–0.6% of overwintering eggs from LACV-endemic sites yield LACV-positive larvae (*26*,*27*). Last, our predicted timing of peak risk for human disease was broadly consistent with observed cases of LACV disease in humans that tend to occur in late summer and early fall (*8*,*13*).

What our dynamic model did not predict was any increase in LACV prevalence in the invaded system (tree-hole/tiger model) over that in the native system (tree-hole model). Even in systems in which LACV survived introduction of the tiger mosquito, the tiger mosquito tended to decrease LACV transmission. For example, both the absolute number of infected mosquitoes and the rate of mosquito infection were lower in the tree-hole/tiger model than in the tree-hole model (Table). Consistent with rates of mosquito infection, we found that rates of host seroprevalence were also lower when tiger mosquitoes were present.

Although the tiger mosquito is a poor amplifying vector for LACV, it may still increase the number of human LACV infections. Indeed, because this species is an aggressive human biter, it has the potential to intensify the rate of disease transfer to human populations, albeit while simultaneously reducing disease spread in wildlife reservoirs (i.e., it may act as a bridge vector). However, this potential is not realized (Table). Although rates at which tiger mosquitoes bite humans (see Basic Model, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html) partially compensated for lower rates of LACV transmission

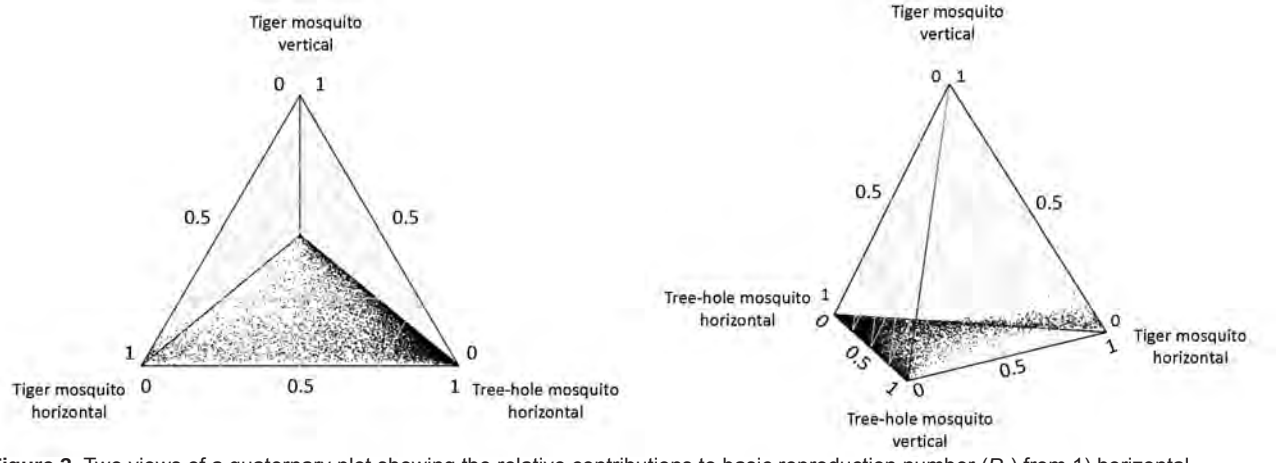

Figure 3. Two views of a quaternary plot showing the relative contributions to basic reproduction number (R_0) from 1) horizontal transmission of La Crosse virus by eastern tree-hole mosquitoes, 2) vertical transmission by eastern tree-hole mosquitoes, 3) horizontal transmission by Asian tiger mosquitoes, and 4) vertical transmission by Asian tiger mosquitoes. This figure plots only the 8,602 replicates (out of 10,000) wherein Asian tiger and eastern tree-hole mosquitoes coexisted. Parameters for each replicate were sampled from the ranges according to our Latin hypercube sampling scheme (http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).

Table. Summary statistics for epidemiologic metrics of LACV, based on Latin hypercube sampling analysis of the full dynamic model*

Variable	Tree-hole model	Tiger model	Tree-hole/tiger model†
Parameter sets with LACV persistence, %	46	0.20	24
End-of-season host seroprevalence rate, %			
Mean	89	79	84
Median	99	88	97
Maximum	100	100	100
Midseason host seroprevalence rate, %			
Mean	65	12	18
Median	74	8.9	12
Maximum	100	38	98
Peak no. infected mosquitoes, per hectare			
Mean	32	58	23
Median	22	50	16
Maximum	331	200	222
Peak mosquito infection rate, %			
Mean	4.5	1.6	1.9
Median	3.5	1.5	1.3
Maximum	27	5.3	15
Average mosquito infection rate, %			
Mean	2.0	0.44	0.80
Median	1.6	0.33	0.57
Maximum	13	1.8	6.8
Maximum human transmission, infections per month per person per hectare			
Mean	15	59	14
Median	8.6	40	7.9
Maximum	251	247	221
Timing of peak human transmission			
Mean	Aug 14	Sep 21	Aug 23
Median	Aug 10	Sep 28	Aug 21
Earliest	Jun 21	Aug 26	Jun 26
Latest	Sep 30‡	Sep 30‡	Sep 30‡
End-of-season egg infection rates, %			
Mean	0.63	0.08	0.28
Median	0.49	0.07	0.20
Maximum	5.0	0.32	2.2

*All metrics beyond the first row are only calculated for the subset of simulations that gave infected mosquitoes. LACV, La Crosse virus.
†We avoid reporting minimum values since these are likely to depend on the threshold that we selected for determining disease persistence (see LAC Dynamics, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).
‡In these systems, the abundance of infected mosquitoes was still increasing at the end of the season, indicating that infection rates do not slow before the decline in mosquitoes at the end of the summer.

among wildlife reservoirs, this compensation was not complete. Thus, human infections were still predicted to occur more commonly in the native system (Table; see also Summary Statistics for Alternate Scenarios at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).

Sensitivity Analysis with Partial Rank Correlation Coefficients

In 1-vector models, positive correlation with R_0 was found for transmission rates, biting rates, mosquito survival rates, mosquito population growth rates, mosquito maturation rates, mosquito carrying capacity, and rates of LACV dissemination among mosquitoes (Figure 4). In contrast, rates of host recovery were negatively correlated with R_0, as was host population size. Although this latter result is somewhat counterintuitive, it is well known for systems with a saturating functional response (*28*).

In the 2-vector model, most PRCC values were reduced but maintained the same sign. This finding reflects the similar effect but lesser role of either mosquito species individually when both species are present. Not surprisingly, PRCC reductions were more severe for the tiger mosquito, which is the less competent vector in the 2-vector system. Although most PRCC values merely exhibited reductions in the 2-vector model, several underwent more striking changes. First, the tiger mosquito population growth rate and the tiger mosquito carrying capacity switched from being positively correlated with R_0 (strongly so in the case of carrying capacity) in the tiger model to being negatively correlated with R_0 in the tree-hole/tiger model. This switch occurs because tiger mosquitoes are generally detrimental to LACV spread in systems in which the native vector is also present, a conclusion that accords with our general finding that tiger mosquitoes should, if anything, reduce LACV transmission. Second, the population growth rate of the tree-hole mosquito actually became more strongly correlated with R_0 when tiger mosquitoes were present. The explanation is as follows. In the tree-hole/tiger model, this parameter helps to influence the outcome of interspecific competition. Specifically,

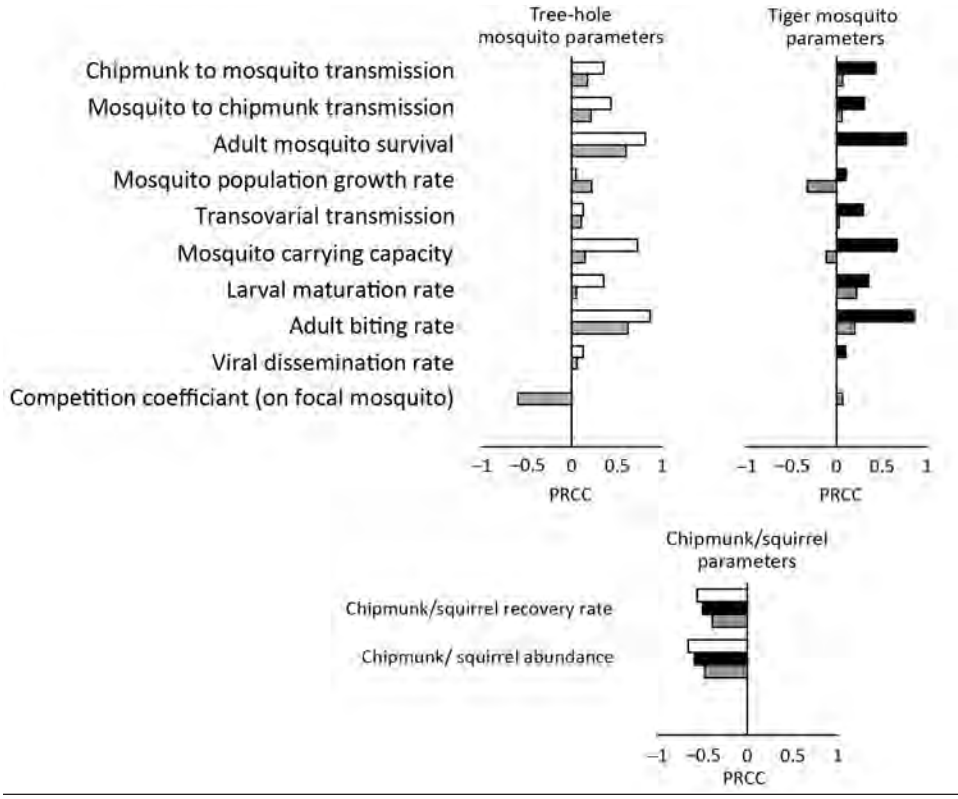

Figure 4. Partial rank correlation coefficients (PRCCs) showing the effect of each model parameter of La Crosse virus transmission on the basic reproduction number (R_0) in the tree-hole model (white), tiger model (black), and tree-hole/tiger model (gray). Positive PRCC values indicate that R_0 is positively correlated with a specific parameter, whereas negative PRCC values indicate the opposite. For specific PRCC values and significance, see Latin Hypercube Sampling and PRCC, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html.

high tree-hole mosquito growth rates give this species an increased chance for survival against the more aggressive, generally more fecund, tiger mosquito population.

Although PRCC analyses can identify correlations between model parameters and disease outcomes, large PRCC values additionally indicate model parameters that contribute a high degree of uncertainty to model predictions. In the tree-hole/tiger model, the largest sources of uncertainty in R_0 were the survival rate of tree-hole mosquitoes, the biting rate of tree-hole mosquitoes, and interspecific competition of tiger and tree-hole mosquitoes. In the tree-hole model and the tiger model, the largest contributions to uncertainty were survival and biting rates but also vector carrying capacities.

Discussion

In contrast to previously published conclusions (*29*), our model suggests that LACV should be sustainable in 46%–60% of scenarios in which the tree-hole mosquito serves as the sole vector. This conclusion still indicates a sizeable number of scenarios in which LACV transmission should not occur. One interpretation is that LACV spread is only marginally favorable and that small changes in system characteristics (e.g., different mosquito or virus strains or environmental conditions) are sufficient to initiate or suppress disease transmission. This marginal favorability could explain the patchy detection of LACV across its native range (*9*) and the sudden appearance of LACV at sites where it was previously absent. The potential for variability in epidemiologic parameters raises the question of how to predict when and where LACV might emerge. Although measuring every system parameter at every local site is not feasible, our PRCC analysis suggests that careful attention to vector survival, competition, biting rates, and carrying capacities would be beneficial.

One factor that does not explain the emergence of novel LACV foci is the invasion of tiger mosquitoes. We predict that the invasive tiger mosquito should actually reduce disease transmission in wildlife reservoirs and human populations (even accounting for the fact that tiger mosquitoes are aggressive human biters). Thus, the presence of the invasive tiger mosquito does not sufficiently explain the dramatic increase in LACV disease cases in Appalachia (*8*,*12*–*14*,*30*) (http://trace.tennessee.edu/utk_gradthes/1788/), suggesting that correlations between tiger mosquito invasion and the epidemiologic risk for LACV disease are driven by other, concomitant, changes. In support of this conclusion is the absence of any increase in LACV disease prevalence in the Midwest, despite the presence of a tiger mosquito infestation. Indeed, reported cases in the region have decreased (*12*), consistent with predictions from our model, but may also be independent of the arrival of tiger mosquitoes (see Midwest LAC Cases, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html).

Having ruled out a straightforward contribution of invading tiger mosquitoes to LACV disease emergence, we

consider the possibility that tiger mosquitoes are responsible for recent changes in LACV epidemiology. One potential mechanism involves indirect effects on the native vector. Tree-hole mosquitoes that survive competition with tiger mosquitoes are generally larger and more competent LACV vectors (*31*), which could increase the likelihood of LACV transmission. Another possible mechanism is niche differentiation. In general, mosquito competition is quantified by raising the larvae of competing species together in 1 container and then assessing growth metrics such as survival or maturation time. Although this approach enables estimation of direct competition, it does not capture spatial (*32*) or temporal niche partitioning that can decrease the strength of interspecific competition. If tiger mosquitoes do not reduce the tree-hole population as severely as our model predicts, then their dampening effect on LACV transmission will, likewise, be diminished (see Conditions Under Which Tiger Mosquitoes Enable LAC Spread http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). Last, our estimates for LACV transmission to and from tiger mosquitoes are based on 1 study that used an LACV strain predating establishment of the tiger mosquito in the United States (*17*). Given that transmission studies can be highly variable and that, since introduction of the tiger mosquito into the United States, local LACV strains may have adapted to be more suitable in this new host, it is also possible that our estimates for tiger mosquito competence are overly low (see Conditions Under Which Tiger Mosquitoes Enable LAC Spread, at http://www.clfs.umd.edu/biology/faganlab/disease-ecology.html). Recent evidence finding substantial infection rates in a tiger mosquito population in Tennessee (*33*) supports this conclusion, although further transmission studies and analyses of virus evolution are warranted.

One final explanation for the recent emergence of LACV, and the explanation that we favor, is that our model predictions are correct and that other factors beyond tiger mosquitoes are responsible for the change. A promising contender is the Asian bush mosquito (*Ochlerotatus japonicus*), hereafter referred to as the bush mosquito. This mosquito is a second container-breeding invasive species that, like the tiger mosquito, seems to have arrived in North America in a shipment of tires (*34*). Because the bush mosquito was introduced more recently than the tiger mosquito (*34*), it has not been studied as extensively, particularly in the context of LACV. Nevertheless, laboratory work has demonstrated its competence as an LACV vector (*35*), and LACV has been isolated from field-collected pools of these mosquitoes (*36*). The role of bush mosquitoes in LACV transmission may be studied by using a model similar to that presented here. However, this study would require additional empirical work, including characterization of transovarial transmission by this species.

Beyond the introduction of novel vectors, other changes (e.g., climatologic variables [*4*], human demographics [*37*], wildlife densities [*38*], and land use [https://vtechworks.lib.vt.edu/handle/10919/64932]) may also contribute to LACV emergence. According to our PRCC analysis, for example, small changes in adult mosquito survival rates could dramatically alter R_0. Mosquito survival rates not only increase the equilibrium size of mosquito populations but also increase the likelihood of mosquitoes surviving to their second or third blood meals, which is necessary for horizontal LACV transmission. Decreases in mosquito predators, varying from birds to spiders (*38*–*41*), could thus strongly affect LACV prevalence. Our PRCC analysis also indicates that mosquito carrying capacities have a substantial effect on LACV persistence. Consequently, even small increases in container availability (e.g., new tire yards or unemptied backyard planters) should have dramatic effects on LACV disease incidence rates. Last, substantial growth has occurred in southern Appalachia over the past 30 years (*37*); thus, even without increased enzootic transmission, absolute cases may have increased from population growth alone. Although purely speculative, such habitat and demographic changes may be the true cause of the recent emergence of LACV.

That tiger mosquito invasion is not predicted to increase LACV transmission or even human cases highlights an important issue at the interface between disease ecology and invasion biology. In particular, this finding shows that predicting whether an invasive vector will exacerbate or dampen the spread of a disease can be complex and can depend on an elaborate network of species interactions. Although this network includes obvious disease interactions like horizontal and vertical transmission, it also includes ecologic interactions that may be relatively independent of the disease itself. In the LACV system, for example, competition between native tree-hole mosquitoes and invasive tiger mosquitoes strongly influences whether or not LACV persists (Figure 4). Indeed, as a consequence of this competition, tiger mosquitoes can drive local extinction of LACV, despite the fact that tiger mosquitoes can acquire and transmit the virus, making them seem to be competent vectors.

Because of the complexity of disease transmission in ecologic systems, it is often hard to identify the causes of altered disease epidemiology. However, faced with increasing climate and landscape change, ongoing introduction of novel invasive species (pathogens and vectors), and emerging or reemerging diseases, an understanding of the effects of these different forms of global change on disease dynamics is essential. We have moved toward this goal by developing a framework for investigating the role of invasive vectors on the transmission of a native disease. Using LACV as an example,

our model highlights the fact that the introduction of a new disease vector does not guarantee increased disease transmission and, in fact, can even drive local extinction of an endemic pathogen.

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Reassortant Eurasian Avian-Like Influenza A(H1N1) Virus from a Severely Ill Child, Hunan Province, China, 2015

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In 2015, a novel influenza A(H1N1) virus was isolated from a boy in China who had severe pneumonia. The virus was a genetic reassortant of Eurasian avian-like influenza A(H1N1) (EA-H1N1) virus. The hemagglutinin, neuraminidase, and matrix genes of the reassortant virus were highly similar to genes in EA-H1N1 swine influenza viruses, the polybasic 1 and 2, polymerase acidic, and nucleoprotein genes originated from influenza A(H1N1)pdm09 virus, and the nonstructural protein gene derived from classical swine influenza A(H1N1) (CS H1N1) virus. In a mouse model, the reassortant virus, termed influenza A/Hunan/42443/2015(H1N1) virus, showed higher infectivity and virulence than another human EA-H1N1 isolate, influenza A/Jiangsu/1/2011(H1N1) virus. In the respiratory tract of mice, virus replication by influenza A/Hunan/42443/2015(H1N1) virus was substantially higher than that by influenza A/Jiangsu/1/2011(H1N1) virus. Human-to-human transmission of influenza A/Hunan/42443/2015(H1N1) virus has not been detected; however, given the circulation of novel EA-H1N1 viruses in pigs, enhanced surveillance should be instituted among swine and humans.

Pigs are well known as genetic mixing vessels for human and avian influenza viruses (*1*,*2*), and swine influenza viruses (SIVs) occasionally infect humans (*3*–*5*). SIV was first reported in humans in 1958 in Czechoslovakia (*6*). The largest outbreak of classical swine influenza A(H1N1) (CS H1N1) virus occurred in Fort Dix, New Jersey, USA, in 1976 (*7*,*8*). Human infections with variant influenza subtype H1N1 and H3N2 viruses with matrix (M) genes derived from swine-origin influenza A(H1N1)pdm09 virus have occurred continuously since the virus was first detected in 2009, and the number of infections has increased substantially in recent years (*6*). Two cases of Eurasian avian-like influenza A(H1N1) (EA-H1N1) infection have been reported in mainland China. The first case, which began in late December 2010 in a 3-year-old boy in Jiangsu Province, resulted in death; however, the child had a history of renal disease (*9*,*10*). The second case, which began in December 2012 in a 3-year-old boy in Hebei Province, caused mild influenza-like illness (*11*).

EA-H1N1 SIVs have been shown to preferentially bind to human-type receptors, and ferrets have been experimentally infected with some EA-H1N1 SIVs via respiratory droplet transmission (*12*). EA-H1N1 SIVs reportedly have the potential to transmit efficiently and cause a pandemic among humans after long-term evolution in pigs (*12*). We report a severe human infection with a reassortant influenza virus in China and the results of genetic, infectivity, and virulence investigations of the novel virus.

Materials and Methods

Case Investigation

On June 30, 2015, a 30-month-old boy was admitted to a hospital in Changsha City, Hunan Province, China. The following data were recorded: demographic characteristics; underlying medical conditions; clinical signs, symptoms and complications; chest radiograph findings; laboratory test results; antimicrobial drug treatment; and clinical outcomes.

Virus Isolation and Titration

We obtained a bronchoalveolar lavage sample from the patient and inoculated it onto MDCK cells for 72 h at 37°C

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before conducting influenza virus testing. We used Sanger sequencing to subtype the hemagglutinin (HA)–positive isolate from the patient, and we evaluated the bronchoalveolar lavage sample by using next-generation sequencing as previously described (*13*). For additional studies, we propagated the virus in 9- to 11-day-old embryonated chicken eggs for 48 h at 37°C. The allantoic fluid was then harvested and stored at -80°C until use.

For infectivity and virulence analyses, we used influenza A/Jiangsu/1/2011(H1N1) (JS/1/11 EA-H1N1) virus, which had been previously isolated from a child in China with fatal infection and was stored in the Chinese National Influenza Center, Chinese Center for Disease Control and Prevention, Beijing, China. To propagate JS/1/11 EA-H1N1 virus, we inoculated 9-day-old embryonated pathogen-free chicken eggs with 0.2 mL of virus stock and incubated the eggs for 48 h at 35°C. The allantoic fluid was then harvested and stored at –80°C until use.

We determined virus titrations for JS/1/11 EA-H1N1 virus and the patient's isolate by using MDCK cells. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated by using the Reed–Muench formula (*14*).

Genetic Analyses

DNA sequences generated by Sanger sequencing of the patient-derived virus were assembled using DNASTAR (http://www.dnastar.com/). All sequences obtained in this study were submitted to the Global Initiative on Sharing Avian Influenza Data (GISAID) database (http://platform.gisaid.org). Multiple sequence alignments were performed with MUSCLE software (http://www.drive5.com/muscle/) using MEGA6 (http://www.megasoftware.net/). To determine the identity of the patient's isolate, we compared sequences for the isolate with those for viruses in the GISAID database. For comparison, we selected representative isolates for each of the following H1N1 lineages: classical swine, Eurasian avian-like swine, Eurasian avian, North American avian, A(H1N1)pdm09, and seasonal human influenza viruses. We also included recent swine isolates from China with sequences available in GISAID. Phylogenetic relationships were estimated for each of 8 gene segments by using the maximum-likelihood method with the general time-reversible plus gamma distribution plus invariable site substitution model, which was implemented in MEGA6 with 1,000 bootstrap replications.

Virulence and Replication Studies in Mice

We used a mouse model to evaluate virulence and replication of the patient-derived virus and JS/1/11 EA-H1N1 virus. All experimental protocols in mice were approved by the Ethics Committee of the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention (approval no. 201509280027). To determine the virulence of the 2 viruses, we used 8- to 10-week-old female C57BL/6J mice (Vital River Laboratories, Beijing, China). The mice were divided into 3 groups and anesthetized with 0.1 mL of pentobarbital sodium. Two groups then were inoculated intranasally with 10-fold serial dilutions (10^1–10^6 $TCID_{50}$ in 50 μL of phosphate-buffered saline [PBS]) of patient-derived virus or JS/1/11 EA-H1N1 virus; a control group was injected with PBS. Bodyweight was measured daily; mice that lost >25% of their original weight were euthanized for humane reasons. At 14 days postinoculation (dpi), we collected blood samples from all surviving mice and separated the serum for antibody testing. Hemagglutination inhibition (HI) assays were performed, according to standard protocols, using 0.5% turkey erythrocytes (*15*,*16*). Serum samples with HI antibody titers of <20 were considered negative.

To determine virus replication in infected mice, we intranasally inoculated all 3 groups of 8- to 10-week-old mice (9 mice/group) with 10^4 or 10^6 $TCID_{50}$ of JS/1/11 EA-H1N1 virus, the patient-derived virus, or PBS (control group). At 1, 4, and 7 days dpi, we euthanized 3 mice in each group. Brain, nasal turbinates, trachea, lung, heart, spleen, kidney, liver, and intestines were collected for virus titer determination using the $TCID_{50}$ assay and MDCK cells.

Results

The Patient

On June 30, 2015, fever (up to 39.5°C), cough, and dyspnea developed in a 30-month-old boy in Hunan Province. On July 2, he was admitted to the intensive care unit of Hunan Provincial People's Hospital in Changsha, China. Complications (i.e., severe pneumonia, respiratory failure, acute respiratory distress syndrome, and heart failure) were observed (Figure 1). A right pleural effusion and a collapsed lower right lung were noted on a chest radiograph from day 2 after illness onset (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/22/11/16-0181-Techapp1.pdf). Oseltamivir was administered on July 3, and on July 10, closed drainage of the thoracic cavity was performed. On July 24, day 24 after illness onset, no obvious abnormalities of the cardiac diaphragm were observed (online Technical Appendix Figure 1). On July 27, the patient was transferred to the general hospital ward, where he recovered and was discharged on August 7 (Figure 1).

Virus Isolation and Genetic Analyses

The bronchoalveolar lavage sample from the patient was positive for subtype H1N1 influenza virus based on deep sequencing; no other virus sequences were found (online Technical Appendix Figure 2). Virus was isolated from the original patient sample, and Sanger sequencing confirmed the pathogen as a subtype H1N1 influenza virus. We termed

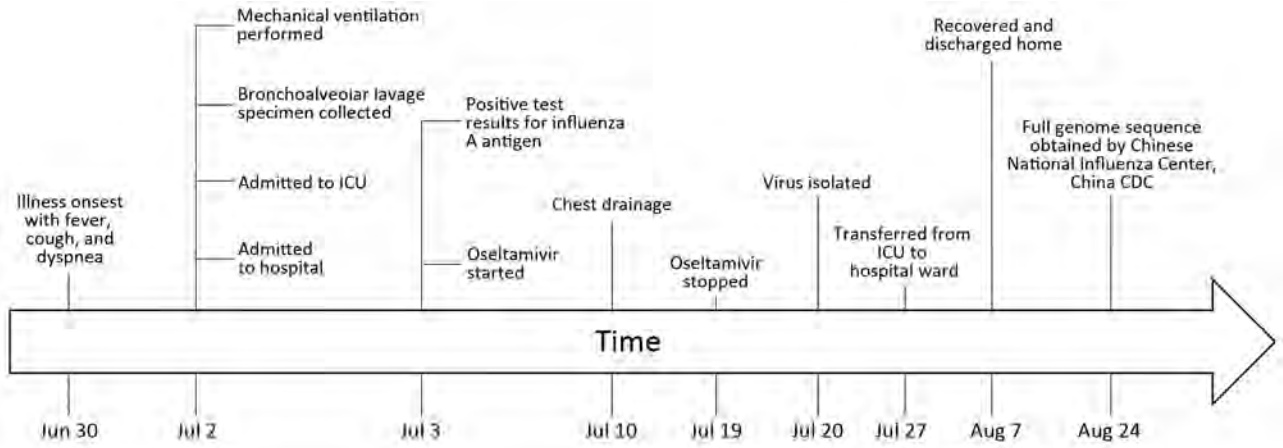

Figure 1. Clinical course for a 30-month-old patient infected with Eurasian avian-like influenza A(H1N1) virus and identification of the causative pathogen, Beijing, China, 2015. China CDC, Chinese Center for Disease Control and Prevention, Beijing; ICU, intensive care unit.

the virus influenza A/Hunan/42443/2015(H1N1) (HuN EA-H1N1) and deposited the nucleotide sequences in the GISAID database (accession nos. EPI691392–EPI691399).

Eight genes from HuN EA-H1N1 virus (PB2, PB1, PA, HA, NP, NA, M, and NS) consisted of 2,280, 2,274, 2,151, 1,701, 1,497, 1,410, 982, and 838 nt, respectively. The amino acid motif PSIQSR↓G at the HA cleavage site indicated that the virus was a low pathogenicity influenza virus, and aa 190D and aa 225E (H3 numbering) in the HA protein suggested preferential binding to human influenza virus-binding receptor SAα-2,6-Gal (a sialyl-galactosyl residue with α-2,6-Gal linkage). Residues in the neuraminidase (NA) protein, which were associated with neuraminidase inhibitory drugs (*17*), implied that HuN EA-H1N1 might be sensitive to neuraminidase inhibitors, but an S31N substitution in M2 suggested resistance to M2 ion channel inhibitors.

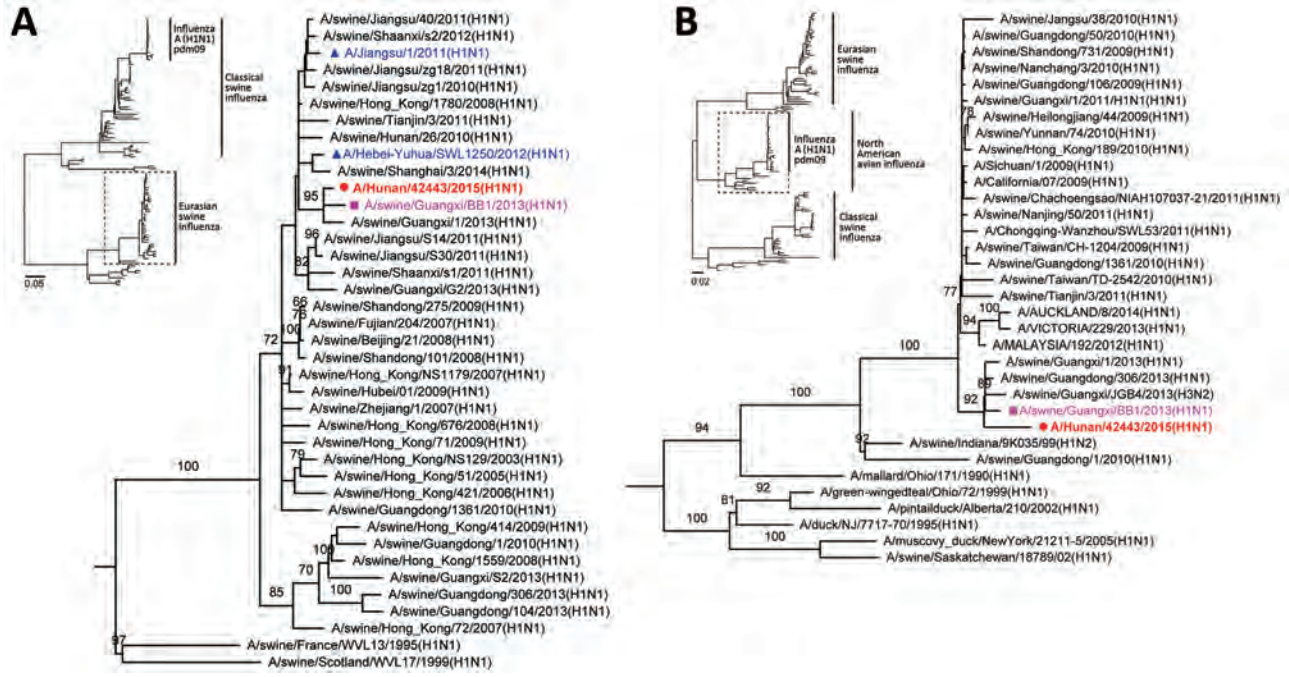

Figure 2. Phylogenetic analysis of Eurasian avian-like influenza A/Hunan/42443/2015 virus (HuN EA-H1N1). A) Analysis of the hemagglutinin gene of representative clades of Eurasian avian-like H1N1 viruses. B) Analysis of the polymerase basic 2 gene of influenza A(H1N1)pdm09 virus. Insets show evolutionary analyses for all lineages of subtype H1N1 viruses. The reliability of the trees was assessed via bootstrap analysis with 1,000 replications; only bootstrap values ≥60% are shown. The horizontal distances are proportional to the genetic distance. Red indicates HuN EA-H1N1 virus, the virus reported in this study; pink indicates A/swine/Guangxi/BB1/2013(H1N1), which shared high similarity with HuN EA-H1N1 virus; and blue indicates 2 human Eurasian avian-like influenza A(H1N1) isolates. Scale bars indicate nucleotide substitutions per site.

Complete sequences of HuN EA-H1N1 virus were 98.2%–99.3% identical in all 8 gene segments with influenza A/swine/Guangxi/BB1/2013(H1N1) virus, which was isolated in 2013 from a swine in Guangxi Province, China (*18*). Phylogenetic analysis of all genes showed that HuN EA-H1N1virus was a reassortment of EA-H1N1, A(H1N1) pdm09, and CS H1N1 viruses (Figure 2) (online Technical Appendix Figure 3). HA, NA, and M genes of the virus shared highest similarity with those of 2 other human EA-H1N1 viruses in China: JS/1/11 EA-H1N1 and A/Hebei-Yuhua/SWL1250/2012 (HB/1250/12). The remaining 5 genes (polybasic [PB] 1 and 2, polymerase [PA], nucleoprotein [NP], and nonstructural protein [NS]) in HuN EA-H1N1 virus differed from those in the 2 other EA-H1N1 viruses: 4 internal genes (PB2, PB1, PA, and NP) clustered with A(H1N1)pdm09 virus (Figure 2, panel B) (online Technical Appendix Figure 3), and the NS gene shared a common ancestor with A(H1N1)pdm09 virus and derived from CS H1N1 virus.

Virulence and Replication in Mice

To evaluate the virulence of JS/1/11 EA-H1N1 and HuN EA-H1N1 viruses, we first determined the MID_{50} (50% mouse infectious dose) and MLD_{50} (50% mouse lethal dose) for each virus by inoculating 3 groups of mice, respectively, with 10-fold serial dilutions (10^1–10^6 $TCID_{50}$/50 μL) of JS/1/11 EA-H1N1 virus, HuN EA-H1N1 virus, or PBS. By 14 dpi, no JS/1/11 EA-H1N1 virus–inoculated mice had lost ≥10% of their bodyweight, and none had died, indicating an MLD_{50} of $\geq 10^{6.5}$ $TCID_{50}$ for the virus (Figure 3, panel A). A similar trend in bodyweight loss was seen in mice inoculated with 10^1–10^4 $TCID_{50}$ of HuN EA-H1N1 virus. However, at 8 dpi, mice inoculated with 10^5 or 10^6 $TCID_{50}$ of HuN EA-H1N1 virus had lost >25% of their bodyweight (Figure 3, panel A), and by 14 dpi, all mice in these 2 groups (5 mice/group) had died, indicating an MLD_{50} value of $10^{4.5}$ $TCID_{50}$ for the virus (Figure 3, panel B).

HI antibody testing showed that the MID_{50} for JS/1/11 EA-H1N1 virus was much higher than that for HuN EA-H1N1 virus ($\geq 10^{4.7}$ vs. $10^{2.9}$ $TCID_{50}$, respectively) (Table 1). The cross-reactive antibody response for JS/1/11 EA-H1N1 and HuN EA-H1N1 indicated similar antigenicity (Table 1).

We also investigated the tissue tropism and replication of HuN EA-H1N1 and JS/1/11 EA-H1N1 viruses in 2 groups of mice. Both viruses replicated in the respiratory tract, but infectivity was substantially divergent (Figure 4). At 1, 4, and 7 dpi, no virus was detected in any tissues of mice inoculated with 10^4 $TCID_{50}$ of JS/1/11 EA-H1N1 virus. However, at the same time points, virus was clearly present in the respiratory tract, including the nasal turbinates, trachea, and lungs, of mice inoculated with 10^4 $TCID_{50}$ of HuN EA-H1N1 virus. These findings were consistent with antibody responses in the mice (Table 1).

Mice inoculated with 10^6 $TCID_{50}$ of JS/1/11 EA-H1N1 virus had limited virus replication in nasal turbinates and tracheal tissues. However, virus was clearly present in lung tissues at 1 and 4 dpi, but titers decreased rapidly by 7 dpi (Figure 4). Compared with JS/1/11 EA-H1N1 virus–

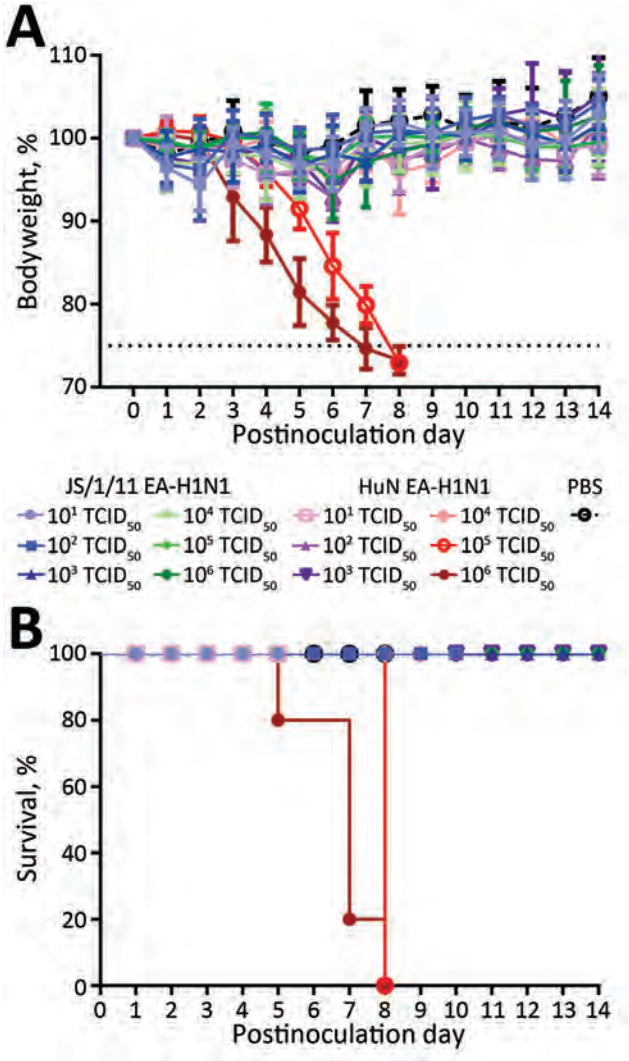

Figure 3. Illness (A) and death (B) among C57BL/6 mice inoculated with Eurasian avian-like influenza A/Jiangsu/1/2011 (JS/1/11 EA-H1N1) virus or Eurasian avian-like influenza A/Hunan/42443/2015 (HuN EA-H1N1) virus. Eight-to-ten week old female C57BL/6J mice (5/group) were inoculated intranasally with various 50% tissue culture infectious doses ($TCID_{50}$) of JS/1/11 EA-H1N1 virus or HuN EA-H1N1 virus (in 50-μL of PBS) or with 50 μL mL of PBS (control group). A) Illness was assessed by weight changes over 14 days and is graphed as a percentage of the average weights on the day of inoculation (day 0). Average bodyweight changes ± SD are shown. Dotted horizontal line indicates a bodyweight loss of 75%. B) Death was investigated by using a survival curve. On postinoculation day 14, mice with a bodyweight loss of >25% and those who died naturally (i.e., not including those that were euthanized) were recorded as fatalities.PBS, phosphate-buffered saline.

Table 1. Seroconversion in C57BL/6 mice inoculated with JS/1/11 EA-H1N1 and HuN EA-H1N1 viruses*

Virus and dose, log_{10} $TCID_{50}$/50 μL	Hemagglutination inhibition titer†										MID_{50}, log_{10} $TCID_{50}$‡
	JS/1/11 EA-H1N1 virus antigen					HuN EA-H1N1 virus antigen					
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	
JS/1/11 EA-H1N1											4.7
6	40	20	≥80	40	40	≥80	40	≥80	40	40	
5	<10	40	20	20	40	10	≥80	20	40	≥80	
4	10	10	<10	<10	<10	20	<10	<10	10	<10	
3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	
2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	
1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	
HuN EA-H1N1											2.9
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
4	≥80	40	≥80	≥80	<10	≥80	≥80	≥80	≥80	<10	
3	≥80	40	40	<10	20	≥80	≥80	80	<10	40	
2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	
1	<10	<10	<10	<10	<10	<10	<10	<10	<10	10	
PBS	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	

*Serum samples were obtained from mice 14 d after inoculation. HuN EA-H1N1, Eurasian avian-like influenza A/Hunan/42443/2015(H1N1) virus; JS/1/11 EA-H1N1, Eurasian avian-like influenza A/Jiangsu/1/2011(H1N1) virus; MID_{50}, mouse infectious dose; ND, not determined due to death of the animal (treated as positive for determination of the MID_{50}); $TCID_{50}$, 50% tissue culture infectious dose.
†Titers <20 were regarded as negative for seroconversion.
‡MID_{50} was determined using the Spearman–Karber method (*19*).

infected mice, those infected with HuN EA-H1N1 virus had substantially higher virus titers in nasal turbinates, trachea, and lungs (Figure 4) and occasionally in extrapulmonary organs, including the liver ($10^{2.0}$ $TCID_{50}$/mL) and kidney ($10^{1.8}$ $TCID_{50}$/mL) of 1 mouse. A high virus titer was persistent in the respiratory tracts of HuN EA-H1N1–infected mice. These findings were consistent with bodyweight and survival data (Figure 3).

Serologic Investigation

We conducted a retrospective study to investigate the potential for human-to-human transmission of HuN EA-H1N1 virus, a reassortant EA-H1N1 virus. A total of 12 close contacts (4 family members and 8 healthcare workers) of the patient were included in this investigation. No family members reported influenza-like symptoms, but 3 healthcare workers showed signs and symptoms of illness. Whole-blood specimens from all close contacts were obtained for HI testing (*15*,*16*). An antibody response to HuN EA-H1N1 virus (HI titer of 40) was detected in only 1 contact, a doctor who exhibited signs of illness (Table 2). However, fever developed in this person on the same day she had contact with the patient, making it unlikely that her symptoms were caused by exposure to the patient. Furthermore, the positive HI antibody result for this contact may have been caused by a cross-reaction with A(H1N1)pdm09 virus (*11*,*20*). Thus, these findings indicate that human-to-human transmission of HuN EA-H1N1 virus did not occur.

Discussion

We identified a reassortant EA-H1N1 virus, HuN EA-H1N1, in a child in China with severe pneumonia. HA, NA, and M genes of the HuN EA-H1N1virus were derived from EA-H1N1 viruses; PB2, PB1, PA, and NP genes were derived from A(H1N1)pdm09 virus; and NS gene was derived from CS H1N1 virus. Our virulence studies in C57BL/6 mice showed that HuN EA-H1N1 virus exhibited greater virulence than JS/1/11 EA-H1N1 virus, which had been previously isolated from a child with fatal infection (Figure 3; Table 1). The full genome of another EA-H1N1 virus, HB/1250/12, which was isolated from a patient with mild illness, shared 98.9–99.6% nt identity with JS/1/11 EA-H1N1 virus, and these viruses caused similar illness and fatality rates in mice (data not shown). These findings suggest that the internal genes of HuN EA-H1N1 virus could be one a cause of the severe clinical syndrome seen in the HuN EA-H1N1 virus–infected child. Furthermore, the presence of renal disease and a history of long-term steroid treatment could have been major contributors to the death of the JS/1/11 EA-H1N1 virus–infected child (*10*).

A previous study showed that EA-H1N1 SIVs preferentially bind to human-type receptors, and some of the tested viruses were transmitted to ferrets by airborne droplets (*12*). That study concluded that EA-H1N1 SIVs have the potential to transmit efficiently and to cause a human influenza pandemic. We report a case of human infection with a reassortant EA-H1N1 swine influenza virus. Four internal genes of the virus were derived from A(H1N1)pdm09 virus. The 190D aa and 225E aa (H3 numbering) in the HA protein of the HuN EA-H1N1 virus suggested preferential binding to the SAα-2,6-Gal receptor. Although human-to-human transmission was not detected in our study, EA-H1N1 viruses have been reported to transmit efficiently via respiratory droplets in the ferret model (*12*). In addition, EA-H1N1 virus gene segments (NA and M genes)

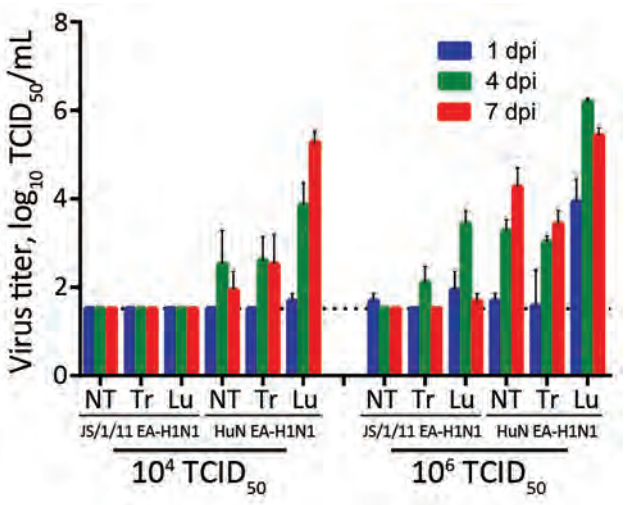

Figure 4. Replication of Eurasian avian-like influenza A/Jiangsu/1/2011 (JS/1/11 EA-H1N1) virus and Eurasian avian-like influenza A/Hunan/42443/2015 (HuN EA-H1N1) virus in the respiratory tracts of C57BL/6 mice. Eight- to ten-week-old female mice (3/group/time point) were inoculated intranasally with 50 μL of PBS containing 10^4 or 10^6 $TCID_{50}$ of JS/1/11 EA-H1N1 or HuN EA-H1N1 virus. Mice from each group were euthanized at 1, 4, and 7 days postinoculation (dpi). Tissues from each animal were homogenized in 1 mL of PBS and then clarified by centrifugation, and virus titers in the supernatant were determined by $TCID_{50}$ assay using MDCK cells. Results are the mean ± SD. NT, nasal turbinate; Tr, trachea; Lu, lung; $TCID_{50}$, 50% tissue culture infectious dose. Dotted horizontal line indicates the detection limit.

have been reported to contribute to the efficient respiratory droplet transmission of A(H1N1)pdm09 viruses in the ferret model (*21*). Our studies in mice also showed that infectivity and virulence were increased in reassortants of EA-H1N1 virus and A(H1N1)pdm09 virus. Thus, given the prevalence of novel EA-H1N1 viruses in pigs and their potential transmissibility to and pathogenicity in humans, enhanced influenza surveillance should be instituted among swine and humans.

Our study had several limitations. First, potential sources of infection for the HuN EA-H1N1 virus–infected patient were not identified. We collected 9 swab samples from pigs raised on the premises of the patient's home, which was in a rural location, but EA-H1N1 virus was not isolated. Second, despite the high similarity of HA (98.9%) and NA (97.7%) genes from JS/1/11 EA-H1N1 and HuN EA-H1N1 viruses (online Technical Appendix Table), we could not conclude that the A(H1N1)pdm09 virus–derived internal genes were associated with the high virulence of HuN EA-H1N1 virus and the patient's severe clinical syndrome. More investigations are needed to verify the virulence factor of HuN EA-H1N1 virus. Third, because an acute-phase serum sample was not available, we could not determine whether a doctor who exhibited signs of illness became infected before or after exposure to the patient; however, the doctor had a fever the same day she had contact with the patient.

In conclusion, EA-H1N1 swine influenza viruses occasionally infect humans. We report on a novel EA-H1N1 virus reassortant, HuN EA-H1N1 virus, which was isolated from a boy in China with severe pneumonia. The virus contained 2 surface genes from an EA-H1N1 virus and 4 internal genes from A(H1N1)pdm09 virus. Compared with JS/1/11 EA-H1N1 virus, the reassortant virus exhibited higher infectivity, virulence, and replication in C57BL/6J mice, demonstrating the need for further evaluation of HuN EA-H1N1 virus to assess the threat it poses to public health. Our results indicate the need for heightened surveillance.

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Table 2. HI antibody titers to HuN EA-H1N1 virus and CA07 virus in serum collected from an infected patient and his close contacts, Hunan Province, China, 2015*

Contact type and age, y	Contact date		Fever	Date of illness onset	Date of serum sample collection	HI titer	
	Initial	Final				HuN EA-H1N1	CA07
Doctor, 26	Unknown	Unknown	No	NA	Sep 21	5	40
Doctor, 28	Unknown	Unknown	No	NA	Sep 21	5	5
Nurse, 33	Unknown	Unknown	No	NA	Sep 21	20	40
Doctor, 36	Jul 2	Jul 11	Yes	Jul 11	Sep 21	5	5
Doctor, 47	Jul 13	Jul 13	Yes	Jul 13	Sep 28	40	80
Doctor, 39	Jul 13	Jul 13	No	NA	Sep 28	5	160
Doctor, 45	Jul 2	Jul 2	Yes	Jul 2	Sep 28	5	40
Nurse, 38	Jul 2	Jul 13	No	NA	Sep 28	5	40
Parent, 43	Jun 30	Jul 1	No	NA	Sep 29	20	40
Parent, 42	Jun 30	Jul 1	No	NA	Sep 29	5	5
Grandparent, 67	Jun 30	Jul 1	No	NA	Sep 29	5	40
Grandparent, 63	Jun 30	Jul 1	No	NA	Sep 29	5	5
Patient, 2	NA	NA	Yes	Jun 30	Sep 29	80	160

*CA07, A/California/07/2009 [A(H1N1)pdm09 virus; HI, hemagglutination inhibition; HuN EA-H1N1, Eurasian avian-like influenza A/Hunan/42443/2015(H1N1) virus; NA, not available.

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Serotype IV Sequence Type 468 Group B *Streptococcus* Neonatal Invasive Disease, Minnesota, USA

Sarah Teatero, Patricia Ferrieri, Nahuel Fittipaldi

To further understand the emergence of serotype IV group B *Streptococcus* (GBS) invasive disease, we used whole-genome sequencing to characterize 3 sequence type 468 strains isolated from neonates in Minnesota, USA. We found that strains of tetracycline-resistant sequence type 468 GBS have acquired virulence genes from a putative clonal complex 17 GBS donor by recombination.

Group B *Streptococcus* (GBS) is an opportunistic pathogen responsible for infections in neonates and adults; infection results in substantial morbidity and mortality worldwide (*1*). Ten serotypes (Ia, Ib, II–IX) have been described on the basis of their capsular polysaccharide antigen (*2*). A widely used multilocus sequence typing (MLST) scheme enables discrimination of GBS strains in >700 sequence types (STs), which are grouped in a few clonal complexes (CCs) (*3*).

We recently reported increased frequency of isolation of both carriage and invasive serotype IV GBS in Minnesota, USA, and increased frequency of isolation of serotype IV strains causing invasive disease in 3 Canadian provinces (*4*–*7*). We also showed that emerging serotype IV GBS organisms are genetically heterogeneous. Although most serotype IV strains belonged to ST459 (CC1) or ST452 (CC23), the population also comprised ST3, ST196, ST710, ST711, ST291, ST682, and ST468 isolates, the latter a single-locus variant of ST452 (*4*–*7*). Little is known about several of these less common serotype IV STs causing human disease, including their genomic makeup and antimicrobial drug resistance profiles. Also unknown are the molecular mechanisms underlying their emergence.

Homologous recombination occurs frequently in GBS and can involve vast areas of the genome in some lineages (*8*,*9*). Although MLST does not permit detection of recombination events with accuracy, the use of now relatively inexpensive next-generation whole-genome sequencing (WGS) has enabled the study of recombination in GBS at the population level (*4*,*9*,*10*). We used WGS analysis of 3 ST468 isolates recovered from neonates in Minnesota to test the hypothesis that recombination is a main driver of genetic diversity among serotype IV GBS.

The Study

We studied 3 ST468 strains, 2 isolated in 2007 and 1 in 2010 from neonates with early-onset or late-onset GBS disease (Table). We prepared genomic DNA by using a QIAGEN DNA MiniKit (QIAGEN, Toronto, ON, Canada) and genomic libraries by using Nextera XT kits (Illumina, San Diego, CA, USA). We sequenced the libraries as paired-end reads (150 bp + 150 bp) by using a MiSeq instrument (Illumina). We determined pili content in silico by aligning short-read WGS data with the sequences of pilus island (PI) 1, PI-2a, and PI-2b and using MOSAIK (*5*). The 3 ST468 strains possessed PI-2b only.

We next aligned the short reads to the genome of serotype IV ST452 strain NGBS572 (GenBank accession no. CP007632.1) and identified polymorphisms by using a variant ascertainment algorithm as previously described (*4*). The 3 ST468 strains differed from the ST452 reference strain by an average of 725 single-nucleotide polymorphisms (SNPs). When we plotted genome-wide SNPs of the ST468 strains against the genome of the reference strain (using BRIG [*11*]), we found a conspicuous area with an overabundance of SNPs (positions 210–320 kbp of the reference genome [Figure 1]). Nonrandom polymorphism distribution suggests recombination. To confirm this hypothesis, we analyzed the polymorphism data with BRAT NextGen (*12*), run with 20 iterations, 100 replicates, and a significance cutoff of 0.05. The analysis defined a region of recombination in strain PF-10 corresponding to positions 211,553–331,548 bp of the reference genome (Figure 1). A slightly narrower area of recombination was defined in strains PF-17 and PF-18 (211,553–323,601 and 249,415–323,601, respectively) (Figure 1).

In all 3 strains, the gene *tkt*, used in the MLST scheme, was found within the region of recombination, which explained the MLST result that ST468 is a single-locus variant of ST452. The common region of recombination also contained genes involved in metabolic pathways, such as *nagA*, encoding an N-acetylglucosamine-6-phosphate deacetylase involved in sialic acid metabolism. The ability of certain

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Table. Strains of neonatal invasive group B *Streptococcus* disease used in genetic diversity study, Minnesota, USA*

Isolate no.	Year cultured	Culture source	Patient age, d	SNPs to NGBS572	SRA accession no.
PF-10	2010	Blood	0	766	SRR4101407
PF-17	2007	Blood	2	706	SRR4101408
PF-18	2007	Blood	9	704	SRR4101409

*All isolates were serotype IV (*6*). SNP, single-nucleotide polymorphism; SRA, Sequence Read Archive.

streptococcal species to use sialic acid might play a role in the persistence and survival of these infecting organisms in vivo (*13*). Additionally, the region contained genes encoding the glycerol kinase GlpK, the aminopeptidase PepC, and the NAD synthetase nicotinamide adenine dinucleotide. This region also contains a bacteriocin (enterocin A) and penicillin-binding proteins PBP2X and PBP1A.

To identify the potential recombination donor, we first built a pseudoreference genome of strain PF-10. In brief, we performed de novo assembly of short reads generated for this strain as previously described (*5*), ordered obtained contigs against the reference genome of the ST452 strain NGBS572 with progressiveMauve (*14*), and concatenated the contigs with a separator that introduced a stop codon in every reading frame. Next, we aligned whole-genome sequence short reads of strains belonging to 12 different STs against the PF-10 pseudogenome and identified polymorphisms as described here. These 12 STs represent the 6 major CCs most frequently associated with GBS disease in humans (*9*). The pattern of polymorphisms strongly suggested that the donor of the recombined fragment was a CC17 strain (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/11/15-2031-Techapp1.pdf). Strains of CC17 are associated with neonatal infections, especially late-onset disease (*15*).

Lateral exchange of antimicrobial drug resistance mediated by mobile genetic elements occurs frequently in GBS. The implications of these events (beyond the obvious gain of resistance to a particular antimicrobial agent) are not yet fully understood. For example, Da Cunha et al. recently presented convincing evidence that tetracycline resistance has contributed to the worldwide expansion of GBS clones causing disease in humans (*9*). Strains of one of the major STs associated with serotype IV GBS invasive disease, ST452, are sensitive to tetracycline (*5*). Our unpublished observations suggest that the genome of

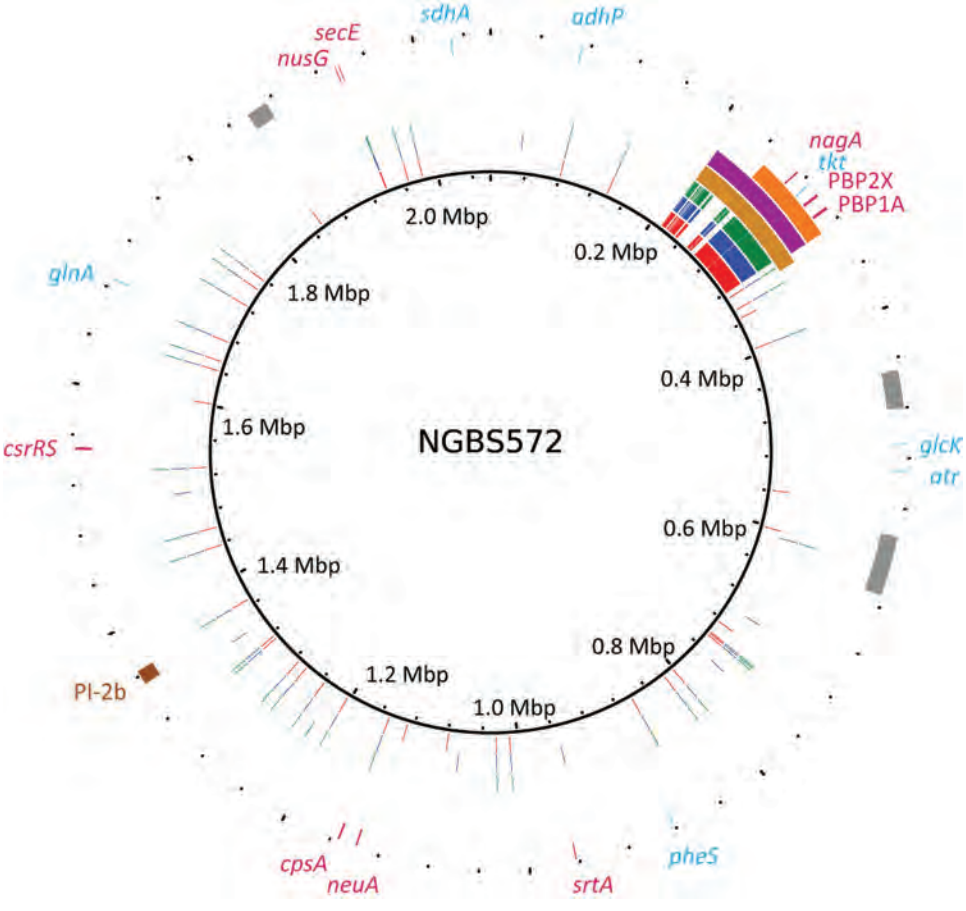

Figure 1. Genome analysis that identified recombination in sequence type (ST) 468 strains of group B *Streptococcus*. Plotting of polymorphisms identified in ST468 strains (PF-10, PF-17, and PF-18 in red, blue, and green, respectively) relative to the genome of the ST452 strain NGBS572 shows areas of densely clustered polymorphisms between positions 210,000 and 320,000 bp. Genome landmarks, such as mobile genetic elements (gray), multilocus sequence typing genes (light blue), pilus (brown), and virulence genes (dark pink), are marked in the outer circle. Precise areas of recombination in ST468 strains defined by BRAT NextGen (*12*) are depicted in gold (PF-10), purple (PF-17), and orange (PF-18). Mbp, megabase pairs.

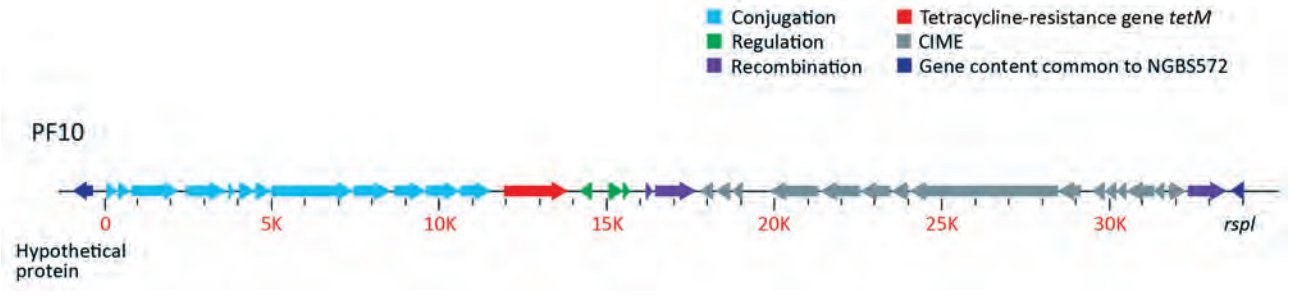

Figure 2. Representative diagram of a *tetM*-containing mobile genetic element (MGE) in group B *Streptococcus* sequence type (ST) 468 strains. Genome analysis discovered that the MGE is integrated at the 3′ end of gene *rspI*. Genes common between ST468 strains and the ST452 strain NGBS572 are shown in dark blue. Genes involved in conjugation are shown in light blue, regulatory genes in green, recombination genes in purple, and the gene encoding tetracycline resistance (*tetM*) in red. Cis integrative and mobilizable element genes (CIME) are shown in gray. Mbp, megabase pairs

ST452 strains could be considered a hybrid genome combining sequences of ST23 and ST17. ST468 strains analyzed here have additional ST17 content and were resistant to tetracycline. We determined that the genomes of all 3 ST468 strains contained gene *tetM*, which was carried on a mobile genetic element (MGE) (Figure 2). Areas of this MGE have 99% identity (blastn, www.ncbi.nlm.nih.gov/blast/Blast.cgi) to transposon Tn*916*. The Tn*916*-like element lies upstream of cis-mobilizable genetic content. Further inspection revealed that the MGE is inserted in the 3′ end of gene *rspI* (positions 232,722–250,539 bp of the ST452 genome), which is a known hot spot for integrative conjugative element integration in GBS (*8*).

Conclusions

WGS-based studies are expanding our understanding of genome reshaping in GBS and beginning to shed light on the population dynamics of this species. In particular, the use of WGS has shown that strains with multiple genome backgrounds are involved in the emergence of serotype IV GBS disease in North America (*5*,*6*). Here, we show that strains of one of these diverse backgrounds, ST468, have acquired additional genetic material from a neonatal-associated lineage by means of recombination. We also show that these isolates have acquired tetracycline resistance by lateral transfer. The changing epidemiology of GBS disease, with the involvement of a diverse array of strain genotypes, warrants continuing monitoring of colonization and invasive serotype IV GBS infections. Our data also support including this serotype in GBS vaccine formulations currently under development.

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Capsular Switching and Other Large-Scale Recombination Events in Invasive Sequence Type 1 Group B *Streptococcus*

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We report several cases of recombination events leading to capsular switching among sequence type (ST) 1 group B *Streptococcus* strains. These strains otherwise shared a common genome backbone with serotype V ST1 strains. However, the genomes of ST1 serotype V strains and those of serotypes VI, VII, and VIII strains differed substantially.

Group B *Streptococcus* (GBS) is a major cause of invasive infections in neonates. GBS also causes invasive disease in adults, with incidence rates that have been increasing in North America, particularly among the elderly (*1*). A serologic reaction directed against the polysaccharide capsule permits the classification of GBS into 10 serotypes: Ia, Ib, and II–IX (*2*). Serotype V strains are most frequently associated with invasive infections in adults in North America (*3*,*4*). Multilocus sequence typing (MLST) permits differentiation of GBS strains into >700 sequence types (STs), which are grouped into a few clonal complexes (CCs) (*5*). A recent study found that 92% of serotype V GBS strains recovered from the blood of nonpregnant adults in Houston, Texas, USA, and Toronto, Ontario, Canada, belonged to ST1 (*4*).

Homologous recombination occurs frequently in GBS and can involve vast areas of the genome in some lineages (*6*,*7*). Recombination leading to capsular switching has been reported numerous times in GBS (*3*,*6*,*8*). Capsular switching might result from a single recombination event that replaces the *cps* locus encoding the capsular biosynthetic machinery, leaving the rest of the genome unchanged. However, it is also possible that receiving strains acquire a donor *cps* locus and additional genetic material from the same or other donor strain(s). Because multiple recombination events can endow the receiving GBS strain with an enhanced virulence arsenal, obtaining information about additional genome changes is important.

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The Study

We investigated 111 ST1 GBS isolates collected by the Toronto Invasive Bacterial Diseases Network during 2009–2015. This network is a population-based surveillance system for invasive bacterial diseases in metropolitan Toronto and Peel Region, Ontario, Canada (total population under surveillance ≈5.5 million) that includes all hospitals (n = 28) providing care to and all laboratories (n = 25) processing sterile site cultures from residents of the population area. MLST and serotyping were performed as previously described (*4*,*5*,*9*). Most (103) of these ST1 strains were serotype V, but 8 ST1 isolates were found to be serotype Ib, II, IV, VI, VII, or VIII. These 8 strains represent an opportunity to investigate in more detail the molecular underpinnings of capsular switching in GBS. To this end, we sequenced the genomes of all 8 strains. We extracted genomic DNA by using the QIAGEN DNA minikit (QIAGEN, Toronto, Ontario, Canada) and prepared genomic libraries by using Nextera XT Kits (Illumina, San Diego, CA, USA). We sequenced libraries as paired-end reads with Illumina HiSeq (101 bp + 101 bp) or MiSeq (150 bp + 150 bp) instruments and deposited whole-genome sequencing data in the Sequence Read Archive of the National Center for Biotechnology Information (Table).

We next performed de novo genome assemblies using the A5 pipeline (*10*) and assessed the *cps* loci of the 8 strains. In all cases, blastn (http://blast.ncbi.nlm.nih.gov) comparisons identified the *cps* locus expected for the serotype identified by latex agglutination. To characterize recombination leading to capsular switching, we first used the variant ascertainment algorithm VAAL (*11*) to identify polymorphisms in each strain relative to the genome of serotype V ST1 strain SS1 (GenBank accession no. CP010867). The number of polymorphisms identified varied greatly between strains of the different serotypes. Strains of serotypes Ib, II, and IV had a relatively small number of polymorphisms, including single-nucleotide polymorphisms and small insertion/deletions, relative to the serotype V ST1 reference. The serotype Ib strain had 1,437 polymorphisms, the 2 serotype II strains had 1,115 and 816, respectively, whereas the serotype IV strain had 256. However, strains of the other 3 serotypes had a substantially higher number of polymorphisms relative to the genome of the serotype V ST1 strain SS1. The 2 serotype VI strains had 12,703 and 9,406 polymorphisms, respectively, the serotype VII strain had 4,117, and the serotype VIII

Table. Characteristics of 8 non–serotype V sequence type 1 group B *Streptococcus* isolates collected by the Toronto Invasive Bacterial Diseases Network, Ontario, Canada, 2009–2015*

Strain name	Serotype	Year isolated	Age group of patient	Source	SRA accession no.
NGBS217	Ib	2011	0–6 d	Blood	SRR3030375
NGBS748	II	2010	7–89 d	Blood	SRR3030378
NGBS814	II	2011	≥60 y	Blood	SRR3030379
NGBS1098	IV	2012	7–89 d	Blood	SRR3030380
NGBS209	VI	2011	≥60 y	Blood	SRR3030374
NGBS537	VI	2012	19–59 y	Blood	SRR3030376
NGBS015	VII	2009	≥60 y	Blood	SRR3030373
NGBS621	VIII	2012	19–59 y	Blood	SRR3030377

*Sequence types as determined by multilocus sequence typing. SRA, Sequence Read Archive.

strain had 3,471. By using custom scripts and the R software environment (https://www.r-project.org), we created plots of polymorphism distribution relative to the genome of the reference strain with a sliding window of 5 kbp. We observed a nonrandom polymorphism distribution in all 8 strains (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/15-2064-Techapp1.pdf).

We next plotted the polymorphisms identified in the strains against the genome of serotype V reference strain SS1 by using BRIG (*12*) and assessed recombination by using BratNextGen (run with 20 iterations and 100 replicates at p = 0.05) (*13*). The analysis showed that serotype Ib, II, and IV strains had a genome background similar to the ST1 serotype V reference strain but that each had experienced

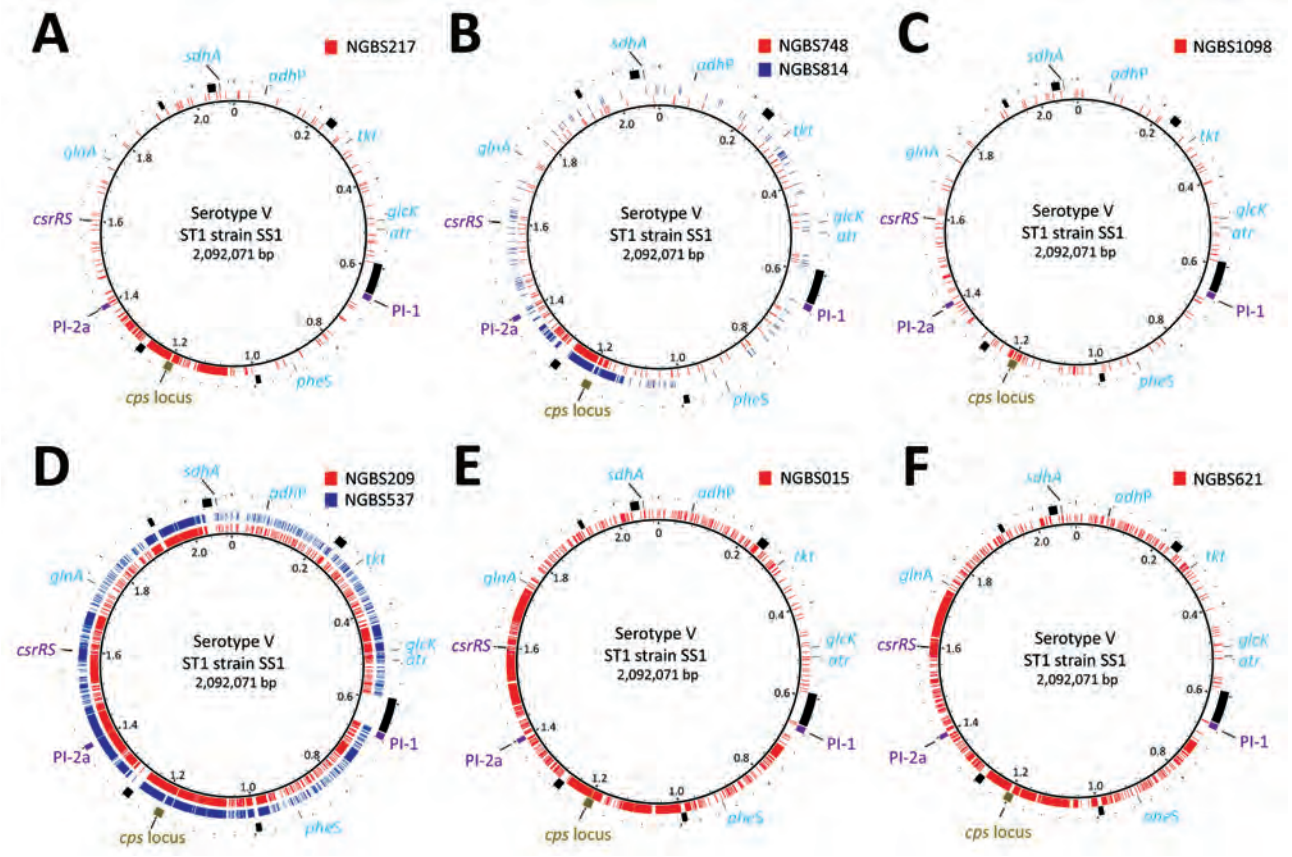

Figure. Extent of recombination among 8 non–serotype V sequence type (ST) 1 group B *Streptococcus* isolates collected by the Toronto Invasive Bacterial Diseases Network, Ontario, Canada, 2009–2015. A) Serotype Ib strain NGBS217. B) Serotype II strains NGBS748 and NGBS814. C) Serotype IV strain NGBS1098. D) Serotype VI strains NGBS209 and NGBS537. E) Serotype VII strain NGBS015. F) Serotype VIII strain NGBS621. Polymorphisms identified in non–serotype V ST1 strains (illustrated in different colors, with each line representing a polymorphism) are plotted against the genome sequence of reference serotype V ST1 strain SS1 (innermost circle). The *cps* locus is shown in gold in the outermost circle. Genome landmarks, such as genes used in the multilocus sequence typing scheme (*adhP, atr, tkt, glcK, sdhA, glnA,* and *pheS*) are shown in light blue. Mobile genetic elements of strain SS1 are depicted in black. Polymorphisms mapping to mobile genetic elements identified in the various non–serotype V ST1 strains were not included in the analysis. ST1, sequence type 1.

horizontal transfer of genome sequences of differing sizes, all of which included the *cps* locus. Namely, serotype Ib strain NGBS217 had exchanged a DNA region of ≈200 kb and acquired a *cpsIb* locus (Figure, panel A). Serotype II strains NGBS814 and NGBS748 had exchanged DNA regions of ≈189 kb and ≈152 kb, respectively, and gained a *cpsII* locus (Figure, panel B). Recombination in the serotype IV strain NGBS1098 was less extensive (≈79 kb) but resulted in acquisition of a *cpsIV* locus (Figure, panel C). Thus, in strains of these 3 serotypes, capsular switching most likely resulted from a single recombination event that replaced the original *cpsV* locus of the receiving strains with those of the donor strains but left the rest of the genome of the receiving strains unchanged. We speculate that these recombination events most likely occurred by conjugation and that DNA exchange took place in the human gut or urogenital tract. These body sites can be colonized by multiserotype GBS populations (*14*).

In contrast with those results, the genomes of strains of serotypes VI, VII, and VIII differed extensively from the reference serotype V strain. Indeed, recombination detected by BratNextGen involved >50% of the genomes of these isolates, even though they were found to be ST1 by MLST (Figure, panels D–F). Based on the available data, it is difficult to ascertain if those strains were originally ST1 strains that acquired a *cps* locus and many other genomic regions by recombination in apparently multiple recombination events or, alternatively, if the isolates under investigation originally possessed the *cps* locus corresponding to their serotype and acquired by recombination from unknown ST1 donors the different portions of the genome that contain all genes used in the MLST scheme. In the latter scenario, and in particular for serotype VI strains, the increased number of polymorphisms in ST1-like areas of the genome suggests that the putative ST1 donor was genetically not closely related to the serotype V ST1 reference strain SS1.

Conclusions

Our data suggest that extensive recombination might be a key contributor to clonal diversification and emergence of GBS serotypes VI, VII, and VIII, which are less often identified in North America but have substantial regional dominance in other geographic areas (*15*). MLST is a useful approach to begin to examine the genetic relationships of GBS strains. In some cases, particularly when temporally and geographically related isolates are evaluated, this approach also permits initial prediction of recombination events (*3*,*5*–*8*). Whole-genome sequencing extends MLST capabilities and enables precise identification and characterization of genetic variation attributable to extensive recombination in this opportunistic pathogen. Although the polysaccharide-based, trivalent conjugate GBS vaccine under development offers great promise, frequent capsular switching suggests that subunit vaccines based on antigens expressed by all GBS serotypes might offer enhanced protection.

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Changing Pattern of *Chlamydia trachomatis* Strains in Lymphogranuloma Venereum Outbreak, France, 2010–2015

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We describe a change in the molecular epidemiology of *Chlamydia trachomatis* strains involved in an outbreak of rectal lymphogranuloma venereum in France during January 2010–April 2015. Until 2012, the *C. trachomatis* L2b strain predominated; however, starting in 2013, most cases involved the L2 strain. We also identified 4 genetic L2b *omp*A variants.

Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by the invasive L genovars of *Chlamydia trachomatis*. Since 2003, outbreaks of LGV have spread across Europe and other high-income countries, mainly among HIV-infected men who have sex with men (MSM) (*1*–*5*). Almost all LGV cases have been caused by the *C. trachomatis* L2b variant, which harbors an A/G substitution on the *omp*A gene at position 485, suggesting a single source of origin for the outbreaks (*6*,*7*). Recently, however, 2 *C. trachomatis* variants co-circulated during an LGV epidemic in Spain (*8*). The objective of this study was to investigate if genetic variations exist within LGV *C. trachomatis* strains circulating in France during 2010–2015 by sequencing of the *omp*A gene.

The Study

In January 2010, France introduced sentinel surveillance for *C. trachomatis* proctitis, approved by the country's Data Protection Authority, to monitor the LGV outbreak. Laboratories perform routine testing for *C. trachomatis*, and positive anorectal specimens are referred to the French National Reference Center for Chlamydiae (Bordeaux, France) for LGV testing, using a real-time PCR targeting a 36-bp deletion in the *pmp*H gene (*9*). For each patient, clinical, biological, and sexual behavior data are collected after written consent is obtained.

During January 2010–April 2015, we retrospectively selected 179 LGV-positive anorectal specimens from the surveillance samples. To ensure that samples were representative of infections in the LGV-infected population, we used the following criteria for selecting samples for each year of the study: 1) the percentage of specimens analyzed each year was the same and corresponded to ≈12% of anorectal LGV cases diagnosed in France per year; 2) the residences of patients were classified as in the Paris area versus other regions of France to respect the geographic distribution of cases during each year of the survey; 3) samples were distributed over the 12 months of each year, except 2015; and 4) specimens had a high bacterial load, defined as a cycle threshold of ≤30 using the *pmp*H real-time PCR. We analyzed 23 *C. trachomatis* specimens from 2010, 24 from 2011, 24 from 2012, 40 from 2013, 49 from 2014, and 19 from January–April 2015.

Amplification of the *omp*A gene was performed directly on specimens. A 1,100-bp fragment was amplified by nested PCR, using the NLO and NRO primers and PCTM3 and SERO2A primers (*10*), and sequenced in both directions. An L genovar was confirmed for all specimens by *omp*A sequencing. Most specimens (52.5%, 94/179) had *omp*A sequences identical to that of the *C. trachomatis* L2b/UCH-1/proctitis reference strain (GenBank accession no. AM884177.1); 61 (34%) specimens had sequences identical to that of reference strain L2/434/BU (GenBank accession no. AM884176.1). In the remaining 24 specimens, we identified 4 genetic L2b *omp*A variants that had nonsynonymous single-nucleotide polymorphisms, compared with nucleotide sequence of the L2b/UCH-1/proctitis reference strain. One variant, L2b *omp*A variant 1 (designated L2bV1; GenBank accession no. JX971936), was found in 19 specimens and featured a C→A substitution at position 517 (Leu173Ile) (*8*,*11*). A second variant, L2bV2 (GenBank accession no. KU518893), was detected in 1 specimen and had an A→C substitution at position 515 (Lys172Thr). The third variant, L2bV3 (GenBank accession no. KU518894), was detected in 2 specimens and shared a C→A substitution at position 493 (His165Asn). The fourth variant, L2bV4 (GenBank accession no. KU18892), was detected in 2 specimens and featured a C→A point mutation at position 286 (Ala96Thr).

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Our analysis of the distribution of the *C. trachomatis ompA* genotype during 2010–2015 showed that the percentage of L2b strain was significantly lower in 2013 (35%, 14/40), 2014 (38.8%, 19/49), and 2015 (31.6%, 6/19) than in 2010 (86.9%, 20/23), 2011 (70.8%, 17/24), and 2012 (75%, 18/24) ($p \leq 0.05$ by χ^2 test) (Figure). Conversely, the percentage of L2 strain was significantly higher in 2013 (52.5%, 21/40), 2014 (44.9%, 22/49), and 2015 (52.6%, 10/19) than in 2010 (4.3%, 1/23), 2011 (20.8%, 5/24), and 2012 (8.3%, 2/24) ($p \leq 0.05$ by Fischer exact test). The percentage of L2b *ompA* variants was similar during each year of the survey (Figure). In 2012 and 2013, the distribution of L2 and L2b strains and L2b *ompA* variants was the same in Paris and other regions of France, showing that the shift was not due to regional variation ($p = 0.86$ by χ^2 test).

All specimens were from men. Symptoms (rectal syndrome, rectal pains, anal discharge, rectal bleeding) were present in all cases, and clinical features were similar. Data about sexually transmitted bacterial co-infections (e.g., *Neisseria gonorrhoeae*, syphilis) were available for 64.2% (115/179) of patients and showed a similar prevalence among those infected with L2b (34.6%, 18/52), L2 (35.3%, 12/34), or L2b *ompA* variants (38.5%, 5/13). Serologic HIV status was documented for 112 patients; results were positive for 86.8% (33/38), 79.7% (47/58), and 68.8% (11/16) of the patients carrying L2 strain, L2b strain, or L2b *ompA* variants, respectively ($p = 0.14$). The percentage of MSM was lower among patients infected with L2b *ompA* variants (76.9%, 10/13) than among those infected with L2 (100%, 24/24) or L2b strains (95.7%, 45/47) ($p = 0.063$). Having an occasional, versus a steady, sex partner was frequently reported, ranging from 76.5% (26/34) in the L2 group to 100% (8/8) in the L2b *ompA* variants group.

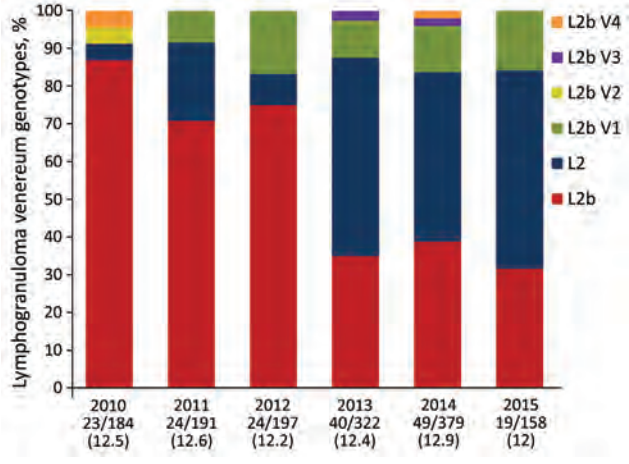

Figure. Distribution of lymphogranuloma venereum–associated *Chlamydia trachomatis* genotypes and variants, according to sequencing results of the *ompA* gene of representative patient samples, France, 2010–2015. Numbers below bars indicate no. samples analyzed/no. cases total (%).

Conclusions

We describe a change in the molecular epidemiology of *C. trachomatis* strains involved in an outbreak of anorectal LGV in France. Our results show that, until 2012, the L2b strain predominated, a finding that is in agreement with reports coming from other countries in Europe (*12*). However, L2b is not a recently emerged strain: the strain was present in 1981 among MSM in San Francisco, California, USA (*12*). Different LGV strains have spread widely in the MSM community since 2013, and prevalence of the L2 genotype has increased. These data suggest that the cocirculation of the 2 predominant LGV strains could be the result of 2 independent introductions. The L2 strain is more prevalent in the United States (*13*), and the last known outbreak was in 1992 in the Caribbean area (*14*). Knowing the country of residence of the partner who was the possible source of infection would potentially enable identification of distinct introduction pathways for L2 and L2b strains.

Our findings show that 4 genetic *C. trachomatis* L2b *ompA* variants have been circulating in France since 2010. Three of the variants have amino acid changes in the variable domain II, which has been described as a common antigenic domain for *C. trachomatis*. The L2bV1 strain was identified during September 2011–March 2012 in 4 specimens collected in New York, USA, and in 1 specimen collected in Spain between 2009 and 2011 (*8,11*). In our study, *C. trachomatis* strain L2bV1 was first identified in 2011 and then each year thereafter; it was the predominant L2b *ompA* variant. The 3 other variants had not been previously described.

Patient characteristics did not differ with regard to clinical data, sexual behavior, or the *C. trachomatis* genotypes involved in LGV infections. Our results are discordant with those of Rodríguez-Domínguez et al. (*8*), who showed less aggressive symptoms among patients infected by *C. trachomatis* L2 strain than those infected by L2b strain.

Our results must be confirmed by genetic characterization of more specimens. However, we observed genetic diversity of LGV *C. trachomatis* strains when testing as few as 12% of reported anorectal LGV patients a year. Future research might examine if the increase of LGV cases in other countries is also associated with an increase in *C. trachomatis* L2 strain.

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ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015

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In England in 2015, *Shigella sonnei* isolates from men who have sex with men produced extended-spectrum β-lactamases and exhibited macrolide resistance. Whole-genome sequencing showed a close relationship among the isolates, which harbored a plasmid that was previously identified in a shigellosis outbreak among this population but has acquired a mobile element.

Historically, shigellosis is an infection associated with travel to countries in which this infection is endemic. In England in 2009, *Shigella flexneri* 3a infections dramatically increased among men who have sex with men (MSM) and were thought to be associated with sexual transmission (*1*). Since 2011, an emerging epidemic of *S. sonnei* infections in England among men has occurred (*2*). We describe an investigation of a cluster of multidrug-resistant *S. sonnei* infections among MSM in England during 2015.

The Study

In November 2015, Public Health England (PHE) identified a cluster of *S. sonnei* isolates by analyzing whole-genome sequencing data from specimens collected during September and October from 4 adult men residing in London and having no known travel history. Clinical reporting data indicated that 2 of the men were MSM. The isolates were later found to exhibit high levels of antimicrobial drug resistance to amoxicillin, ceftriaxone, tetracycline, sulfonamides, trimethoprim, and azithromycin and to produce extended-spectrum β-lactamase (ESBL) (Table 1). The isolates were sensitive to quinolones. PHE convened an outbreak control team to identify additional cases, characterize cases, capture exposure information, rule out a point source for the outbreak, and provide evidence for additional targeted public health efforts.

Since August 2015, PHE has conducted whole-genome sequencing of ≈70% of *S. sonnei* isolates (those voluntarily referred from hospital laboratories) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-0653-Techapp1.pdf). Single nucleotide polymorphism (SNP) clustering is performed, as previously described (*3*). Readings (in FASTQ format) from all sequences in this study can be found at the PHE Pathogens BioProject (National Center for Biotechnology Information project no. PRJNA315192).

DNA from isolate 183660 was used to elucidate plasmid sequencing (online Technical Appendix). The multidrug-resistance phenotype was explained by the presence of an 89,000-bp IncFII plasmid designated p183660 (GenBank accession no. KX008967). This plasmid had >95% nt identity to pKSR100, a multidrug-resistance plasmid from an MSM case of *S. flexneri* 3a infection (GenBank accession no. LN624486) that had acquired the previously described pKSR100 integron (*bla*$_{\text{TEM-1}}$, *dfrA17*, *dfrA1*, *sul1*, *aadA5*) (*4*) and a novel mobile element harboring *bla*$_{\text{CTX-M-27}}$ (Figure 1). Macrolide resistance was conferred by *erm*B and *mph*A.

For this investigation, we defined a case-patient as a resident of England with laboratory-confirmed *S. sonnei* infection belonging to a 5 SNP cluster with the SNP address 1.3.3.9.207.212.%, a resistance profile characterized by the presence of the plasmid p183660, and a specimen collected during September 1, 2015–February 29, 2016. We identified 9 case-patients (Figure 2) from fecal specimens; all had no known travel history in the 2 weeks before illness. Median age was 33 (range 28–83) years. Gastrointestinal symptom onset ranged from early September 2015 to late December 2015. No patients were bacteremic. Treatment failure was reported for 2 patients; both were hospitalized and 1 died, although *Shigella* was not recorded as a cause of death. Duration of illness was not systematically collected. Diagnoses occurred in general practice (n = 5), sexual health clinics (n = 2), and hospitals (n = 2). Seven patients

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Table 1. Results of the phenotypic antimicrobial drug resistance profile of 4 isolates of *Shigella sonnei*, England, 2015*

Isolate ID	MIC for antimicrobial drugs tested, μg/mL†																	
	AMP	AMC	CTX	CRO	CAZ	CFP	GEN	TOB	TMP	SEP	ATM	CIP	COL	ERT	MER	FOS	TEM	AZT
182834	>1,052	1	>32	>32	2	0.38	0.5	0.38	>32	64	0.38	<0.032	0.19	0.008	0.06	1	<1	>256
184494	>1,052	1	>32	>32	2	0.5	0.38	0.38	>32	128	0.5	<0.032	0.125	0.008	0.06	1.5	<1	>256
183660	>1,052	1	>32	>32	1	0.38	0.38	0.38	>32	128	0.38	<0.032	0.125	0.008	0.06	1	<1	>256
164679	>1,052	1	>32	>32	1	0.38	0.38	0.38	>32	64	0.38	<0.032	0.19	0.008	0.06	1	<1	>256

*TEM was included in the panel to aid detection of OXA-48–like carbapenemases. AMC, amoxicillin/clavulanate; AMP, ampicillin; ATM, aztreonam; AZT, azithromycin; CAZ, ceftazidime; CFP, cefepime; CIP, ciprofloxacin; COL, colistin; CRO, ceftriaxone; CTX, cefotaxime; ERT, ertapenem; FOS, fosfomycin; GEN, gentamicin; ID, identification; MER, meropenem; SEP, trimethoprim/sulfamethoxazole; TEM, temocillin; TMP, trimethoprim; TOB, tobramycin.
†Phenotypic antimicrobial drug susceptibility testing was performed by using Etest (bioMérieux, Marcy l'Etoile, France) and interpreted by using breakpoints for *Enterobacteriaceae* established by the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org/). Production of extended-spectrum β-lactamase was confirmed by using CTX + clavulunic acid and CAZ + clavalunic acid in combination (bioMérieux).

resided in London; 2 resided elsewhere in England, 1 of whom reported recent travel to London. Of 8 patients with reported ethnicity, 6 self-identified as white.

In-depth questionnaires administered by telephone interviews collected information on sexual history, food exposures, and travel history; 8 patients completed the interviews. Seven answered detailed sexual history questions (including the 2 patients identified as MSM from clinical reporting data); all 7 reported having male partners. Six reported exposures considered to be high risk for *Shigella* transmission during the incubation period (e.g., fisting, sex under the influence of recreational drugs [*6*], oral-anal contact, attendance at sex parties, or use of sex toys with a partner) (Table 2). Reported number of sexual partners during the 2 weeks before illness was 0–5. The 7 patients who completed the sexual history questions reported previous high-risk sexual exposures; 6 of these patients and 1 patient who partially completed the questionnaire reported a history of other sexually transmitted infections, including gonorrhea, chlamydia, syphilis, nonspecific urethritis, hepatitis C, and pubic lice. Five reported using mobile apps to meet partners at some time in the past; none reported a prior shigellosis diagnosis. All had visited sexual health services (Table 2). Some patients were HIV positive (frequency is omitted to prevent deductive disclosure).

No epidemiologic links were identified among patients, and no point sources were identified, although food exposure data was collected for 5 patients only. Two reported attending the same sex-on-premises venue in London 3 months apart (1 reported no sexual contact at the venue). None

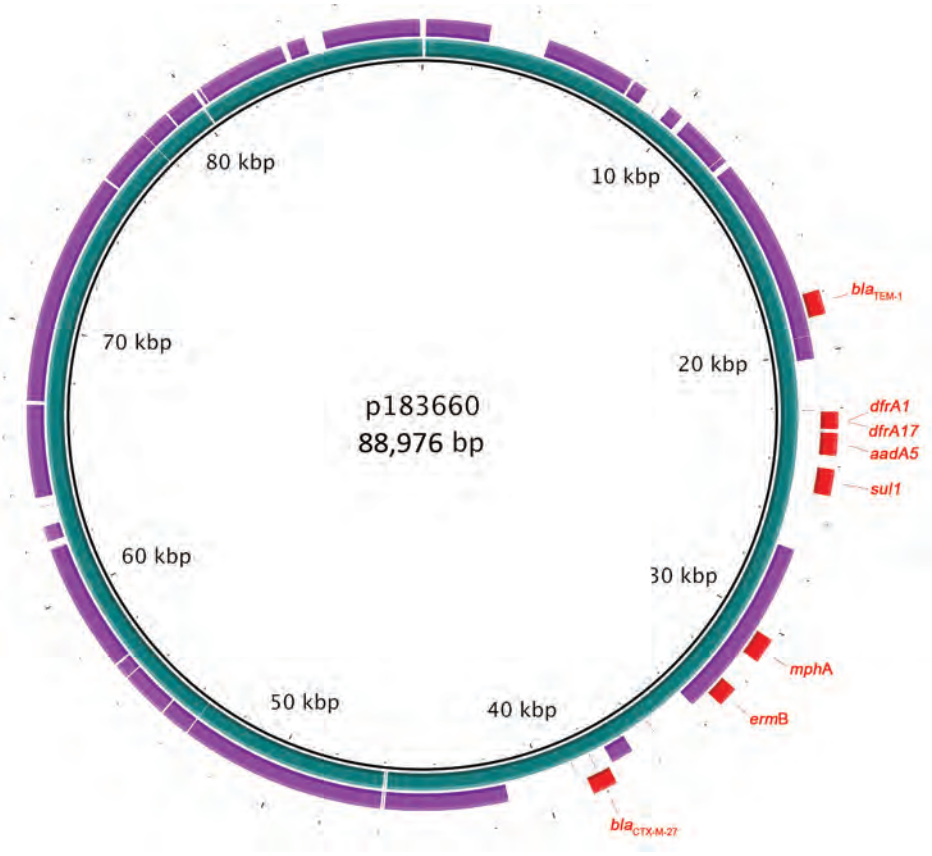

Figure 1. Genomic plot of multidrug-resistance plasmid p183660 (inner ring, blue) from a man in England infected with *Shigella sonnei* compared with pKSR100 (outer ring, purple), a multidrug-resistance plasmid from a case of *S. flexneri* 3a infection occurring among men who have sex in men (*4*). Drug-resistant elements from p183660 are shown in red. Plot produced by using BLAST Ring Image Generator (*5*).

Figure 2. Maximum-likelihood phylogeny showing isolates from a cluster of 9 cases (gray shading) of *Shigella sonnei* infection among men who have sex with men in England, 2015. For context, 246 *S. sonnei* isolates that are representatives from each t25 cluster were included in the comparison. Isolates are labeled by single nucleotide polymorphism address.

reported awareness of possible infection with *Shigella* spp. or of contact with others with gastrointestinal symptoms. Three thought they had acquired their infections from food. Patients reported that they accessed health promotion messages at sexual health clinics and on the internet.

Conclusions

We describe a phylogenetic cluster of ESBL-producing *S. sonnei* infections in MSM in England that raises concerns about the ability to manage the spread of resistant *Shigella* infection in this population. Whole-genome sequencing revealed that the multidrug-resistance phenotype is conferred by acquisition of an IncFII plasmid (p183660) known to be circulating in *S. flexneri*–affected MSM (*4*); this plasmid has acquired $bla_{\text{CTX-M-27}}$.

Patient-reported behaviors were similar to those reported in an earlier *S. flexneri* epidemic among MSM: high numbers of sexual partners, high levels of condomless sex, attendance at sex parties, sex under the influence of recreational drugs, and prior HIV infection (*6*). We identified no point source, further indicating that sexual contact was the dominant mode of transmission.

Although *S. sonnei* causes self-limiting diarrhea in most patients, life-threatening invasive infections in patients co-infected with HIV have occurred (*7*). Quinolone and azithromycin resistance have been observed in recent

Table 2. Reported exposure history for 9 case-patients with *Shigella sonnei* infection in the cluster with ESBL production and macrolide resistance, England, 2015*

Risk factor	No. (%) exposed	No. (%) unexposed	No. unknown†
Exposure ever before onset			
Self-identified as a man who has sex with men	7 (100)	0	2
Fisting	2 (29)	5 (71)	2
Sex under the influence of recreational drugs	4 (57)	3 (43)	2
Scat play	0	7 (100)	2
History of other sexually transmitted infections	7 (88)	1 (13)	1
Use of apps to meet partners	5 (71)	2 (29)	2
Awareness of *Shigella* infections	0	7 (100)	2
Previous *Shigella* diagnosis	0	8 (100)	1
Known to sexual health services	7 (100)	0	2
Exposure during the 2 weeks before onset			
Fisting	1 (14)	6 (86)	2
Sex under the influence of recreational drugs	3 (43)	4 (57)	2
Oral–anal contact	3 (43)	4 (57)	2
Condomless sex‡	5 (71)	2 (29)	2
Attended sex parties or live sex premises	3 (43)	4 (57)	2
Use of sex toys with partner	1 (14)	6 (86)	2
Scat play	0	7 (100)	2

*ESBL, extended-spectrum β-lactamase; scat play, sexual arousal or activity linked to feces.
†Unknowns were not included in percentage calculations; 7 of 9 patients completed and 1 partially completed interviews about sexual behavior.
‡Any condomless sexual contact.

outbreaks of *S. sonnei* infections in MSM; cepaholsporins are recognized as a suitable therapeutic choice for invasive or prolonged infections (*8*,*9*).

Primary public health concerns include possible treatment failure for severe shigellosis in immunocompromised patients; further spread of the multidrug-resistant IncFII plasmid to other enteric pathogens in MSM, with possible implications for treatment among the immunocompromised; rapid spread of drug-resistant *S. sonnei* infections among MSM, including outside the United Kingdom, as happened with *S. flexneri* 3a (*4*), especially among the HIV infected; and transmission through food handling and childcare centers, with potential to cause outbreaks of drug-resistant *S. sonnei* infections in other populations. Low awareness of *Shigella* infections among patients in our study suggests that prior awareness campaigns targeting high-risk MSM in England (*10*) have not been fully successful. Future campaigns being planned to improve awareness of *Shigella* infection and transmission routes will target social media, sexual health clinics, and primary care workers to increase awareness among MSM and healthcare staff. Frontline sexual health clinicians and microbiologists in England have been made aware of emerging drug resistance in *Shigella* spp. among this vulnerable group and of the need to perform antimicrobial drug susceptibility testing if treatment is considered necessary.

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Early Growth and Neurologic Outcomes of Infants with Probable Congenital Zika Virus Syndrome

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We report the early growth and neurologic findings of 48 infants in Brazil diagnosed with probable congenital Zika virus syndrome and followed to age 1–8 months. Most of these infants had microcephaly (86.7%) and craniofacial disproportion (95.8%). The clinical pattern included poor head growth with increasingly negative *z*-scores, pyramidal/extrapyramidal symptoms, and epilepsy.

The first reports of Zika virus infection in Brazil were in early 2015 (*1*). Shortly thereafter, Zika virus was associated with microcephaly (*2*). In February 2016, the World Health Organization (WHO) declared the potential association between Zika virus and microcephaly, a public health emergency of international concern (*3*).

Zika virus is able to cross the placental barrier. A growing body of evidence suggests that Zika virus causes cell death in neurons in vitro (*4*), brain anomalies, and microcephaly, resulting in what has been called congenital Zika virus syndrome (*5*). Cortical and subcortical atrophy, brain calcifications, ventriculomegaly, cerebellum anomalies, and abnormal neuronal migration have been described (*6*). The main reported signs and symptoms include abnormalities in neurologic examination, dysphagia, microcephaly (*7*–*9*), and a phenotype characterized as fetal brain disruption sequence (*10*).

Because this congenital infection is newly recognized, its full spectrum is not completely described, and little is known about the growth and neurologic outcomes of infants with congenital Zika virus syndrome in the first months of life. We reviewed the records of 48 infants born from September 2015 onwards that were enrolled at the Reference Center for Neurodevelopment, Assistance, and Rehabilitation of Children during January–May 2016 in Sao Luis, Brazil.

The Study

Because isolating Zika virus from human tissues is difficult, we used the following definition by Franca et al. (*6*), which was developed based on a protocol of the Brazil Ministry of Health (*11*) to identify highly probable cases of congenital Zika virus syndrome: 1) central nervous system abnormalities detected by cranial computed tomography (CT) scan, with or without microcephaly; and 2) negative results for syphilis, toxoplasmosis, rubella, cytomegalovirus, and herpes (STORCH) on serologic tests of the infant after delivery. Microcephaly was defined as head circumference (HC) 2 SD below the mean for gestational age and sex based on the INTERGROWTH-21st standards (*12*). Severe microcephaly was defined as HC 3 SD below the mean (*12*). The mothers were asked about the month of appearance of rashes during pregnancy. Birthweight and birth length *z*-scores were also classified according to the INTERGROWTH-21st criterion (*12*). The weight, length and HC after birth were classified according to the WHO standards (*13*). The initial status and rate of change of weight, length, and HC were estimated in a random-intercept multilevel linear regression model by using age in months as an explanatory variable. The Research Ethics Board of the Federal University of Maranhão approved the study (1510305).

Rash during pregnancy was reported by 73.9% (34/46) of mothers, mostly in the first trimester (52.2%). Most infants (52.1%) were male, and 87.2% were born at term. The HC *z*-score at birth was considered normal for 13.3% of the infants, whereas for 22.2% of the infants, the HC was >2 but <3 SD below the mean. However, most infants had an HC ≥3 SD below the mean (64.5%). The birth length *z*-score was compromised for 43.2%, and the birthweight

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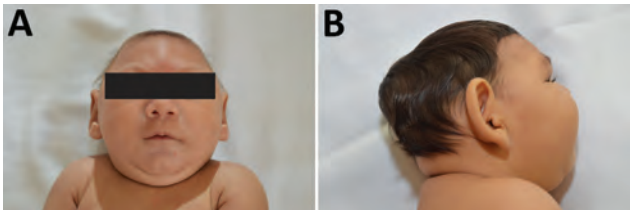

Figure 1. Characteristic phenotype of fetal brain disruption sequence in infants with probable congenital Zika virus syndrome, Sao Luís, Brazil, 2015–2016. A) Craniofacial disproportion and biparietal depression. B) Prominent occiput.

was ≥2 SD below the mean for 19.6% of infants. The mean age at last visit to the reference center was 4.4 months. Nearly all infants had a phenotype characteristic of fetal brain disruption sequence (Figure 1), including craniofacial disproportion (95.8%), biparietal depression (83.3%), prominent occiput (75.0%), and excess nuchal skin (47.9%) (Table).

Of the 48 infants, 85.4% had irritability, making irritability the most common symptom described, followed by pyramidal/extrapyramidal syndrome (56.3%), epileptic seizures (50.0%), and dysphagia (14.6%). Pyramidal syndrome included hypertonia, clonus, hyperreflexia, and increased archaic reflexes. Extrapyramidal symptoms were characterized by tonus fluctuation and asymmetric dyskinesias in the extremities that were absent during sleep. Some infants also had clubfoot (10.4%) and arthrogryposis (10.4%), and 1 infant (2.1%) had cleft lip/cleft palate. Among the 27 infants who underwent electroencephalography, 48.1% had abnormal brain activity without epileptiform discharges, 29.6% had focal discharges, and 22.2% had multifocal epileptiform discharges. All infants had abnormal cranial CT scan imaging findings. The most common were brain calcifications (91.7%), cortical malformations (87.5%), and secondary ventriculomegaly (77.1%). Brain stem and cerebellum hypoplasia and white matter attenuation were less common (Table).

For each infant, we noted weight, length, and HC *z*-scores at birth and each postnatal visit up to 8 months of age (Figure 2). The mean HC *z*-score at birth was –3.61, and it decreased –0.46 per month. The mean weight *z*-score was –1.12 at birth, and it decreased –0.08 per month. The mean length *z*-score was –1.57 at birth, and it decreased –0.16 per month.

Conclusions

We describe the early growth and neurologic outcomes of infants with probable congenital Zika virus syndrome in the first 8 months of age. In total, 64.5% of infants were born with severe microcephaly, and 95.8% had a phenotype of fetal brain disruption sequence.

The most common clinical symptom noted was irritability, characterized by hyperexcitability (clonus following external stimulation), irritable and impatient cry, and sleep

Table. Clinical characteristics of probable congenital Zika virus syndrome in infants from birth to 1–8 months of age, Sao Luis, Brazil, 2015–2016

Characteristic	No. (%)
Rash in mother during pregnancy, n = 46	
First trimester	24 (52.2)
First month	1 (2.2)
Second month	12 (26.1)
Third month	11 (23.9)
Second trimester	10 (21.7)
Fourth month	9 (19.6)
Sixth month	1 (2.2)
No rash	12 (26.1)
Sex, n = 48	
M	25 (52.1)
F	23 (47.9)
Gestational age at birth, n = 47	
Preterm	4 (8.5)
Term	41 (87.2)
Postterm	2 (4.3)
Head circumference *z*-score at birth,* n = 45	
≥–2	6 (13.3)
Microcephaly, <–2	10 (22.2)
Severe microcephaly, <–3	29 (64.5)
Birth length *z*-score,* n = 3	
≥–2	21 (56.8)
<–2	11 (29.7)
<–3	5 (13.5)
Birthweight *z*-score,* n = 46	
≥–2	37 (80.4)
<–2	8 (17.4)
<–3	1 (2.2)
Age at last visit, mo, n = 48	
1	2 (4.2)
2	6 (12.5)
3	7 (14.6)
4	10 (20.8)
5	10 (20.8)
6	7 (14.6)
7	5 (10.4)
8	1 (2.1)
Phenotype, n = 48	
Craniofacial disproportion	46 (95.8)
Biparietal depression	40 (83.3)
Prominent occiput	36 (75.0)
Excess nuchal skin	23 (47.9)
Signs and symptoms, n = 48	
Irritability	41 (85.4)
Pyramidal/extrapyramidal syndrome	27 (56.3)
Epileptic seizures	24 (50.0)
Dysphagia	7 (14.6)
Congenital clubfoot	5 (10.4)
Arthrogryposis	5 (10.4)
Cleft lip/cleft palate	1 (2.1)
Electroencephalogram findings, n = 27	
Abnormal activity, no epileptiform discharges	13 (48.1)
Focal epileptiform discharges	8 (29.6)
Multifocal epileptiform discharges	6 (22.2)
Cranial computed tomography imaging findings, n = 48	
Calcifications in the brain parenchyma	44 (91.7)
Malformation of cortical development	42 (87.5)
Ventriculomegaly	37 (77.1)
White matter attenuation	15 (31.3)
Brain stem and cerebellum hypoplasia	6 (12.5)

*Reported as deviations of the raw *z*-score from the mean measured in SD units.

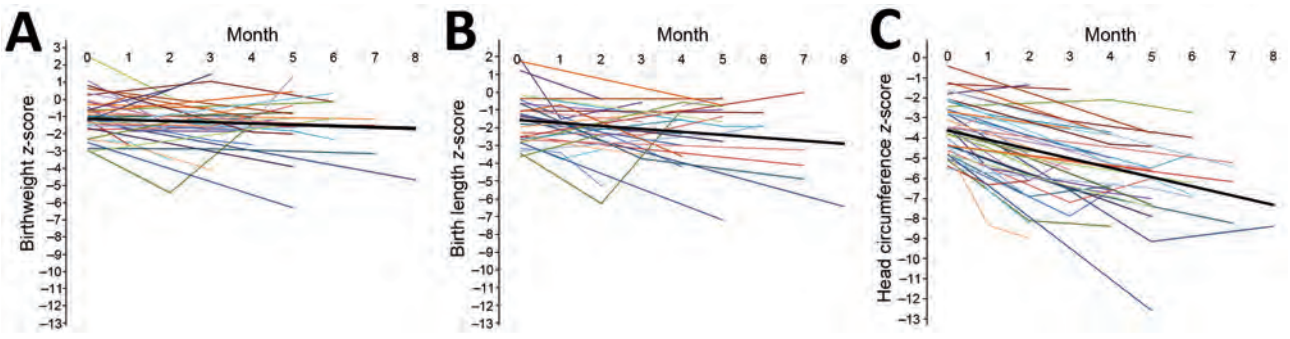

Figure 2. Weight (A), length (B), and head circumference (C) z-scores from birth to 1–8 months of age among infants with probable congenital Zika virus syndrome, Sao Luís, Brazil, 2015–2016. The thick black line depicts the mean z-score at birth and the mean rate of change in the z-score over time, estimated in a random-intercept multilevel linear regression model.

disorders. The infants were difficult to calm down even when fed. As the infants aged, neurologic symptoms began to emerge, usually from the second to the third month onwards with pyramidal/extrapyramidal syndrome, epileptic seizures, and dysphagia, although some infants had ≥1 of these symptoms much earlier. All infants who underwent electroencephalography had some abnormality, including brain activity maturation disorders and focal or multifocal epileptiform discharges. In 9 infants, brain activity maturation disorders evolved into focal or multifocal epileptiform discharge patterns over time. Focal or multifocal patterns were associated with epileptic seizures that did not respond to medication. Five infants initially had hypsarrhythmia, indicating highly disorganized brain activity, and had spasms and neuromotor delays. These 5 infants subsequently had a multifocal epileptiform pattern.

Early head growth was severely compromised, suggesting a very disruptive brain insult (*10*). In addition, as the infants aged, the HC *z*-scores dropped even further, suggesting that most of these infants would not be able to show catch-up growth. The HC *z*-score was substantially compromised (–5.45) at 4 months of age, whereas the weight *z*-score was in the normal range (–1.44), and the length *z*-score was affected (–2.21) but not as substantially.

Notably, 6 infants with probable congenital Zika virus syndrome who had abnormal imaging findings and a characteristic phenotype were not born with microcephaly. However, 3 infants had microcephaly postnatally. This finding suggests that microcephaly at birth is only 1 of the manifestations of this syndrome (*5*). Therefore, screening should be based not only on HC measurement at birth but also on the phenotype associated with fetal brain disruption sequence and cranial CT scan imaging findings.

Our findings are subject to a few limitations. For some infants, data were missing for some variables. A higher likelihood of selection bias exists because infants with more severe cases tended to be referred to the rehabilitation center. Zika virus infection was not confirmed in any mother, and only 1 infant was IgM positive. Because specific laboratory tests were still ongoing, the case definition might have included patients without Zika virus infection. However, we ruled out the 5 most common causes of congenital infection. Chikungunya incidence was low in the area in 2015 (1.3 cases/100.000) (*14*), and congenital infection caused by this pathogen occurs almost exclusively peripartum and is associated with maternal viremia (*15*). No mother in our case series reported fever or arthralgia near delivery.

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Severe Fever with Thrombocytopenia Syndrome Complicated by Co-infection with Spotted Fever Group Rickettsiae, China

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During 2013–2015 in central China, co-infection with spotted fever group rickettsiae was identified in 77 of 823 patients infected with severe fever with thrombocytopenia syndrome virus. Co-infection resulted in delayed recovery and increased risk for death, prompting clinical practices in the region to consider co-infection in patients with severe fever with thrombocytopenia syndrome.

In recent years, new tickborne pathogens have increasingly emerged, creating public health challenges. Co-infection may occur in humans either through the bite of 1 tick co-infected with multiple pathogens or bites of multiple ticks, each carrying a different pathogen (*1*).

In 2009, severe fever with thrombocytopenia syndrome virus (SFTSV) was identified in humans in China, and since then, the virus has been detected in 19 provinces (*2*). The most highly affected region is in central China, where over one third of cases have been reported. Another tickborne pathogen, *Candidatus* Rickettsia tarasevichiae, classified among the spotted fever group rickettsiae (SFGR), was first identified in 2012 in the northeastern area of China, but is now infecting humans in the more densely populated central region (*3*). SFGRs have been detected in *Haemaphysalis longicornis* ticks (*3*,*4*), which also serve as a competent vector for SFTSV (*5*). In 2014, *Candidatus* R. tarasevichiae infection was detected in SFTSV-infected persons in eastern central China, indicating that co-infection with SFGR might be common among SFTSV-infected persons in the region (*3*). To determine the effects of co-infection with SFGR in SFTSV-infected persons, we compared clinical characteristics and laboratory findings for patients with SFTSV infection only with those for patients co-infected with SFTSV and *Candidatus* R. tarasevichiae.

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The Study

During 2013–2015, we conducted a retrospective investigation at the 154 Hospital of the People's Liberation Army in Xinyang City, Henan Province, China. All patients meeting the criteria for having suspected severe fever with thrombocytopenia syndrome (SFTS) were enrolled (*6*). Serial serum and anti-coagulated blood samples were collected from patients throughout hospitalization and during convalescence.

RNA detection by reverse transcription PCR and serologic testing by ELISA were performed for diagnosis of SFTSV infection (*6*). SFTSV infection was determined by the detection of viral RNA in serum, seroconversion, or a 4-fold increase in SFTSV-specific IgG titers in paired serum samples collected ≥2 weeks apart. We used an indirect immunofluorescence assay (Focus Diagnostic, Cypress, CA, USA) to detect *Rickettsia rickettsii* IgG. Acute SFGR infection was defined as seroconversion or a 4-fold increase in *R. rickettsii* IgG titers in paired serum samples. We measured serum levels of cytokines and chemokines by using a Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad, Hercules, CA, USA).

For the study, we recruited 823 SFTS patients who had paired serum samples available for testing (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/11/16-1021-Techapp1.pdf). Of those patients, 77 (8.5%) also had serologic evidence of SFGR infection: 45 showed seroconversion, and 32 had a 4-fold increase in IgG titers. Those 77 patients represented the SFTSV–SFGR co-infection group (online Technical Appendix Table 2); the other 746 patients represented the SFTSV single-infection group.

Influenza-like symptoms were the most common clinical manifestations in both groups, and, except for fever, which was more prolonged in the co-infection group ($p = 0.039$), symptoms were comparable in the groups (online Technical Appendix Table 3). Ascites and hemorrhagic signs were more common in the co-infection than the single-infection group ($p = 0.002$ and $p = 0.003$, respectively). The frequencies of other complications, including gastrointestinal, respiratory, and neurologic syndromes, were similar in the 2 groups.

At hospital admission, the co-infection group had longer prothrombin times (Table). For both groups, thrombocytopenia occurred starting at 4 days after symptom onset and persisted for as long as 2 weeks (Figure 1). Using

[1]These authors contributed equally to this article.

Table. Laboratory test results for patients with severe fever with thrombocytopenia syndrome with and without co-infection with spotted fever group rickettsiae

Characteristics	Single infection, n = 77	Co-infection, n = 746	p value
Laboratory parameters on admission, no. (%) patients			
Leukocyte count <4 × 10^9/L	60 (77.9)	613 (82.2)	0.358
Platelet count <100 × 10^9/L	64 (83.1)	624 (83.7)	0.905
Neutrophils >70%	36 (46.8)	348 (46.7)	0.986
Lymphocytes <20%	27 (35.1)	276 (37.0)	0.738
Hemoglobin <110 g/L	9 (11.7)	113 (15.2)	0.416
Aspartate aminotransferase >40 U/L	62 (80.5)	621 (83.4)	0.527
Alanine aminotransferase >40 U/L	40 (52.0)	421 (56.6)	0.435
Albumin <35 g/L	10 (13.0)	90 (12.1)	0.817
Alkaline phosphatase >150 U/L	3 (3.9)	48 (6.4)	0.378
Gamma-glutamyl transpeptidase >50 U/L	22 (28.6)	161 (21.6)	0.164
Lactate dehydrogenase >245 U/L	60 (85.7)	599 (83.9)	0.691
Creatine kinase >200 U/L	49 (63.6)	465 (62.3)	0.822
Blood urea nitrogen >7.14 mmol/L	22 (28.6)	244 (32.9)	0.442
Total bilirubin >17.1 μmol/L	10 (13.0)	77 (10.3)	0.469
Ceatinine >97 μmol/L	14 (20.0)	130 (18.2)	0.716
Serum amylase >115 U/L	30 (56.6)	242 (52.4)	0.560
Calcium <2.1 mmol/L	34 (61.8)	389 (61.8)	0.997
Coagulation parameters, median (interquartile range)			
Prothrombin time, s	11.5 (10.4–12.3)	10.9 (10.2–11.6)	0.017
Thrombin time, s	20.6 (17.4–22.7)	19.7 (17.6–22.4)	0.318
Activated partial thromboplastin time, s	45.5 (40.5–58.5)	46.2 (38.8–56.6)	0.797
Fibrinogen, g/L	2.8 (2.2–3.2)	2.7 (2.3–3.2)	0.775
International normalized ratio	1.0 (0.9–1.1)	1.0 (0.9–1.0)	0.136
Prothrombin time activity, %	84.0 (76.2–92.3)	87.7 (79.7–98.1)	0.124
D-dimer, ng/mL	1,014 (356–1,432)	647 (381–1,373)	0.603

$\log_{10}$-transformed data with the generalized estimating equation model, we showed that platelet count and leukopenia recovery were delayed in the co-infection group compared with the single-infection group ($p = 0.045$ and $p = 0.027$, respectively). The generalized estimating equation model also showed that the co-infection group had higher levels of serum creatine kinase ($p = 0.047$) and lactate dehydrogenase ($p = 0.022$) during those recovery processes.

Based on the dynamic patterns at 2-day intervals, virus loads in the single-infection group peaked at day 5 after symptom onset and gradually decreased thereafter. Virus loads in the co-infection group peaked at day 7 and then deceased at a lower rate than that for the single-infection group after we adjusted for sex, age, and time from symptom onset to hospital admission ($p = 0.028$) (Figure 2, panel A).

At weeks 1 and 2 after symptom onset, SFTSV-specific IgG titers and positivity rates were not significantly different between the 2 groups (Figure 2, panels C, D). At week 3, the co-infection group had a significantly lower rate of SFTSV positivity ($p = 0.007$). Antibody titers at week 4 were not significantly different between the groups (Figure 2, panel C).

We conducted laboratory testing for 34 patients with SFTSV–SFGR co-infection, 30 sex- and age-matched patients with SFTS only, and 25 controls who were negative for both pathogens by molecular and antibody testing. Levels of interleukin (IL)–1 receptor agonist, IL-8–10, IL-17, interferon-γ, monocyte chemoattractant protein 1, monocyte chemoattractant protein α1, granulocyte colony-stimulating factor, fibroblast growth factors, and tumor necrosis factor–α were similar in the single-infection and co-infection groups and significantly elevated compared with levels in the control group (online Technical Appendix Figure). IL-6 and IL-15 levels were elevated in both infection groups, but they were significantly higher in the SFTSV single-infection group. Platelet-derived growth factor–BB and RANTES (regulated on activation, normal T cell expressed and secreted) were decreased in both groups, but we observed intergroup differences only for RANTES.

Altogether, 87 (10.6%) patients died. The case-fatality rate in the co-infection group (16.9% [13/77]) was insignificantly higher than that in the single-infection group (9.9% [74/746]) ($p = 0.058$). The association between co-infection and higher case-fatality rate was significant after adjustment for sex, age, and interval from disease onset to hospital admission (odds ratio 1.992, 95% CI 1.025–3.873; $p = 0.042$) (online Technical Appendix Table 4).

Conclusions

Our retrospective investigation in an SFTSV-endemic region of China identified SFTSV–SFGR co-infection in ≈8.5% of SFTSV-infected patients and a higher frequency of fatal outcome and delayed recuperation in the co-infected patients. These findings highlight the importance of considering SFGR infection in the differential diagnosis for patients in SFTSV-endemic regions.

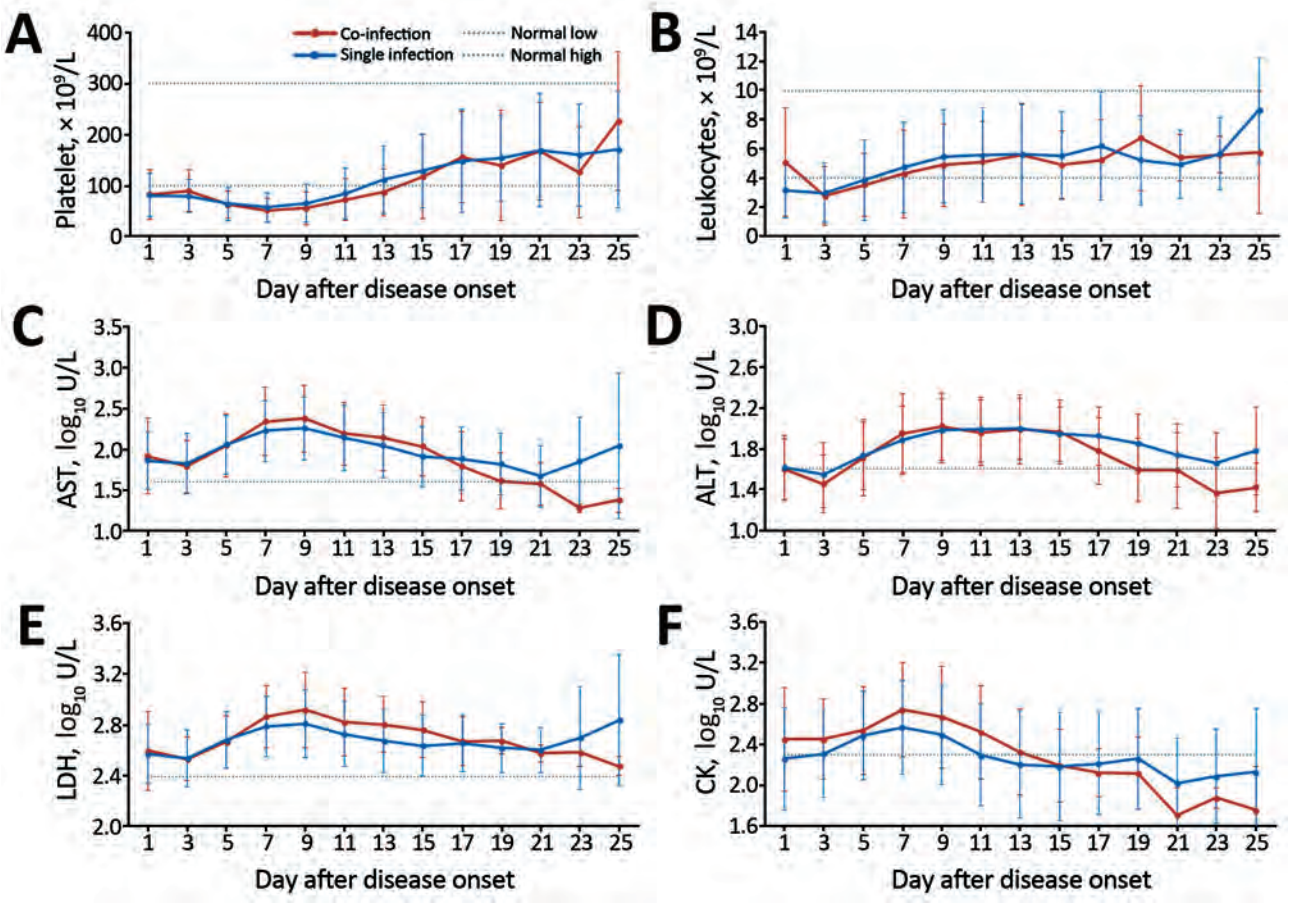

Figure 1. Dynamic profiles for 6 selected laboratory parameters for hospitalized patients with severe fever with thrombocytopenia syndrome virus (SFTSV) infection only or with SFTSV and spotted fever group rickettsiae co-infection, China, 2013–2015. A–B) Mean counts and 95% CIs (error bars) for platelets (A) and leukocytes (B). C–F) $\log_{10}$-transformed median level of and interquartile ranges (error bars) for aspartate aminotransferase (AST) (C); alanine aminotransferase (ALT) (D); lactate dehydrogenase (LDH) (E); and creatine kinase (CK) (F). Dashed lines indicate the reference level for each parameter. Parameters were compared by using the generalized estimating equation model.

SFTSV infection can cause a wide variety of signs and symptoms, ranging from influenza-like illness to more severe complications and even life-threatening disease (*7*). Complications usually involve neurologic and hemorrhagic manifestations, which can progress to multiple organ dysfunction in critically ill patients. Rickettsial infections are clinically difficult to distinguish from many virus infections (*8*), and our results showed that symptoms common to SFTSV- and SFGR-infected patients (e.g., influenza-like illness, gastrointestinal symptoms) are not intensified in co-infected patients. In contrast, less common hemorrhagic signs, especially gastrointestinal hemorrhages, are exacerbated in co-infected patients. Previous studies have shown that in patients with SFTS, blood coagulation parameters are prolonged, as characterized by activated partial thromboplastin time and thrombin time (*9*,*10*). Thrombocytopenia, a common laboratory finding in patients with SFTS, can contribute to hemorrhage, and hemorrhagic signs have also been observed in patients infected with SFGR species (e.g., *R. rickettsii* and *R. conorii*) (*12*–*14*); however, SFGR mainly invade the vascular endothelial cells, causing vascular inflammation and increased vascular permeability (*11*). Also, based on prolonged thrombocytopenia and longer prothrombin times that have been observed in co-infected persons, we hypothesize that the additive effect from 2 pathogens might lead to aggravated hemorrhage.

Doxycycline is the recommended therapeutic regimen for rickettsia infection (*15*) and could be administered in cases of SFTSV–SFGR co-infection. From a public health perspective, intensified efforts should be made to detect SFTSV–SFGR co-infection in regions where *H. longicornis* ticks predominate and carry both SFTSV and SFGR.

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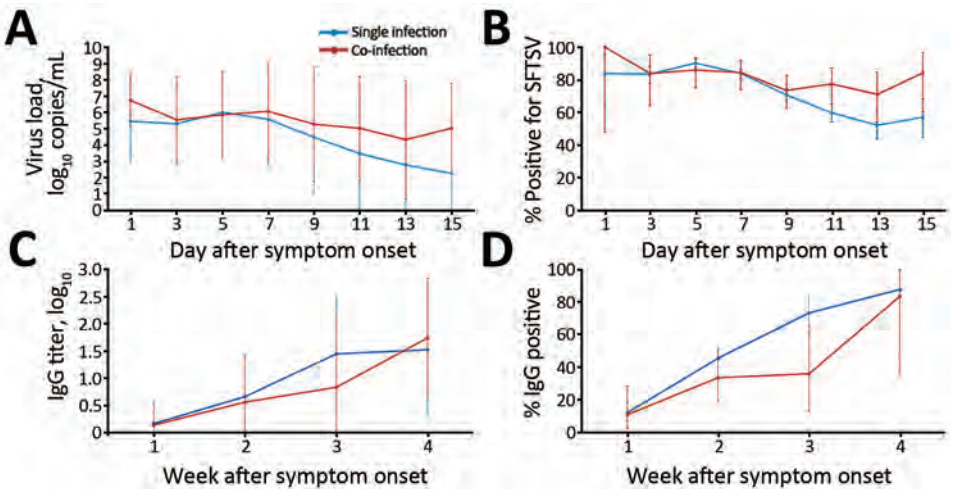

Figure 2. Dynamic profiles for severe fever with thrombocytopenia syndrome virus (SFTSV) RNA and SFTSV-specific IgG in hospitalized patients with SFTSV infection only or with SFTSV and spotted fever group rickettsiae co-infection, China, 2013–2015. A) log_{10}-transformed SFTSV virus loads. B) Percentage of patients positive for SFTSV. C) log_{10}-transformed SFTSV IgG titers. D) Percentage of patients positive for SFTSV IgG. Comparisons were performed using the generalized estimating equation model. The error bars, which show the standard deviation for log_{10}-transformed SFTSV virus loads and log_{10}-transformed SFTSV IgG titers, represent the 95% CI for the percentage of patients positive for SFTSV and SFTSV IgG.

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The authors had the following roles in the study and preparation of the manuscript: Q.-B.L., W.L., and W.-C.C. conceived and designed the experiments; Q.-B.L., H.L., P.-H.Z., N.C., Y.-D.F., X.-M.C., J.-G.H., C.-T.G., and X.-A.Z. performed the experiments; Q.-B.L., H.L., P.-H.Z., N.C., and W.L. analyzed the data; N.C. and Z.-D.Y. contributed materials; and Q.-B.L., H.L., P.-H.Z., N.C., and W.L. prepared the manuscript.

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Guinea Worm (*Dracunculus medinensis*) Infection in a Wild-Caught Frog, Chad

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A third-stage (infective) larva of *Dracunculus medinensis*, the causative agent of Guinea worm disease, was recovered from a wild-caught *Phrynobatrachus francisci* frog in Chad. Although green frogs (*Lithobates clamitans*) have been experimentally infected with *D. medinensis* worms, our findings prove that frogs can serve as natural paratenic hosts.

The peculiar epidemiology of *Dracunculus medinensis* (Guinea worm), the causative agent of dracunculiasis (Guinea worm disease), in Chad has led to speculation that a paratenic host is involved in the life cycle, most likely an animal with an aquatic stage that would feed upon copepods and harbor the infection for subsequent transmission to a human or dog definitive host (*1*). Recent experiments demonstrated that *D. medinensis* worms, like the closely related parasite *D. insignis*, could utilize green frog (*Lithobates clamitans*) tadpoles as a paratenic host (*2*). During June and July 2016, a survey of potential *D. medinensis* worm paratenic hosts was conducted in Chad. The study area was located in southern Chad near the small village of Marabe (Moyen Chari region, Kyabe district), along the upper reaches of the Chari River, where many infections in dogs have been recorded; the closest large town to Marabe is Sarh (*3*).

The Study

We used standard procedures, as previously described (*1*), to examine muscle and viscera of 88 frogs from the study area; the frogs, which were of several sizes and species (i.e., Ranidae, Pipidae, Phrynobatrachidae, Bufonidae), were collected by local villagers and fishermen. In brief, the viscera was removed and placed in water for at least 1 h before being examined by microscope for motile nematode larvae. The musculature and carcass were bluntly dissected and similarly placed in water for at least 1 h before the solution was examined for motile nematode larvae.

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We observed 1–5 nematode larvae in 6 (7%) of the 88 frogs. Morphologically similar larvae were collected from the viscera washing of 5 of the 6 frogs; these larvae were identified as pinworms, based on morphologic characteristics and comparison to larvae released by a female oxyurid collected from the gut. However, upon subsequent microscope examination, 1 larva from the muscle and carcass washings of a single mature frog was found to be morphologically consistent with *Dracunculus* species, including size, distinct cuticular striation, and, most notably, a 3-lobed tail (Figure). We preserved the larva in ethanol and then extracted DNA and amplified a partial cytochrome c oxidase subunit I gene by PCR (*4*). Partial sequencing (187 bp) showed that the larva shared 99.5% similarity with *D. medinensis* isolates in GenBank (accession nos. LK978189 and KF770021–KF770024), confirming its identity as *D. medinensis*. The sequence shared only 95.2% similarity with *D. insignis* and 91.9% similarity with *D. lutrae* (GenBank accession nos. EU646534 and EU646602, respectively).

To confirm the species identity of the frog, we extracted DNA from ethanol-fixed tissue and amplified the 16S ribosomal RNA gene (*2*). The sequence (450 bp) indicated that the frog was a ranid species in the genus *Phrynobatrachus*, most likely *P. francisci* because it shared 99% similarity with *P. francisci* sequences in GenBank (accession nos. GU457546–GU457549, EU71820, and AY902377).

Conclusions

Tadpoles and frogs have long been known to experimentally support infective larvae of *D. insignis* (*5*–*7*), and just recently, they have been shown to experimentally support infective larvae of *D. medinensis* (*2*); however, natural infection with *Dracunculus* species has not previously been documented in any wild-caught amphibian. The finding of a wild-caught frog harboring a natural infection with a *D. medinensis* larva validates the findings of these experimental infections and demonstrates that such a paratenic host is likely involved in the transmission of *D. medinensis* larvae in Chad. This finding is especially noteworthy at this point because the Guinea worm eradication program has reduced the number of countries with endemic Guinea worm disease from 20 to 4 and the number of persons infected each year from >3 million in 1986 to <20. To be uncovering this aspect of the *D. medinensis* life cycle, the description of which was published >145 years ago (*8*) and remained relatively unchanged to date, further highlights the need to continue field research, even at the end of an eradication campaign.

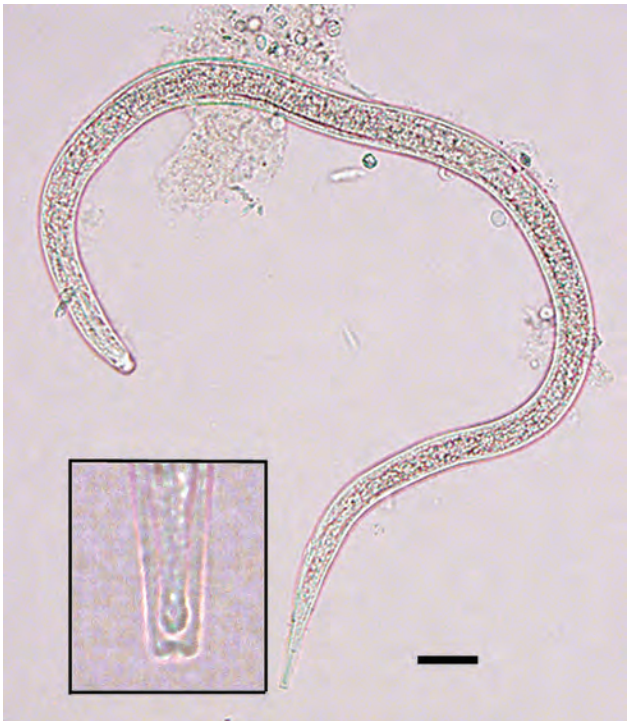

Figure. Size and shape of a *Dracunculus medinensis* third-stage larva recovered from a *Phrynobatrachus francisci* frog from Chad. Scale bar indicates 25 μm. Inset shows detailed morphology of the tip of the tail of the larva, including the characteristic 3-lobed tip.

Given the diversity of frog species (i.e., families Ranidae, Pipidae, and Phrynobatrachidae) that can be infected with *D. medinensis* or *D. insignis* worms, it seems probable that natural *Dracunculus* infections are not limited to frogs of the genus *Phrynobatrachus* but may well include numerous other ranids and highly aquatic *Xenopus* species frogs (African clawed frogs), which are common and native to Chad. Additional surveillance is needed to detail the prevalence and burden of infection among frogs in Chad as well as the diversity of natural hosts. These data do not address whether all transmission occurring in humans and dogs in Chad are a result of consumption of a paratenic host, such as a frog, but the peculiar epidemiology of *D. medinensis* worms in Chad clearly suggests that traditional drinking water sources are not the primary source of infection.

Our findings confirm that an appropriate wild-caught paratenic host in Chad was infected with a *D. medinensis* larva, and they corroborate findings of experimental studies that suggested the possible inclusion of an amphibian paratenic host in the maintenance of *D. medinensis* worms in nature. We conclude that paratenic hosts, specifically frogs, may facilitate transmission of *D. medinensis* worms to humans and dogs in Chad via consumption of poorly cooked or raw food items.

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Dog-Mediated Human Rabies Death, Haiti, 2016

Ryan M. Wallace, Melissa D. Etheart, Jeff Doty, Ben Monroe, Kelly Crowdis, Pierre Dilius Augustin, Jesse Blanton, Natael Fenelon

Haiti has experienced numerous barriers to rabies control over the past decades and is one of the few remaining Western Hemisphere countries to report dog-mediated human rabies deaths. We describe the circumstances surrounding a reported human rabies death in 2016 as well as barriers to treatment and surveillance reporting.

Rabies kills 59,000 persons each year worldwide, more than any other zoonotic disease (*1*). In the Western Hemisphere, deaths caused by dog-mediated human rabies have been nearly eliminated. However, these deaths still occur in Haiti, where researchers estimate that up to 130 persons die from dog-mediated rabies each year (*1*). Disease surveillance in Haiti since the 2010 earthquake has improved, but the capacity for detecting and responding to rabies cases is still limited. We describe an investigation of a suspected human rabies case, including the patient's clinical signs and symptoms and the healthcare and public health response to dog-mediated human rabies. We also highlight challenges faced by Haiti's public health system.

Case Report

On January 14, 2016, a woman with behavioral changes and hydrophobia visited a regional hospital in Cap-Haïtien, Nord Department, Haiti. Her husband reported that a dog had bitten her 3 months before symptoms developed. A local clinic treated the wound on the day the bite occurred but did not offer rabies vaccination. Healthcare workers at the regional hospital made a presumptive diagnosis of rabies but were unable to offer palliative care. The couple left without providing additional contact information. The hospital administrator reported the suspected rabies case to the national Department of Epidemiology and Laboratory Research (DELR), as required by Haiti's national surveillance system. Without contact information, DELR was unable to investigate further.

Haiti's Ministry of Health (Ministère de la Santé Publique et de la Population [MSPP]), with assistance from the US Centers for Disease Control and Prevention (CDC), has developed a robust surveillance system for 44 conditions, 13 of which are immediately reportable, including suspected human rabies. Under this surveillance system, health alerts for suspected human rabies cases are investigated to confirm clinical cases of rabies, identify persons or animals exposed to a rabid animal, and identify healthcare and community contacts of the person suspected of having rabies. Since February 2015, CDC has assisted DELR in 3 human rabies investigations, which identified 27 rabies-exposed persons in addition to patients.

The person with suspected rabies in this case report was not admitted to the hospital, and no contact information was obtained. Therefore, public health investigators could not determine her health outcome, gather potential human and animal exposures, or complete classification of this case on the basis of the World Health Organization's clinical case definition for rabies (*2*).

On March 14, 2016, three months after the woman with suspected rabies had visited the regional hospital, a CDC-trained veterinarian who was conducting a rabies survey among mongooses was alerted by community members to a potential human rabies death. Initial reports led the veterinarian to believe that the person who died was the same woman who sought care at the Cap-Haïtien hospital. A team of healthcare workers from CDC and the Pan American Health Organization (PAHO) had already planned training on integrated bite case management (IBCM) in this area. In addition to the training, during March 30–April 10, CDC and PAHO assisted Haiti's Ministry of Agriculture, Natural Resources and Rural Development (MARNDR) and DELR in investigating the suspected human rabies case.

On April 5, 2016, the investigation team conducted a verbal autopsy with the decedent's husband. The investigation confirmed that the person who died was a 54-year-old woman who was bitten on the left hand on November 30, 2015, while fending off a dog that was acting aggressively toward her goats. The woman visited a local healer, who administered 1 shot of an unknown substance. Except for residual pain in the hand, the woman remained healthy until January 10, 2016, when her husband recognized signs of confusion; notably, she had placed common household items in unusual locations. During January 11–13, fevers,

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hypersalivation, agitation, and incoherent speech developed. On January 14, the woman accused her husband of trying to kill her when he offered her water (presumed hydrophobia). On that day, the husband and wife traveled to a health clinic and were immediately referred to the regional referral hospital in Cap-Haïtien. The husband reported that palliative care was denied, and they left the hospital without providing contact information. The wife died later that night. According to WHO clinical rabies case definitions, the woman's illness was a probable rabies case.

The investigators verified that neighbors had killed an abnormally aggressive dog on approximately November 30, 2015. Neighbors reported that the dog had attempted to bite several persons, but it was killed without further human exposures. The dog had bitten 1 pig, which could not be located because of the delay between the bite event and the case investigation.

Conclusions

Few cases of rabies in Haiti are reported to health authorities; in 2015, only 7 cases were documented, 5 of which were detected through the veterinary sector. Lack of recognition of rabies has been attributed to low awareness, unique cultural beliefs, and a high incidence of numerous conditions (i.e., cerebral malaria, meningitis, viral encephalitis, and tetanus) that may confound rabies diagnosis (*3*,*4*). Furthermore, diagnosis of human rabies is not performed in Haiti due to limitations in diagnostic capacity and cultural aversion to collection of postmortem samples.

The healthcare team identified no other human rabies exposures in this investigation. Given the delay in investigating this case, if any persons had been exposed, they likely would have already succumbed to rabies, underscoring the importance of timely reporting and investigation. In 2015, in an effort to improve healthcare provider recognition of rabies cases and surveillance reporting, PAHO and MSPP developed a rabies training course for healthcare providers. Trainings in Cap-Haïtien are planned for 2016.

Although animal rabies is a reportable condition to MARNDR and bite events are reportable to MSPP, neither the rabid dog nor the bite event were reported in this situation. A 2014 survey estimated that 95,000 animal bites occur annually in Haiti (1% bite rate) (*5*). However, only 6,500 bites (6.8% of estimated bites) were reported through the national surveillance program that year. To improve bite detection and healthcare-seeking behaviors, CDC and PAHO collaborated with MARNDR, DELR, and MSPP to develop an IBCM system to assist in reporting bites to MARNDR for animal investigation. Results are reported to bite victims and to the responsible healthcare sector. Since its inception, the IBCM system has increased detection of animal rabies cases 18-fold and improved patient healthcare-seeking behavior. However, the system's success depends on reliable and timely reporting. The IBCM program is now operational in 3 of Haiti's 10 departments but is not yet available in Cap-Haïtien. Therefore, even if the bite had been reported through appropriate surveillance channels, follow-up likely would not have occurred. To improve reporting in Nord Department (Cap-Haïtien), the investigation team has trained 11 veterinary professionals to use the IBCM program (Figure).

Figure. A team consisting of workers from the US Centers of Disease Control and Prevention; the Pan American Health Organization; Haiti's Ministry of Agriculture, Natural Resources and Rural Development; and Christian Veterinary Mission trained 11 veterinary professionals on principles of animal rabies surveillance. Here, trainees gain experience drawing up sedative medications into a pole syringe, which is used to sedate suspected rabid animals from a safe distance. (Photograph courtesy of R.M. Wallace.)

Haiti has made considerable strides in controlling dog-mediated human rabies deaths through efforts such as dog vaccination, the implementation of the IBCM system, and medical provider training. These advances have been made through collaborative work with Haiti's government institutions and international partners. Through the continued support and expansion of these programs, cases like the one reported in this article will eventually be eliminated. This case is an unfortunate reminder that dog-mediated human rabies deaths continue to occur in some Western Hemisphere countries; however, this death provided stimulus for training local health officials and served as a reminder of why Haitians and international partners seek elimination of this disease.

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Staphylococcus aureus Colonization and Long-Term Risk for Death, United States

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To examine the association of colonization by *Staphylococcus aureus* and general population mortality, we followed 10,598 adults for 8.5 years on average. Methicillin-susceptible *S. aureus* colonization was not associated with death. Methicillin-resistant *S. aureus* carriage predicted death in a crude analysis but not after adjustment for socioeconomic status and co-morbidities.

Staphylococcus aureus is a common cause of mild to life-threatening infections. It is differentiated into methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA); the emergence of isolates resistant to vancomycin has raised concern that the bacterium might become untreatable with current antimicrobial drugs (*1*). Whether *S. aureus* colonization influences general population mortality remains unclear (*2*,*3*). Therefore, we examined the association in a US population representative sample followed for nearly a decade.

The Study

We used data from the 2001–2004 National Health and Nutrition Examination Survey (NHANES) and the NHANES 2001–2004 Mortality-Linked File. NHANES is a survey of the US noninstitutionalized civilian population conducted by the Centers for Disease Control and Prevention's National Center for Health Statistics. We selected 10,598 NHANES participants using a complex multistage sampling design and followed them through December 31, 2011, to track mortality. NHANES protocols were approved by the institutional review boards of NCHS and CDC, and all participants provided informed consent.

Nasal swabs collected from participants were tested for *S. aureus* and methicillin resistance during 2001–2004 following the National Committee for Clinical Laboratory Standards disk-diffusion method. We additionally tested isolates resistant to oxacillin (MRSA) and intermediately resistant to oxacillin and every tenth isolate sensitive to oxacillin (MSSA) for staphylococcal cassette chromosome *mec* (SCC*mec*) typing, genes encoding enterotoxins, toxic shock syndrome toxin-1, and Panton-Valentine leukocidin (PVL) toxin (details on the methods are available at http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l35_b_doc.pdf). We collected data on age, sex, race/ethnicity, family income, smoking, and medical conditions using questionnaires.

Univariate and multivariate Cox proportional hazards regressions were used to estimate the hazard ratio (HR) with corresponding 95% CIs for MSSA and MRSA association with mortality. Analyses were performed in STATA version 11 (StataCorp LP, College Station, TX, USA), taking into account the survey design and sampling weights, to produce nationally representative estimates. We considered p values <0.05 as statistically significant.

A total of 27.3% of participants were colonized with MSSA and 1.3% with MRSA (Table 1). During an average of 8.5 years of follow-up, the total death rate per 1,000 person-years was 10.3: 10.8 for noncolonized participants, 8.8 for those colonized with MSSA, and 25.6%for those colonized with MRSA. MRSA-colonized participants tended to be older and non-Hispanic white, have low poverty income ratio, smoke cigarettes, have co-morbidities, or have been hospitalized and/or have had dialysis in the previous 12 months (Table 1). Approximately 58.6% of MRSA isolates were of SCC*mec* type II, suggestive of hospital-associated MRSA, and 41.4% had MRSA isolates of SCC*mec* type IV, indicative of community-associated MRSA.

In Cox regression, MSSA-colonized participants had a significantly lower risk for death than did noncolonized participants in univariate analysis (hazard ratio [HR] 0.82, 95% CI 0.68–0.98) but not after adjustment for covariates. MRSA colonization was associated with higher risk for death than no *S. aureus* colonization in univariate analysis (HR 2.40, 95% CI 1.56–3.69) and after adjustment for sociodemographic factors (HR 1.54, 95% CI 1.06–2.34); MRSA colonization also carried higher risk for death than MSSA colonization (HR 2.90, 95% CI 1.92–4.40). However, after additional adjustment for smoking, body mass index, and co-morbidities (asthma, diabetes, hypertension, cardiovascular diseases, stroke, liver disease, and cancer), these associations were no longer significant (Table 2).

Likewise, colonization with SCC*mec* type II MRSA more strongly predicted long-term mortality than did no *S. aureus* ((HR 3.38, 95% CI 2.00–5.72) or MSSA

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Table 1. Characteristics of study participants by *Staphylococcus aureus* colonization status, National Health and Nutrition Examination Survey, United States, 2001–2004*

Characteristic	No *S. aureus*	MSSA	MRSA	p value†	All participants, N = 10,598
Prevalence	71.4	27.3	1.3		100
Median age, y (IQR)	43 (31–56)	41 (30–54)	54 (34–67)		43 (31–55)
Sex				<0.001	
M	46.1	54.0	36.4		48.1
F	53.9	46.0	63.6		51.9
Race/ethnicity				<0.001	
Non-Hispanic white	70.3	74.4	80.8		71.5
Non-Hispanic black	12.1	8.4	11.7		11.1
Hispanic	12.4	12.8	4.5		12.4
Other	5.2	4.5	3.0		5.0
Body mass index				.28	
Normal or underweight	34.6	32.6	33.8		34.0
Overweight or obese	62.4	65.0	63.6		63.2
Missing	3.0	2.4	2.5		2.8
Poverty income ratio				<0.001	
<1.85	30.4	29.4	42.7		30.3
≥1.85	64.1	64.3	47.7		63.9
Missing	5.5	6.3	0.6		5.8
Smoking	49.2	44.6	63.8	<0.001	48.1
Co-morbidities					
Asthma	11.9	12.9	20.5	.07	12.3
Diabetes	8.1	9.4	13.5	.13	8.6
Hypertension	33.0	30.3	39.5	.05	32.4
Cardiovascular diseases‡	6.1	4.6	12.5	.01	5.7
Stroke	2.7	1.9	4.9	.03	2.5
Liver disease	3.4	3.1	2.6	.76	3.3
Cancer	8.9	7.3	16.7	.02	8.5
Hospitalization and/or dialysis in past 12 mo	10.5	10.6	19.8	<0.001	10.6
Mean survival, y (SE)	8.47 (0.11)	8.67 (0.10)	7.5 (0.28)	<0.001	8.52 (0.10)
Death during follow up	9.1	7.7	19.3	<0.001	8.9

*All values are percentages except as indicated. IQR, interquartile range; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin resistant *S. aureus*.
†p values for comparison of non–*S. aureus* colonization vs. MRSA colonization vs. MSSA colonization; p values calculated using χ^2 test for categorical variables and analysis of variance for continuous variables.
‡Coronary heart disease, congestive heart failure, and myocardial infarction.

colonization (HR 4.05, 95% CI 2.42–6.79) in univariate analyses but not after adjustment for sociodemographic characteristics. Colonization with SCC*mec* type IV MRSA was not associated with death in univariate or adjusted analysis, unlike no *S. aureus* or MSSA colonization (Table 2). However, the small number of participants with MRSA SCC*mec* types II (83) and IV (53) limited our analysis.

Conclusions

In this population-based study, we found that MSSA is not associated with long-term mortality. MRSA is associated with risk for death in crude analysis but not after adjustment for socioeconomic factors, co-morbidities, or both.

Regarding MSSA, our results are consistent with studies conducted in inpatient and nursing home participants that also failed to find an association (*4*–*7*). Many of these studies were underpowered, calling for larger scale investigations. In line with the inverse association in our unadjusted analysis for MSSA and mortality, Wertheim et al. found that MSSA-colonized participants had longer survival than did noncolonized participants among patients with *S. aureus* bacteremia (*3*). They suggested that colonized patients might be more immunologically adapted to the endogenous *S. aureus* strains that caused the bacteremia or that exogenous strains in noncolonized patients were more virulent. In our case, we hypothesized that the negative association was imputable to some confounders, such as race/ethnicity, smoking, and co-morbidities (hypertension, cardiovascular diseases, stroke), instead of an independent association. We found that persons with MSSA were more likely to be non-Hispanic white and have asthma or diabetes than were persons without *S. aureus* nasal carriage, who were more likely be non-Hispanic black; have a lower poverty income ratio; be a smoker; and have hypertension, cardiovascular diseases, stroke, liver disease, or cancer. Concerning MRSA, Chan et al. observed that MRSA colonization was associated with higher mortality than non-MRSA colonization in nursing home residents in China followed for 2 years (*8*). Previous research also found that methicillin resistance in *S. aureus* bacteremia did not predict long-term mortality after adjustment for age, comorbidities, severity of acute illness, metastatic infections, and long-term facility resident status (*1*).

Table 2. Cox proportional hazards regression for *Staphylococcus aureus* colonization and long-term mortality, United States*

Exposure variable‡	No. deaths	No. patients	Unadjusted HR† (95% CI)	Unadjusted p value	Model 1 HR† (95% CI)§	Model 1 p value	Model 2 HR† (95% CI)¶	Model 2 p value
S. aureus colonization								
No *S. aureus*	1,051	7,794	1 (referent)		1 (referent)		1 (referent)	
MSSA	279	2,668	0.82 (0.68–0.98)	0.035	1.03 (0.85–1.24)	0.79	1.06 (0.88–1.27)	0.52
MRSA	41	136	2.40 (1.56–3.69)	<0.001	1.54 (1.06–2.34)	0.026	1.38 (0.88–2.17)	0.15
MRSA SCC*mec* II	36	83	3.38 (2.00–5.72)	<0.001	1.42 (0.88–2.26)	0.14	1.40 (0.87–2.24)	0.16
MRSA SCC*mec* IV	5	53	1.07 (0.37–3.12)	0.9	2.05 (0.60–7.03)	0.25	1.96 (0.55–6.92)	0.29
In colonized participants								
MSSA	279	2,668	1 (referent)		1 (referent)		1 (referent)	
MRSA	41	136	2.90 (1.92–4.40)	<0.001	1.48 (0.94–2.32)	0.09	1.27 (0.76–2.13)	0.35
MRSA SCC*mec* II	36	83	4.05 (2.42–6.79)	<0.001	1.36 (0.84–2.21)	0.2	1.17 (0.66–2.08)	0.58
MRSA SCC*mec* IV	5	53	1.30 (0.44–3.84)	0.63	1.90 (0.54–6.64)	0.3	1.97 (0.58–6.70)	0.26

*HR, hazard ratio; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; SCC*mec*: staphylococcal cassette chromosome *mec*.
†Generated by using Cox proportional hazards regression.
‡Categorized into no *S. aureus* colonization (0), MSSA colonization (1) and MRSA colonization (2), to generate hazard ratios for MSSA colonization (1) and MRSA colonization (2) in reference to no *S. aureus* colonization (0). In the analysis by SCC*mec* types, exposure was categorized into: no *S. aureus* colonization (0), MSSA colonization (1), MRSA SCC*mec* II (2), and MRSA SCC*mec* IV (3) and hazard ratios for MRSA SCC*mec* II (2), and MRSA SCC*mec* IV were reported. In colonized participants, the analysis was repeated with MSSA colonization being the reference category.
§Adjusted for age, sex, race/ethnicity, and Poverty Income Ratio.
¶Adjusted for age, sex, race/ethnicity, poverty Income ratio, smoking, body mass index, and co-morbidities (asthma, diabetes, hypertension, cardiovascular diseases, stroke, liver disease, and cancer).

Delayed treatment initiation, virulence factors such as PVL, or both have been hypothesized to be responsible for excess mortality in MRSA infections and bacteremia. Subsequent research found that PVL was associated with decreased time from colonization to bacteremia but not to mortality (*9*). Again in confirmation of our results, Han et al. studied the effect of SCC*mec* type on the 30-day in-hospital mortality in 184 patients with MRSA and found SCC*mec* II significant in univariate analysis, but not after co-variates adjustment (*10*). That study also concluded that SCC*mec* II was a marker for disease severity instead of a predictor of mortality.

Our study had several limitations. The carriage of *S. aureus* could be transient in some cases and not always reflect the carriage pattern over time. Although the nares are the most common site for *S. aureus* carriage, extranasal sites can also harbor the organism (*11*). *S. aureus* carriage was tested in 2001–2004 and might not reflect the current epidemiology and MRSA strain types. Data on co-morbidities, such as cardiovascular or liver diseases and cancer, were not available for participants 18–19 years of age and were coded as missing in the analyses. We did not compare death for the genes encoding enterotoxins because of their limited numbers. However, these NHANES data are the only national data available, and the major strengths of our study include the sample size representative of the US population with generalizable findings and higher power for statistical inferences.

Our findings are of public health and clinical importance because a lack of independent association between colonization by *S. aureus* and mortality implies that decolonization might not prevent death. Some studies indicate that decolonization of patients at high risk for infections could reduce the number of subsequent *S. aureus* infections, but evidence remains limited that it prevents death (*5*).

Dr. Mendy is a physician completing a PhD in epidemiology at the University of Iowa. His research focuses on infectious causes of chronic diseases.

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Group B *Streptococcus* Serotype III Sequence Type 283 Bacteremia Associated with Consumption of Raw Fish, Singapore

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We conducted a retrospective study of 40 case-patients and 58 controls as part of a nationwide investigation of a group B *Streptococcus* outbreak in Singapore in 2015. Eating a Chinese-style raw fish dish (yusheng) was a major risk factor for bacteremia, particularly caused by serotype III sequence type 283.

Group B *Streptococcus* (GBS) disease is caused by *Streptoccocus agalactiae*, a common commensal bacteria found in the gastrointestinal and genital tracts of 15%–30% of healthy adults (*1*). GBS can cause invasive infections, especially in elderly persons and those who have underlying medical conditions, such as diabetes mellitus (*2*). The serotype III sequence type 283 (ST283) strain has been associated with invasive disease in adults (*3*). GBS infection is not a reportable disease in Singapore, and positive culture isolates are not routinely serotyped.

GBS can also cause bovine mastitis (*4*) and disease in saltwater and freshwater fish (*5*–*7*). GBS serotypes Ia and III ST283 and ST491 have also been detected in tilapia from Southeast Asia (*8*).

In June 2015, the Ministry of Health (MOH) in Singapore was alerted to an increase in the number of GBS bacteremia cases among adults admitted to 6 acute-care public hospitals. Seven acute-care public hospitals in Singapore account for >80% of beds for acute-care patients; no increase in cases was observed at a women's and children's hospital (Figure).

Anecdotal links between eating raw fish and illness were reported for some patients. Although 1 study in the United States found a link between eating fish and risk for GBS colonization in adults (*9*), and fish and humans can be infected with the same strains (*5*,*8*), links between eating fish and GBS infection in humans have not been established. To identify risk factors associated with this purportedly foodborne outbreak, the MOH conducted a case–control study in July 2015 as part of a national outbreak investigation.

The Study

The retrospective case–control study comprised 40 case-patients and 58 healthy contacts. Case-patients were defined as patients from the 6 hospitals who had GBS isolated from blood cultures during June 1–July 14, 2015, the period of highest GBS bacteremia incidence. Healthy household members or colleagues of case-patients were selected as controls because they were more likely to have had similar dietary patterns.

Using a standardized interviewer-administered questionnaire, we obtained demographic data, medical history, and 2-week food and exposure histories. Information was also obtained on consumption of raw fish, seafood, or unpasteurized dairy products; exposure to live fish; or invasive medical procedures. Data were collected over a period of 1 week.

Positive blood culture isolates from 36 of the 40 case-patients were obtained from hospital laboratories. Capsular typing was conducted by the National Public Health Laboratory (NPHL) of the MOH by using a PCR-based method (*10*). The NPHL was unable to obtain positive blood culture isolates from 4 case-patients for further typing. After the case selection period, the public hospitals were requested to report all cases of GBS bacteremia to the MOH and submit positive blood culture isolates for capsular typing by the NPHL. Sequence types were determined by using multilocus sequence typing (*11*,*12*).

Mean age of case-patients (61.5 years) was higher than that of controls (44.8 years) (Table 1). A larger proportion of case-patients (52.5%) were male than controls (31.0%). Most case-patients and controls were of Chinese ethnicity; none had occupational exposure to live animals. A total of 72.5% of case-patients reported underlying medical conditions, more than twice the total for controls (31.0%).

Univariable logistic regression analysis showed that eating raw fish was associated with GBS bacteremia (Table 2). Eating yusheng, Chinese-style raw fish dish usually prepared with freshwater fish, such as Asian bighead carp or snakehead fish, or saltwater fish, such as wolf herring, was a major risk factor (odds ratio [OR] 5.11, 95% CI, 1.93–13.52).

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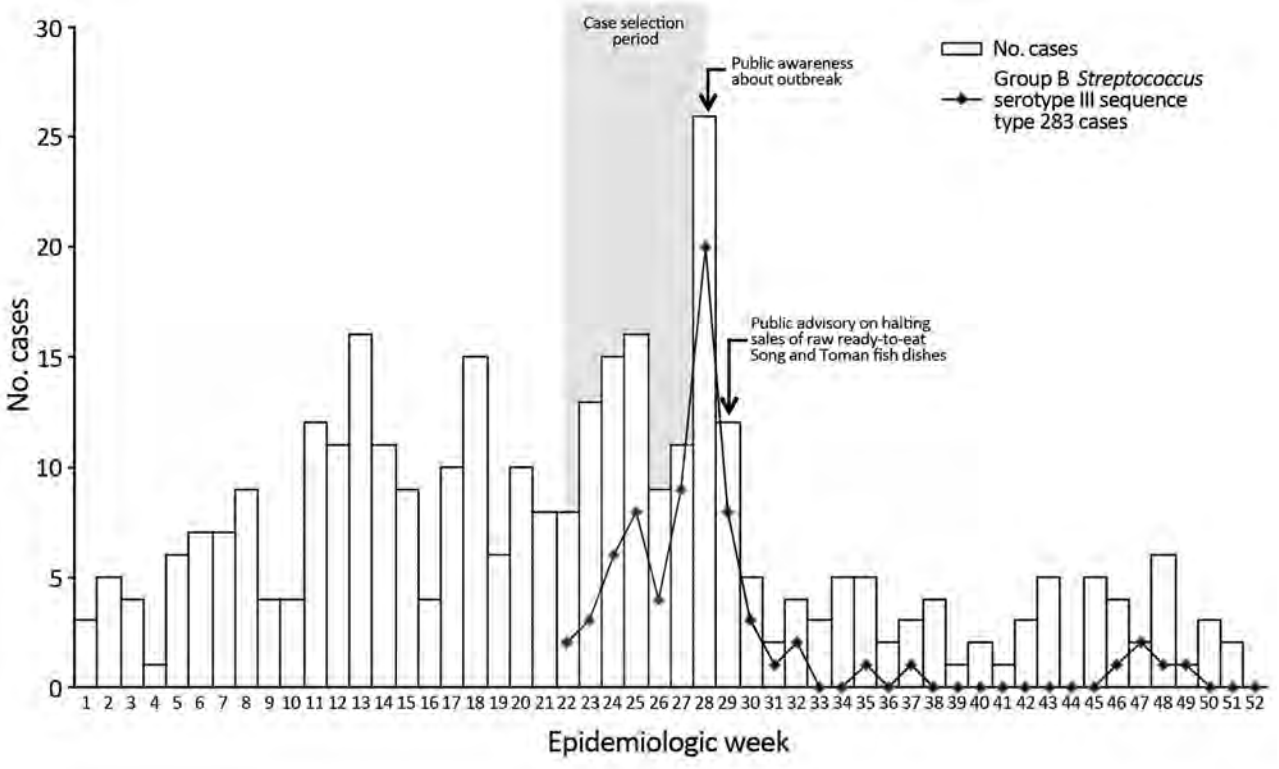

Figure. Timeline of group B *Streptococcus* bacteremia cases reported in 6 public hospitals, Singapore, epidemiologic weeks 1–52, 2015

The odds of GBS bacteremia among case-patients ≥65 years of age were 17.73 times that of those 14–44 years of age (95% CI 4.73–66.52). We found no association between patient age and eating yusheng or between GBS and other exposures. However, diabetes mellitus (OR 6.36, 95% CI 2.08–19.45) and cardiovascular disease (OR 4.23, 95% CI 1.76–10.17) were major risk factors.

A multivariable model that controlled for age group, sex, and concurrent medical conditions showed that the odds of GBS bacteremia among those who had eaten raw fish were 8.58 times that of those who had not eaten raw fish (95% CI 2.25–32.69). Eating yusheng remained a major risk factor in separate analysis by type of raw fish food items consumed (adjusted OR 11.38, 95% CI 2.76–46.98) (Table 2).

Mean and median durations between eating raw fish and onset of illness were 3.5 and 4.5 days, respectively. Mean and median durations between eating yusheng and onset of illness were 3.7 and 4.0 days, respectively.

Of 36 isolates tested, all from case-patients who ate raw fish (n = 19) were serotype III ST283. Isolates from case-patients who did not eat raw fish had various serotypes: Ia (n = 1), II (n = 4), III (n = 7), VI (n = 4), and VII (n = 1). Restricted analysis of 25 case-patients with serotype III ST283 bacteremia and 58 controls showed a strong association between eating yusheng and serotype III ST283 bacteremia (adjusted OR 25.92, 95% CI 5.38–124.96).

Mean age of case-patients infected with ST283 was significantly younger than patients infected with non-ST283 (57.4 vs. 68.6 years; p = 0.014). A significantly lower proportion of patients infected with ST283 than non-ST283 reported having underlying medical conditions (56% vs. 100%; p = 0.003).

Conclusions

We found a strong association between eating yusheng and GBS serotype III ST283 bacteremia. These findings are further supported by the presence of GBS serotype III ST283 in fish samples tested by the Agri-Food and Veterinary Authority of Singapore and the National Environment Agency (NEA) (*13*). Other major risk factors for GBS bacteremia, such as age ≥65 years, diabetes mellitus, and cardiovascular disease, were consistent with known risk factors.

After a public advisory was issued on July 24, 2015, to halt sales of raw fish dishes made with Asian bighead carp and snakehead fish (*14*), the weekly number of invasive GBS cases decreased rapidly in ≤2 weeks, from a peak of 26 cases/week to an average of <5 cases/week. This decrease supported the hypothesis of a short incubation period after eating raw fish. The proportion of serotype III ST238 cases also decreased rapidly after the advisory (Figure).

This study might be limited by recall bias, in which case-patients were more likely to recall exposure to raw fish, given the increased public awareness at the time of

Table 1. Characteristics of case-patients and controls in group B *Streptococcus* outbreak investigation, Singapore, 2015*

Characteristic	No. (%) case-patients, n = 40	No. (%) controls, n = 58	p value
Age group, y			<0.0005
0–4	0	0	
5–14	0	1 (1.7)	
15–24	0	5 (8.6)	
25–34	0	9 (15.5)	
35–44	6 (15.0)	13 (22.4)	
45–54	8 (20.0)	9 (15.5)	
55–64	7 (17.5)	11 (19.0)	
≥65	19 (47.5)	5 (8.6)	
Unknown	0	5 (8.6)	
Mean age, y	61.5	44.8	
Median age, y	61.5	44.0	
Age range, y	33–68	14–95	
Sex			0.038
M	21 (52.5)	18 (31.0)	
F	19 (47.5)	40 (69.0)	
Ethnicity			0.527
Chinese	36 (90.0)	47 (81.0)	
Malay	3 (7.5)	6 (10.3)	
Indian	0	2 (3.4)	
Other	1 (2.5)	3 (5.2)	
Housing type†	7 (17.5)	10 (17.2)	0.991
3-room flats and smaller‡	17 (42.5)	22 (37.9)	
4-room HDB flats	8 (20.0)	13 (22.4)	
Executive/5-room HDB flats	4 (10.0)	7 (12.1)	
Private housing	4 (10.0)	6 (10.3)	
Concurrent illness			<0.0005
No	11 (27.5)	40 (69.0)	
Yes	29 (72.5)	18 (31.0)	
Diabetes mellitus	15 (37.5)	5 (8.6)	0.001
Cancer	3 (7.5)	0	0.065
Cardiovascular disease	22 (55.0)	13 (22.4)	0.001
Renal disease	5 (12.5)	0	0.010
Liver disease	3 (7.5)	0	0.065
Gastrointestinal disease	1 (2.5)	0	0.408
Respiratory disease	3 (7.5)	0	0.065
Blood disease	2 (5.0)	1 (1.7)	0.565

*HDB, Housing Development Board.
†Housing type was used as a proxy for socioeconomic status; >80% of the population in Singapore live in public housing managed by the HDB.
‡HDB flats are considered public housing by the HDB.

the study. Not all patients given a diagnosis within the case selection period were interviewed because some were not available or too ill to be interviewed. We obtained a case:control ratio of only 1:1.45 because of difficulties in recruiting participants who were willing to be interviewed. Case-patients detected before June 1 were not recruited because they would have been unlikely to provide an accurate food history. Nonetheless, we still obtained statistical power >80% in detecting a 4-fold increased odds of GBS bacteremia after eating yusheng. Although we found no strong association between GBS bacteremia and consumption of sashimi, sushi, or raw shellfish or exposure to fish-related activities, power to detect statistical significance was limited because of small sample sizes.

Although we found a strong epidemiologic link between eating raw fish, particularly yusheng, and GBS

Table 2. Association between bacteremia and risk factors in a case–control study of a group B *Streptococcus* outbreak, Singapore, 2015*

Risk factor	No. (%) case-patients, n = 40	No. (%) controls, n = 58	Univariable model		Multivariable model†	
			Crude OR (95% CI)	p value	Adjusted OR (95% CI)†	p value
Raw fish	19 (47.5)	11 (19.0)	3.87 (1.57–9.54)	0.003	8.58 (2.25–32.69)	0.002
Yusheng‡	18 (45.0)	8 (13.8)	5.11 (1.93–13.52)	0.001	11.38 (2.76–46.98)	0.001
Sashimi/sushi	5 (12.5)	3 (5.2)	2.62 (0.59–11.65)	0.206	4.39 (0.74–25.91)	0.103
Raw shellfish	1 (2.5)	1 (1.7)	1.46 (0.09–24.07)	0.791	5.18 (0.23–114.95)	0.299
Fish-related activities§	2 (5.0)	6 (10.3)	0.46 (0.09–2.39)	0.352	0.71 (0.10–5.07)	0.736

*OR, odds ratio.
†Included age group (14–44, 45–54, 55–64, and ≥65 y), sex, and medical conditions as independent variables in a logistic regression model.
‡Chinese-style raw fish dish.
§Included fishing, fish spas, fish rearing, and gutting of fish.

serotype III ST283 bacteremia, further studies are needed to understand the precise mechanism of transmission from raw fish to humans and the pathologic process that takes place for infection to occur. In the interest of public health, the NEA has since banned use of freshwater fish in all ready-to-eat raw fish dishes sold by retail food establishments (*15*). The NEA and the Agri-Food and Veterinary Authority of Singapore have also increased measures to ensure that raw saltwater fish intended for consumption are closely monitored and tested for safety.

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Group B *Streptococcus* Sequence Type 283 Disease Linked to Consumption of Raw Fish, Singapore

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An outbreak of invasive group B *Streptococcus* (GBS) disease occurred in Singapore in mid-2015. We conducted a case–control study of 22 adults with invasive GBS infections during June 21–November 21, 2015. Consumption of raw fish was strongly associated with invasive sequence type 283 infections, but not with non–sequence type 283 infections.

Group B *Streptococcus* (GBS) disease is caused by *S. agalactiae*, a commensal bacterium that can be isolated from genitourinary and gastrointestinal tracts of up to 30% of healthy adults. GBS can cause skin and soft tissue infections, urinary tract infections, bacteremia, and meningitis in adults; pregnancy-associated infections can lead to invasive disease in newborns (*1*).

In mid-2015, an outbreak of invasive GBS disease was reported in acute-care hospitals in Singapore. An increase in GBS bacteremia rate was observed for all adult acute-care hospitals, including Tan Tock Seng Hospital (TTSH), a 1,600-bed adult tertiary-care hospital in central Singapore, which reported an increase in GBS bacteremia rate from 3.5 cases/month in January–December 2014 to 6.5 cases/month in January–June 2015. Some infected men and nonpregnant women reported consuming raw fish before infection.

On the basis of detection of GBS in fish samples during joint investigations by the National Environmental Agency, the Agri-Food and Veterinary Authority of Singapore, and the Ministry of Health, sales of raw fish dishes containing Asian bighead carp and snakehead fish were suspended on July 24, 2015 (during epidemiologic week 29) (*2*). Concurrently, serotyping of GBS isolates collected from hospitals showed an increase in serotype III, and multilocus sequence type 283 (ST283) was isolated from a patient with GBS meningitis (*1*). We conducted a study at TTSH to compare the epidemiology of invasive ST283 and non-ST283 infections and to assess factors associated with invasive ST283 infections.

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The Study

We conducted a prospective case–control study (case:control ratio 1:3.5) of patients admitted to TTSH during June 21–November 21, 2015 (epidemiologic weeks 25–46). Using a standardized, interviewer-administered questionnaire, we collected information on food exposure history, in particular consumption of raw or undercooked fish, beef, eggs, and vegetables, during the 2 weeks before admission. Information on demographics, clinical history, and laboratory results were obtained from medical records. All GBS isolates were sent to the National Public Health Laboratory and serotyped by PCR (*3*) and genotyped by multilocus sequence typing using a standard *S. agalactiae* strain (*4*).

We defined case-patients as inpatients at TTSH during epidemiologic weeks 25–46 who had laboratory-confirmed GBS infections detected in samples from any sterile site (blood, synovial fluid, cerebrospinal fluid) (*5*) within 48 hours after admission (*6*). Controls were defined as inpatients at TTSH with negative culture results for any sterile site (i.e., without invasive disease) within 48 hours after admission. We compared characteristics, food and nonfood exposures, and clinical presentation of persons infected with ST283 with those infected with non-ST283 and controls (Table 1). We constructed a multivariable logistic regression model based on major variables of interest from univariable analysis to assess for independent factors associated with ST283 and non-ST283 infections (Table 2).

A total of 22 case-patients (17 with bacteremia, 2 with septic arthritis, and 3 with bacteremia complicated by meningitis, epidural space abscess, and septic arthritis) and 76 controls (73 provided blood, 2 provided joint fluid, 1 provided both) were included in this study. Among 22 case-patients, 11 had serotype III GBS infections, of whom 9 had ST283 infections. None of the other serotype III strains, namely ST17 (*1*) and a new ST not previously reported (*1*), were single-locus variants of ST283. Other serotypes not typed were Ia (*3*), II (*5*), V (*2*), and VII (*1*).

Most (8/9) ST283 infections were identified during epidemiologic weeks 25–29, before suspension of

Table 1. Characteristics of patients with invasive group B *Streptococcus* ST283 or non-ST283 infections and controls, Singapore*

Characteristic	ST283, n = 9	Non-ST283, n = 13	Controls, n = 76	Comparison and p value		
				ST283 vs. non-ST283	ST283 vs. controls	Non-ST283 vs. controls
Demographic						
Sampled during epidemiologic wk 25–29	8 (88.9)	3 (23.1)	37 (48.7)	**<0.01**	**0.02**	0.09
Median age, y (IQR)	59.4 (25–70)	74 (46–88)	77.5 (42–99)	**0.03**	**0.002**	0.37
Age < 65 y†	7 (77.8)	3 (23.1)	18 (23.7)	**0.01**	**<0.001**	0.96
Female sex†	6 (66.7)	6 (46.2)	45 (59.2)	0.34	0.67	0.38
Chinese ethnicity†	9 (100.0)	11 (84.6)	58 (76.3)	0.22	0.10	0.51
Concurrent condition						
Diabetes mellitus†	1 (11.1)	5 (38.5)	33 (43.4)	0.16	0.06	0.74
Malignancy†	1 (11.1)	1 (7.7)	11 (14.5)	0.78	0.78	0.51
Cardiovascular disease†	1 (11.1)	4 (30.8)	29 (38.2)	0.28	**<0.01**	0.61
Renal disease†	1 (11.1)	2 (15.4)	25 (32.9)	0.77	0.18	0.20
Liver disease†	0	2 (15.4)	5 (6.6)	0.22	0.43	0.28
Gastrointestinal disease†	1 (11.1)	2 (15.4)	14 (18.4)	0.77	0.59	0.79
Respiratory disease†	0	2 (15.4)	16 (21.1)	0.22	0.13	0.64
Blood disorder†	0	1 (7.7)	28 (36.8)	0.39	**0.03**	**0.04**
Skin wound†	0	2 (15.4)	12 (15.8)	0.22	0.20	0.97
Charlson Comorbidity Score, median (range)	0 (0–9)	2 (0–7)	3.5 (0–12)	0.21	**0.03**	0.26
Any concurrent condition	3 (33.3)	11 (84.6)	69 (90.8)	**0.01**	**<0.001**	0.50
Hospitalized during 6 mo before admission†	2 (22.2)	6 (46.2)	31 (40.8)	0.25	0.28	0.72
Food history						
Ate raw or undercooked fish ≤2 wk before admission†	7 (77.8)	0	3 (4.0)	**<0.001**	**<0.001**	0.47
Ate raw or undercooked beef ≤2 wk before admission†	2 (22.2)	0	0	0.07	**<0.001**	NC
Ate raw vegetables†	1 (11.1)	0	4 (5.3)	0.22	0.48	0.40
Ate raw eggs†	0	0	13 (17.1)	NC	0.18	0.11
Other exposure history						
Fish-related activities (fishing, fish spa)†	0	0	0	NC	NC	NC
Clinical presentation						
Fever (temperature ≥38°C)	7 (77.8)	9 (69.2)	23 (30.3)	0.66	**<0.01**	**<0.01**
Musculoskeletal pain	7 (77.8)	4 (30.8)	20 (26.3)	**0.03**	**<0.01**	0.74
Fever and musculoskeletal pain	5 (55.6)	3 (23.1)	8 (10.5)	0.12	**<0.001**	0.20
≤2 d between symptom onset and admission	4 (44.4)	11 (84.6)	39 (51.3)	0.05	0.70	**0.03**

*Values are no. (%) unless otherwise indicated. Bold indicates statistical significance. IQR, interquartile range; NC, not calculable.
†Data collected by using questionnaire.

sale of raw fish dishes. During epidemiologic weeks 30–46, only 1 ST283 case was identified, compared with 10 (77%) of 13 of non-ST283 cases. Persons infected with ST283 tended to be younger than persons infected with non-ST283 (median age 59.4 years vs. 74.0 years) (p = 0.033), and they were also less likely to have a preexisting medical condition (33.3% vs. 84.6%; p = 0.014). Seven (77.8%) of 9 ST283-infected patients had eaten raw or undercooked fish ≤2 weeks before admission, compared with 0 of non-ST283–infected patients (p<0.001) and 3 controls (p<0.001). Two of the ST283-infected patients who had eaten raw or undercooked fish had also eaten raw or undercooked beef. ST283-infected persons were more likely than controls to have high fever (temperature ≥38°C) and musculoskeletal pain (55.6% vs 10.5%; p<0.001); however, this comparison was not significant for persons with non-ST283–infection (23.1%; p = 0.12). Only 45 (60%) controls had a noninvasive infection.

Multivariate analysis showed that eating raw or undercooked fish during the 2 weeks before admission was independently associated with ST283 infection, but not with non-ST283 infection (Table 2), when compared with controls. None of the non-ST283–infected patients had eaten raw or undercooked fish during the timeline.

We also conducted a subanalysis that compared ST283-infected patients with onset up to epidemiologic week 29 with controls (who had similar infection onset

Table 2. Multivariable analysis of risk factors associated with invasive group B *Streptococcus* ST283 and non-ST283 infections, Singapore*

Factor	ST283		Non-ST283	
	aOR (95% CI)	p value	aOR (95% CI)	p value
Consumption of raw or undercooked fish ≤2 weeks before hospitalization	100.11 (6.21–1612.91)	**0.001**	NC	NC
Age <65 y	13.34 (1.14–156.56)	**0.04**	1.04 (0.25–4.27)	0.96
Charlson Comorbidity Score	1.02 (0.63–1.64)	0.95	0.85 (0.65–1.11)	0.23

*Bold indicates statistical significance. aOR, adjusted odd ratio; NC, not calculable; ST, sequence type.

and opportunity to purchase and eat raw fish). We observed a stronger association with eating raw fish among persons who could purchase raw fish dishes (adjusted odds ratio 12,423.62, 95% CI 1.51 to 1.02 × 10^8; p = 0.04). However, 2 of the ST283-infected patients did not report eating raw fish, which suggests other means of acquiring ST283 infections.

Conclusions

Several population-based studies have raised concerns about the increasing incidence of invasive GBS disease in men and nonpregnant women (*7*,*8*). We report an outbreak of invasive GBS serotype III ST283 infections in men and nonpregnant women. Molecular epidemiology studies have showed that ST283 strains from fish in Asia had the same virulence gene profile as human invasive isolates, which suggests potential exposure of humans and fish to common environmental sources of ST283 or transmission of the bacterium between different host species (*9*). Fish consumption was associated with an increased risk for acquisition of GBS in a prospective cohort study in the United States (*10*). However, data are limited for the likelihood and routes of interspecies transmission of this strain associated with fish and invasive disease in humans.

We found a strong association between consumption of raw or undercooked fish and invasive ST283 infections in men and nonpregnant women. Being older and having severe concurrent conditions were negatively associated with infection, contrary to findings in previous studies (*11*,*12*). Our findings suggest that non-ST283 strains caused symptomatic infection in more susceptible populations (those with more concurrent conditions), whereas ST283 might be more invasive and affected less susceptible persons.

An additional contributing factor could be differences in food consumption in local populations; elderly persons and those with severe concurrent conditions might avoid eating raw or undercooked foods (*13*). The finding that no non-ST283–infected patients had eaten raw or undercooked fish before admission strengthened our hypothesis of a link between the ST283 and consumption of raw or undercooked fish.

After isolation of ST283 from 4% of freshwater fish samples from restaurants, markets, stores, and fisheries, use of freshwater fish in all ready-to-eat raw fish dishes sold at retail food establishments was banned in Singapore on December 5, 2015 (*14*,*15*). The number of reported invasive GBS infections in Singapore has decreased, although sporadic invasive ST283 infections have been identified. TTSH has seen no new invasive ST283 infections identified since the study ended on November 21, 2015, despite continued active case-finding.

The relatively small number of ST283 and non-ST283 infections in this study is a limitation that precludes drawing definitive conclusions. However, isolation of ST283 from fish samples, the strong association of raw fish consumption with ST283 infection, and the sharp decrease of ST283 infections after the food ban strongly suggest a food-related outbreak. Although the outbreak of GBS disease in Singapore has been controlled, further studies on the virulence, transmissibility, and epidemiology of ST283 and risk factors are warranted to better manage future infections.

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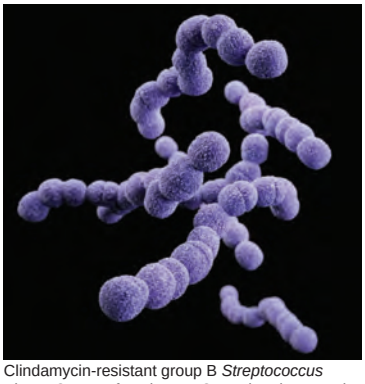
etymologia

Streptococcus [strep"to-kok'əs]

From the Greek *streptos* (“chain”) + *kokkos* (“berry”), streptococcal diseases have been known since at least the 4th century BCE when Hippocrates described erysipelas (Greek for “red skin”). The genus *Streptococcus* was named by Austrian surgeon Theodor Billroth, who in 1874 described “small organisms as found in either isolated or arranged in pairs, sometimes in chains” in cases of erysipelas or wound infections. Over subsequent decades, as microscopy and staining techniques improved, many different researchers characterized the bacteria now known as *Streptococcus pyogenes* (Lancefield group A β-hemolytic streptococcus), *S. pneumoniae*, and other species.



Clindamycin-resistant group B *Streptococcus*
Photo: Centers for Disease Control and Prevention

Source

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Novel Levofloxacin-Resistant Multidrug-Resistant *Streptococcus pneumoniae* Serotype 11A Isolates, South Korea

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Of 608 *Streptococcus pneumoniae* clinical strains isolated at a hospital in South Korea during 2009–2014, sixteen (2.6%) were identified as levofloxacin resistant. The predominant serotype was 11A (9 isolates). Two novel sequence types of multidrug-resistant *S. pneumoniae* with serotype 11A were identified, indicating continuous diversification of resistant strains.

Streptococcus pneumoniae is a common respiratory pathogen that is the leading cause of community-acquired pneumonia (*1*). Although β-lactam antibiotics have long been used for the treatment of respiratory diseases, the increasing prevalence of antibiotic-resistant *S. pneumoniae* strains has hampered treatment in recent decades (*2*,*3*). Resistance to fluoroquinolones has emerged in *S. pneumoniae* and is caused by mutations within short DNA sequences of *gyrA* and *parC* genes that encode the type II topoisomerase subunits known as quinolone-resistance determining regions (QRDRs) (*1*). Previous studies have shown that most of the *S. pneumoniae* strains with reduced susceptibility to the fluoroquinolone levofloxacin exhibit a multidrug-resistant (MDR) phenotype (*2*,*4*). Levofloxacin resistance was closely associated with epidemic MDR clones (*3*). Although fluoroquinolone resistance rates remain low in *S. pneumoniae* in most countries, some extensively drug-resistant (XDR) *S. pneumoniae* isolates have emerged; this resistance is defined as nonsusceptibility to ≥1 agent in all but ≤2 antimicrobial categories (*2*,*4*). We examined *S. pnemoniae* isolates from patients in South Korea to determine antimicrobial resistance. We found novel sequence types (STs) of MDR serotype 11A *S. pneumoniae* that exhibit resistance to second-line antibiotics such as levofloxacin, ceftriaxone, and meropenem.

The Study

During January 2009–December 2014, we isolated 608 *S. pneumoniae* clinical strains at a 698-bed, university-affiliated hospital in South Korea. We determined MICs by using the broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (*5*). We performed antimicrobial resistance tests for levofloxacin, ofloxacin, ciprofloxacin, penicillin, amoxicillin, ceftriaxone, meropenem, erythromycin, clindamycin, vancomycin, linezolid, tetracycline, and tigecycline. We used *S. pneumoniae* ATCC 49619 as a control strain. We defined MDR as resistance or intermediate resistance to ≥3 antimicrobial agents.

We determined serotypes by using the multiplex PCR assay recommended by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/pcr.htm). Reactions also included an internal positive control targeting all known pneumococcal *cpsA* regions (*6*). We sequenced QRDRs of the *gyrA*, *gyrB*, *parC*, and *parE* genes in each isolate (*7*). We performed multilocus sequence typing to investigate the genetic backgrounds of fluoroquinolone-resistant pneumococci (*8*) and assigned allele numbers and STs by using the PubMLST database (http://pubmlst.org/spneumoniae).

Of the 608 clinical *S. pneumoniae* isolates, 16 (2.6%) were levofloxacin resistant (MIC ≥8 μg/mL). We collected 1 resistant isolate in 2009, 3 in 2012, 5 in 2013, and 7 in 2014. Thirteen isolates were from sputum, and 3 isolates were from bronchial lavage. The mean age of patients was 71 years; 14 were male, and 2 were female.

Serotype 11A (n = 9) was most common among the levofloxacin-resistant isolates, followed by serotypes 13 (n = 2), 19F (n = 2), 23F (n = 2), and 6B (n = 1) (Table 1). The most common STs were ST9875 (n = 5), ST8279 (n = 3), and ST9876 (n = 3), which together accounted for 11 of the 16 levofloxacin-resistant isolates. Nine isolates of ST9875, ST9876, and ST10300 were novel STs and had not been identified before this study.

All 16 levofloxacin-resistant isolates contained at least 2 amino acid alterations in the QRDRs of the *gyrA*, *parC*, and *parE* genes. Four QRDR mutations occurred with high frequency: Ser81Phe in *gyrA* was present in all 16 isolates; Ser79Phe and Lys137Asn in *parC* were present in 14 and 11 isolates, respectively; and Ile460Val in *parE* was found in 15 isolates. However, Lys137Asn in *parC* and Asp435Val and Ile460Val in *parE* are mutations not involved in resistance, according to previous reports (*9*,*10*). Isolate HM-854, which was penicillin susceptible, had Ser81Phe in *gyrA* and Asp79Asn in *parC* mutations.

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Table 1. Select characteristics of 16 levofloxacin-resistant *Streptococcus pneumoniae* clinical isolates identified from patients at a hospital in South Korea, 2009–2014*†

Strain	Age, y/sex of patient	Specimen type	Respiratory disorders	Underlying disorders	Serotype	Sequence type
HM-646	36/M	Sputum	Pneumonia	CVA	11A	9875‡
HM-669	70/M	Sputum	Pneumonia	CVA	11A	9875‡
HM-683	77/M	Sputum	Pneumonia	COPD	6B	3173
HM-688	81/M	Sputum	Pneumonia	Cardiac infarction	23F	9876‡
HM-730	77/M	Sputum	Dyspnea with fever	Cervical pain	13	189
HM-762	76/F	Sputum	Pneumonia	Lung cancer	13	8279
HM-781	70/M	Sputum	Pneumonia	CVA	23F	6721
HM-787	35/M	Sputum	Pneumonia	CVA	11A	9875‡
HM-809	58/M	Sputum	Pneumonia	CVA	11A	9875‡
HM-854	77/M	BL	Pneumonia	Lung cancer	11A	99
HM-878	67/M	BL	Pneumonia	ALS	11A	8279
HM-953	82/M	Sputum	Pneumonia	COPD	11A	9875‡
HM-970	68/M	Sputum	Dyspnea with fever	Bronchiectasis	19F	9876‡
HM-1017	85/M	BL	Dyspnea	Lung cancer	11A	8279
HM-1050	62/M	Sputum	Postop atelectasis	CVA	19F	9876‡
HM-1055	89/F	Sputum	Pneumonia	CVA	11A	10300‡

*ALS, amyotrophic lateral sclerosis; BL, bronchial lavage; COPD, chronic obstructive pulmonary disorder; CVA, cerebrovascular accident.
†Among the 16 isolates, 1 (HM-646) was collected in 2009; 3 (HM-669, HM-683, and HM-688) in 2012; 5 (HM-730, HM-762, HM-781, HM-787, and HM-809) in 2013; and 7 (HM-854, HM-878, HM-953, HM-970, HM-1017, HM-1050, and HM-1055) in 2014.
‡Novel sequence type found in our study.

All isolates had ≥1 mutation in *parC*. The 2 isolates without the Ser79Phe mutation in *parC* instead carried Asp-83Gly or Asp83Asn. The 4 isolates without the Lys137Asn mutation in *parC* instead carried the Asn91Asp mutation. Isolate HM-1017 (serotype 11A, ST-8279) had 7 QRDR mutations and exhibited the highest resistance against all antimicrobial agents, including levofloxacin (MIC 64 μg/mL). ST-8279 was associated with 2 different serotypes, 11A (n = 2) and 13 (n = 1). The 3 isolates of novel ST-9876 had the same QRDR amino acid changes but had different serotypes, 19F (n = 2) and 23F (n = 1).

The 16 levofloxacin-resistant isolates were also resistant to ofloxacin (MIC ≥8 μg/mL) and ciprofloxacin (MIC ≥8 μg/mL) (Table 2). All isolates except 3 had MICs ≥16 μg/mL against amoxicillin and ceftriaxone. Fourteen isolates were meropenem-resistant (MIC ≥1 μg/mL); all these isolates were susceptible to vancomycin and linezolid. Only 3 STs (ST-99, ST-189, and ST-3173) exhibited the lowest levofloxacin MIC (8 μg/mL); all these isolates were susceptible to amoxicillin (MIC ≤2 μg/mL).

Most of the 16 isolates in our study were of serotype 11A (n = 9): 5 isolates of ST-9875, 2 of ST-8279, and 1 each of ST-10300 and ST-99. An XDR ST-8279 (serotype 13) clone described in 2014 (*2*) was closely related to the 9 serotype 11A isolates in our study. ST-8279 is a double-locus (*aroE* and *xpt*) variant of ST-156, which is closely related to global clone Spain9V-3 (*2*). Spain9V-3 is related to 3 ST-3642 isolates (serotype 11A) reported in Taiwan in

Table 2. Antimicrobial susceptibilities of 16 levofloxacin-resistant *Streptococcus pneumoniae* clinical isolates identified from patients at a hospital in South Korea, 2009–2014*

Strain	MIC, μg/mL (resistance)												
	LEV	OFL	CIP†	PEN	AMX	CRO	MER	ERY	CLI	VAN	LZD	TET	TIG†
HM-646	16 (R)	32 (R)	32	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-669	16 (R)	32 (R)	32	>16 (R)	>16 (R)	>16 (R)	8 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-683	8 (R)	16 (R)	16	4 (I)	2 (S)	2 (I)	1 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-688	16 (R)	32 (R)	32	>16 (R)	>16 (R)	>16 (R)	8 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-730	8 (R)	16 (R)	8	4 (I)	2 (S)	2 (I)	0.5 (I)	>16 (R)	>16 (R)	0.5 (S)	0.5 (S)	>16 (R)	0.03
HM-762	32 (R)	64 (R)	32	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	0.5 (S)	>16 (R)	0.015
HM-781	16 (R)	32 (R)	16	16 (R)	16 (R)	>16 (R)	8 (R)	>16 (R)	>16 (R)	0.5 (S)	0.5 (S)	16 (R)	0.03
HM-787	16 (R)	32 (R)	64	16 (R)	16 (R)	>16 (R)	8 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-809	16 (R)	32 (R)	64	16 (R)	>16 (R)	>16 (R)	4 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-854	8 (R)	16 (R)	16	0.06 (S)	0.06 (S)	0.5 (S)	<0.015 (S)	8 (R)	0.06 (S)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-878	16 (R)	32 (R)	32	16 (R)	>16 (R)	>16 (R)	8 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	4 (R)	0.03
HM-953	16 (R)	32 (R)	64	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-970	32 (R)	64 (R)	32	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-1017	64 (R)	128 (R)	64	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03
HM-1050	32 (R)	64 (R)	64	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	0.5 (S)	0.03
HM-1055	16 (R)	32 (R)	128	>16 (R)	>16 (R)	>16 (R)	16 (R)	>16 (R)	>16 (R)	0.5 (S)	1 (S)	>16 (R)	0.03

*AMX, amoxicillin; CIP, ciprofloxacin; CLI, clindamycin; CRO, ceftriaxone; ERY, erythromycin; I, intermediate; LEV, levofloxacin; LZD, linezolid; MER, meropenem; OFL, ofloxacin; PEN, penicillin; R, resistant; S, susceptible; TET, tetracycline; TIG, tigecycline; VAN, vancomycin.
†No susceptibility breakpoints are established for ciprofloxacin and tigecycline.

2010 (*11*) and to 3 MDR ST-166 isolates (serotype 11A) reported in South Korea in 2013 (*12*). In our study, 3 novel STs of MDR *S. pneumoniae* were identified (ST-9875, ST-9876, and ST-10300). All the ST-8279, ST-9875, and ST-10300 isolates in our study were serotype 11A, with the exception of 1 of the ST-8279 isolates. The ST-9875 and ST-10300 isolates were single-locus variants (in the *spi* and *gki* genes, respectively) of ST-8279. ST-9876 is a 1-locus (*aroE*) variant of an ST-3384 (serotype 9V) clone registered in the PubMLST database.

Serotypes 19F and 23F are included in the 13-valent pneumococcal conjugated vaccine (PCV13), but serotype 11A is not included in PCV13. Serotype 11A is, however, included in the 23-valent pneumococcal polysaccharide vaccine (PPSV23). The US CDC currently recommends the PPSV23 for all adults ≥65 years of age and all persons 2–64 years of age who are at high risk for pneumococcal disease (*13*). Through national vaccine programs in South Korea, since 2013, PPSV23 has been provided to all adults ≥65 years of age, and since 2014, 10-valent pneumococcal conjugated vaccine or PCV13 have been provided to young children free of charge (*14*).

Conclusions

In South Korea, serotype 11A was the most predominant serotype of the 16 levofloxacin-resistant and XDR *S. pneumoniae* isolates we found. Seven levofloxacin-resistant *S. pneumoniae* strains were isolated in 2014 alone; the dominant serotype was again 11A (n = 5). All except 1 of these 7 serotype 11A isolates were resistant to the 9 different antimicrobial agents tested. We identified 3 novel STs of MDR serotype 11A *S. pneumoniae* in our study. *S. pneumoniae* serotype 11A isolates with novel STs require careful monitoring to combat the increasing prevalence and diversification of MDR pneumococcal strains, especially those with resistance to fluoroquinolones, β-lactams, and third-generation cephalosporins.

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Imported Chikungunya Virus Strains, Taiwan, 2006–2014

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We identified 78 imported chikungunya cases in Taiwan during 2006–2014. Sixty-six (84.6%) cases were initially suspected to be dengue, which indicates the necessity for laboratory diagnostics in differentiation between dengue and chikungunya. Results also emphasize the need for active surveillance of febrile illness at points of entry.

Chikungunya is a mosquitoborne viral disease characterized by symptoms, including fever, rash, myalgia, and polyarthralgia that are usually self-limiting and rarely fatal; however, arthralgia might persist (*1*). The causative agent of this disease is chikungunya virus (CHIKV), which belongs to the family *Togaviridae*, genus *Alphavirus* (*2*). Genotypes of CHIKV include West African, East/Central/South African (ECSA), and Asian.

Since 2000, CHIKV has caused unanticipated, large outbreaks in Africa and Asia and become a major public health concern (*3*). In late 2013, CHIKV reached the Americas and caused several explosive outbreaks (*4*). CHIKV is predominantly transmitted by *Aedes aegypti* and *Ae. albopictus* mosquitoes (*1*). Although chikungunya is not endemic to Taiwan, *Aedes* mosquitoes are found throughout Taiwan (*5*). Infected travelers with high viremia who arrive or return from disease-endemic areas could lead to local transmission and outbreaks in Taiwan.

The Study

To reduce the risk for importation and subsequent spread of arboviruses in Taiwan, active (e.g., fever screening at airports and expanded screening for contact with confirmed cases) and passive (e.g., clinician- or hospital-based) surveillance systems were implemented by the central and local health departments. Serum samples from persons with suspected cases were submitted to the Taiwan Centers for Disease Control (Taipei, Taiwan) for confirmation of arboviral infection.

Because symptoms of different arboviral infections might be similar, we performed multiplex real-time reverse transcription PCR (RT-PCR) with flavivirus consensus primers, alphavirus consensus primers, and virus-specific primers and CHIKV and dengue virus IgM and IgG ELISAs for samples collected from all persons with suspected arboviral infections (*6*,*7*). CHIKV infection was confirmed by detection of CHIKV RNA, isolation of CHIKV, seroconversion, or ≥ 4-fold increase in IgM or IgG titers against CHIKV in paired serum samples (*8*). An imported case of chikungunya was defined as disease in an infected patient who had been traveling abroad >2 weeks before onset of illness.

A total of 78 laboratory-confirmed chikungunya patients who satisfied the definition of an imported case were identified during 2006–2014. Among these patients, 6 persons had suspected CHIKV infections and 4 had suspected CHIKV and dengue virus infections. Two persons were identified though contacts of persons with confirmed cases of chikungunya. The remaining 66 persons were initially reported as having suspected dengue infections.

We determined frequencies of imported chikungunya cases by year and country of origin (Table 1). The first imported case was detected in November of 2006. Since then, imported cases have been detected every year in Taiwan. The most frequently reported countries of origin were Indonesia, the Philippines, and Malaysia.

We found no clear seasonal difference in importation frequency among chikungunya cases (online Technical Appendix Figure, http://wwwnc.cdc.go/EID/article/22/11/16-0404-Techapp1.pdf). Most (57/78, 73%) cases were identified by screening for fever at airports, and 86% (67/78) were reported ≤7 days of illness onset. Main purposes of travel were business trips of foreign workers, tourism, and family visits (Table 2).

Nucleotide sequences of complete structural protein genes C-E3-E2-6K-E1 (capsid–envelope–6K) of 56 imported CHIKV strains were analyzed. A phylogenetic tree was generated by using the maximal-likelihood method and a general time-reversible model in MEGA version 6 (http://www.megasoftware.net/) (*9*).

Imported CHIKV strains were divided into 2 genotypes: ECSA and Asian. The ECSA genotype was the most common genotype of imported CHIKV strains before 2010; strains with this genotype were mainly from Bangladesh, Malaysia, India, and Thailand. The Asian genotype was the most common genotype after 2011; strains with this genotype were mainly from Indonesia, the Philippines, and Singapore (Table 1). A phylogenetic

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Table 1. Annual number of imported cases of chikungunya, by country of origin and genotype distribution of chikungunya virus strains from imported cases, Taiwan, 2006–2014*

Country	Year									Total
	2006	2007	2008	2009	2010	2011	2012	2013	2014	
Indonesia	0	3 (0,3,0)	4 (0,4,0)	4 (1,3,0)	12 (5,5,2)	0	1 (0,1,0)	17 (0,9,8)	5 (0, 5, 0)	46 (6,30,10)
Philippines	0	0	0	0	0	1 (0, 1, 0)	3 (0, 0, 3)	8 (0,0,8)	1 (0, 1, 0)	13 (0,2,11)
Malaysia	0	0	3 (3,0,0)	2 (2,0,0)	1 (0,0,1)	0	0	0	0	6 (5,0,1)
Thailand	0	0	0	2 (2,0,0)	0	0	0	2 (1,0,1)	0	4 (3,0,1)
Singapore	1 (1,0,0)	0	0	1 (1,0,0)	0	0	0	2 (0,1,1)	0	4 (2,1,1)
Bangladesh	0	0	1 (1,0,0)	0	0	0	0	0	0	1 (1,0,0)
India	0	0	1 (1,0,0)	0	0	0	0	0	0	1 (1,0,0)
Myanmar	0	0	0	0	0	1 (0,0,1)	0	0	0	1 (0,0,1)
Cambodia	0	0	0	0	0	0	1 (0,0,1)	0	0	1 (0,0,1)
Guatemala	0	0	0	0	0	0	0	0	1 (0,0,1)	1 (0,0,1)
Total	1 (1,0,0)	3 (0,3,0)	9 (5,4,0)	9 (6,3,0)	13 (5,5,3)	2 (0,1,1)	5 (0,1,4)	29 (1,10,18)	7 (0,6,1)	78 (18,33,27)

*Values in parentheses indicate number of East/Central/South African genotypes, Asian genotypes, and unidentified chikungunya virus strains, respectively, identified in each country per year.

tree of Asian genotype strains was constructed (Figure, panel B).

Most of the imported strains could be grouped into 3 clusters. Cluster 1 contains CHIKV strains imported from Indonesia during 2007–2013 and Singapore during 2013. Cluster 2 contains CHIKV strains imported from the Philippines during 2011–2014. These strains are closely related to those isolated from Yap and St. Martin during 2013 and from the British Virgin Islands during 2014. Cluster 3 contains strains imported from Indonesia during 2010–2014. These data suggest that strains from Indonesia have shifted from cluster 1 to cluster 3 in recent years. A phylogenetic tree of CHIKV strains with the ECSA genotype was constructed (Figure, panel A). A total of 18 imported CHIKV strains from India and Southeast Asia also had the ECSA genotype. All of these strains were grouped together and are closely related to strains from islands in the Indian Ocean.

Although most isolates from Indonesia had the Asian genotype, there were 6 E1–226V variants imported from Indonesia during 2009–2010 that had the ECSA genotype. Relevant amino acid changes in complete structural proteins of imported CHIKV strains are shown in the online Technical Appendix Table.

Conclusions

A total of 78 imported chikungunya cases were identified in Taiwan during 2006–2014. With the exception of 1 imported chikungunya case from Guatemala, countries of origin for all imported cases were in southern and Southeast Asia. The location of these countries reflects the frequency of air travel between Taiwan and these countries and might also reflect the frequency and intensity of chikungunya outbreaks in these countries during the same period.

CHIKV has caused major outbreaks in southern and Southeast Asia since late 2005 (*10*–*14*). Our results are consistent with the ECSA genotype being prevalent in Singapore, Indonesia, Malaysia and Thailand during 2006– 2010. The Asian genotype was prevalent in Indonesia and the Philippines during 2007–2014. We found that the E1-226V variants of the ECSA genotype were imported from Indonesia in 2009 (*15*). Our data are concordant with the chikungunya epidemic status in Southeast Asia, including Indonesia and the Philippines, in recent years (*12*,*13*). However, the low number of imported chikungunya cases in Taiwan does not allow for a meaningful statistical analysis.

Because symptoms of many arboviral infections are similar, surveillance strategies in Taiwan for different arboviruses are also similar. Most of the confirmed chikungunya cases were initially reported as suspected dengue cases, which indicates that it is necessary to perform diagnostic tests for chikungunya and dengue in suspected cases.

During 2006–2014, a total of 36,150 suspected cases of arboviral infections were initially screened for CHIKV by RT-PCR and IgM/IgG ELISA (using the first blood sample). Most case-patients were in the acute phase of the

Table 2. Characteristics of 78 patients with chikungunya, Taiwan, 2006–2014

Characteristic	No. (%)
Case reporting system	
Fever screening at airports	57 (73.0)
Expanded screening for contacts of confirmed case-patients	2 (3.0)
Clinician- or hospital-based	19 (24.3)
Age group, y	
<20	9 (11.5)
20–39	43 (55.1)
40–59	17 (22.0)
≥60	9 (11.5)
Onset day	
≤7	67 (86.0)
>7	11 (14.1)
Laboratory confirmation test	
Viral RNA or virus isolation	59 (76.0)
Serologic analysis	19 (24.3)
Travel purpose	
Foreign labor	26 (33.3)
Tourism	25 (32.0)
Family visit	14 (18.0)
Business trip	8 (10.3)
Other	5 (6.4)

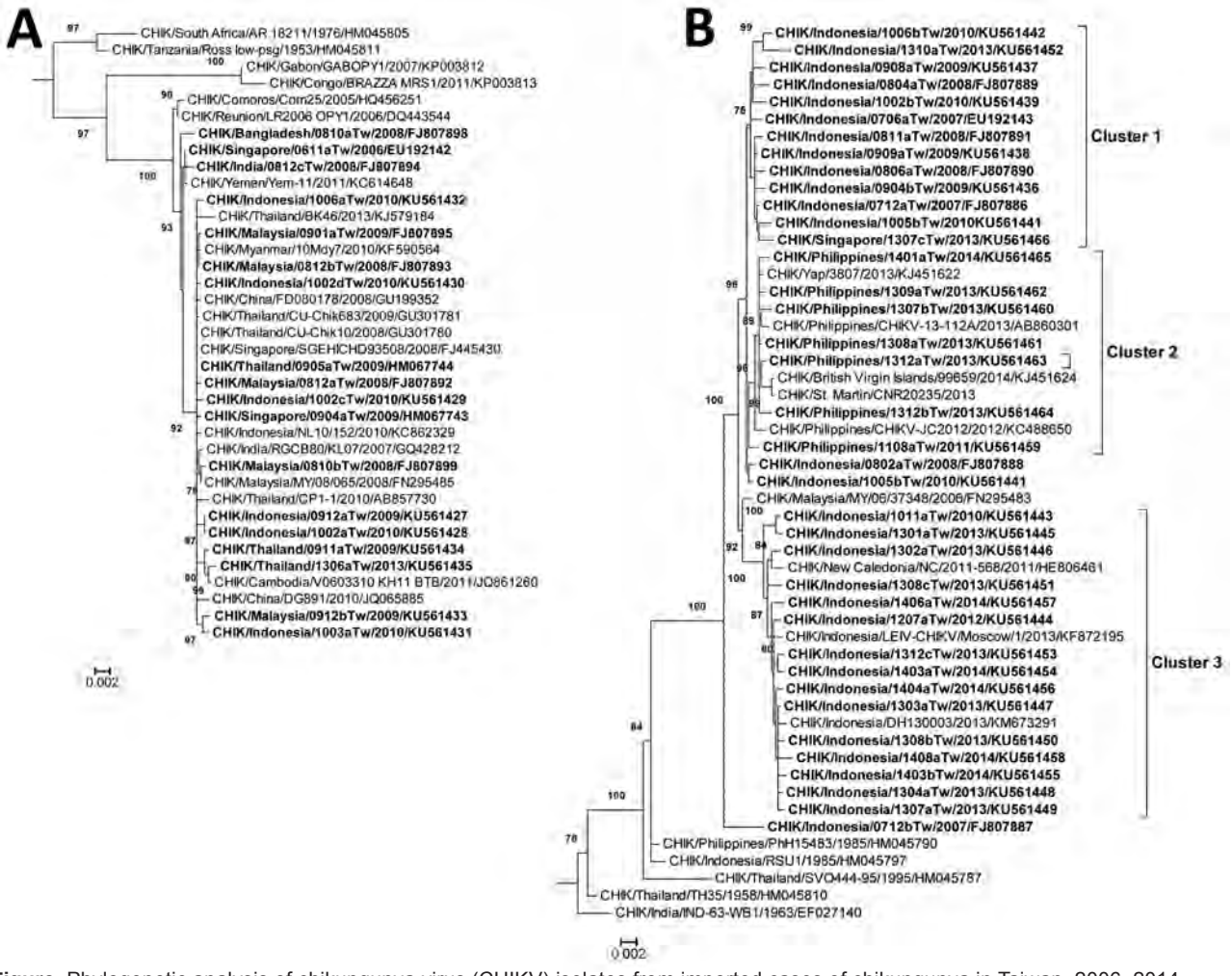

Figure. Phylogenetic analysis of chikungunya virus (CHIKV) isolates from imported cases of chikungunya in Taiwan, 2006–2014. Trees show genetic relationships of the East/Central/South African genotype (A) and the Asian genotype (B) and of CHIKV isolates; clusters are shown in panel B. Trees were generated by using nucleotide sequences (3,747 bp) of complete structural protein genes C-E3-E2-6K-E1 (capsid–envelope–6K) of CHIKV strains. Sequences obtained in this study are indicated in bold. Viruses are identified by virus/country/strain/year of isolation/GenBank accession no. Strains reported in this study were submitted to GenBank under accession nos. FJ807886–FJ807895, FJ807898, FJ807899, EU192142, EU192143, HM067743, HM067744, and KU561427–KU561466. Analysis was performed by using MEGA version 6 software (http://www.megasoftware.net/) and the maximal-likelihood method (general time-reversible model). Bootstrap support values ≥75 are shown (1,000 replicates) along branches. Scale bars indicate nucleotide substitutions per site.

disease. Among the remaining suspected case-patients, convalescent-phase serum samples were collected from 320 patients for testing by ELISA. Results suggested that the positive rate for CHIKV (78/36,150, 0.22%) was low among persons with suspected cases of arboviral infection. In addition, no indigenous chikungunya cases have been identified in this study.

Most confirmatory testing was performed by using RT-PCR. However, this testing could lead to a lower rate of laboratory-confirmed chikungunya cases because there was limited serologic testing completed for paired serum samples. In addition, the population not captured (e.g., those with subclinical or nonacute infections) could result in an underestimation of the number of imported chikungunya cases.

To prevent spread of arbovirus diseases, well-organized integrated disease and vector surveillance systems must be properly implemented and executed. Detection of imported chikungunya cases by active and passive surveillance at an early stage is needed to implement early response activities and reduce risk for local transmission.

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Neutralizing Antibodies to Severe Fever with Thrombocytopenia Syndrome Virus 4 Years after Hospitalization, China

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Severe fever with thrombocytopenia syndrome is an emerging hemorrhagic fever disease in eastern Asia, caused by a tickborne bunyavirus. Of 25 patients hospitalized with this disease in China, 100% produced and maintained neutralizing antibodies to severe fever with thrombocytopenia syndrome virus for the study period of 4 years.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging hemorrhagic fever disease in eastern Asia (*1*–*3*). SFTS is caused by SFTS virus (SFTSV), a tickborne bunyavirus that is transmitted through tick bites (*1*,*3*,*4*) and person to person by contact with patient blood (*5*,*6*). Clinically, SFTS is characterized by a sudden onset of fever, thrombocytopenia, hemorrhagic tendency, gastrointestinal symptoms, and multiple organ dysfunction and a high case-fatality rate of 12%–30% (*1*). SFTSV is a relatively new bunyavirus, and information regarding its neutralizing antibodies is scarce. In this study, we determined the prevalence and duration of neutralizing antibodies in serum samples of SFTS patients in China.

The Study

For this study, patients with a clinical diagnosis of SFTS were those who had fever, thrombocytopenia, or leukopenia without another known acute infectious disease; patients with laboratory-confirmed SFTS had SFTSV antibodies or RNA detected by ELISA or reverse transcription PCR (RT-PCR). Acute-phase (within 2 weeks after onset of illness) and convalescent-phase serum samples obtained during hospitalization of the patients were tested for total antibodies against SFTSV by using a double-antigen sandwich ELISA kit (Xinlianxin Biomedical Technology Limited, Wuxi, China). The study was approved by the ethics committee of Shandong University. Informed consent was obtained from all participants.

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The ELISA plates were coated with recombinant SFTSV nucleoprotein (*7*). Undiluted patient serum samples were used for ELISA; SFTSV antibodies were detected with horseradish peroxidase–labeled recombinant SFTSV protein. Serum samples were considered positive for SFTSV when absorbance of the sample was >2.1 times that of a negative control at 450 nm (*8*). Nested RT-PCR amplification of the SFTSV RNA large segment (900 bp) and small segment (600 bp) have been described previously (*8*). PCR products were confirmed to be SFTSV RNA by DNA sequencing.

During June 26, 2011–August 26, 2012, a total of 46 patients were hospitalized and given a clinical diagnosis of SFTS in a local hospital in Yiyuan County, Shandong Province, China. We confirmed by ELISA or RT-PCR that 33 (71.7%) of these 46 patients were infected with SFTSV. Of the confirmed cases of SFTS, 22 occurred in 2011 and were reported previously (*8*). Two (6.1%) patients with confirmed SFTS died.

Among the 31 laboratory-confirmed living patients with SFTS, 25 agreed and 6 refused to donate blood samples for neutralization assay after discharge. Thirteen (52%) volunteers were male and 12 (48%) were female; their ages ranged from 42 to 75 years (median age 62 years).

Blood samples were obtained from the 25 SFTS volunteers 2 or 3 times during a 4-year period. Serum samples were heat inactivated at 56°C for 30 min and diluted in 2-fold increments from 1:5 to 1:1,280. Each dilution of serum was mixed with an equal volume of solution containing SFTSV (1,000 pfu/mL) at 37°C for 1 hour. Culture medium was used as a control for serum. Samples were tested by using the 50% plaque reduction neutralization test ($PRNT_{50}$). The titer obtained is the reciprocal of the highest serum dilution that reduces the number of plaques by 50% relative to the average number of plaques in viral control wells.

At first, SFTSV does not form clear plaques on Vero cells. SFTSV is passaged on Vero cells until plaques are clearly visible. Initially, 10^6 SFTSV is inoculated into cells in 1 well of a 6-well plate. When a cytopathic effect is visible, cells with the cytopathic effect are aspirated with a pipette tip and transferred to a new well. A single plaque is picked and used for viral stock when the plaques are clearly visible on the fifth passage.

To determine the viral titer with a plaque assay, the viral stock is diluted from 10^{-2} to 10^{-6} in 10-fold increments.

Each dilution of viral stock is used to infect 2 wells of cells. (The negative control contains maintenance medium without virus.) Infected cells are incubated at 37°C in 5% CO_2 for 1 h; then, viral inoculum is replaced with Dulbecco's modified Eagle medium containing 1.5% methylcellulose, 1% fetal bovine serum, 10 mmol/L HEPES, penicillin (100 units/mL), and streptomycin (100 μg/mL). Plates are incubated at 37°C in 5% CO_2 for 10 d. The monolayer is fixed with 4% paraformaldehyde and stained with crystal violet. Plaques in each well are counted to determine the plaque-forming unit.

$PRNT_{50}$ results showed that all 25 patients developed neutralizing antibodies against SFTSV at titers from 20 to 640; the neutralizing antibodies lasted for the entire study period of 4 years (Table). We also performed $PRNT_{90}$ tests for all 25 patients; these showed similar results to $PRNT_{50}$, but the titers were less in extent than those of $PRNT_{50}$ (data not shown).

In general, the titer of neutralizing antibodies decreased over time in all but 2 patients (nos. 5 and 11), who had a higher $PRNT_{50}$ titer in the last year than in the first year; this increase may have been caused by reinfection with SFTSV. However, our previous studies indicate that, in the local area, the incidence of SFTSV infection is <5 cases/100,000 population and the seroprevalence rate of SFTSV in the healthy population is <1% (*8–10*), suggesting that the chance of reinfection of a patient with SFTSV is low. We cannot exclude, however, that these 2 patients could have been infected with other phleboviruses that have not yet been isolated in China. Serum samples from 2 healthy persons were also tested for neutralizing antibodies as controls; neither of them had any neutralization activity against SFTSV.

Table. Neutralizing antibody titers for convalescent-phase serum samples from 25 patients with SFTS, Yiyuan County, Shandong, China, 2011–2014*

Patient no.	Titer by $PRNT_{50}$			
	First year	Second year	Third year	Fourth year
1	640	NT	320	80
2	80	NT	40	80
3	NT	NT	40	40
4	20	NT	40	40
5	20	NT	40	40
6	80	NT	80	20
7	40	NT	80	40
8	5	NT	20	40
9	40	NT	40	20
10	160	NT	80	80
11	160	NT	20	80
12	160	NT	40	40
13	40	NT	20	NT
14	NT	NT	320	NT
15	160	NT	160	80
16	320	NT	40	40
17	160	NT	40	40
18	NT	NT	80	40
19	640	NT	160	160
20	320	80	80	NT
21	640	320	80	NT
22	20	20	20	NT
23	320	80	NT	NT
24	NT	80	40	NT
25	640	80	160	NT

*Convalescent-phase serum samples were obtained during the first, second, third, and fourth years after discharge of the patients from the hospital. NT, no test performed because serum was not available; $PRNT_{50}$, plaque reduction neutralization test indicating the serum titer that reduced 50% of plaque-forming units of SFTS virus; SFTS, severe fever with thrombocytopenia syndrome.

Conclusions

We found that hospitalized patients with SFTS produced long-lasting neutralizing antibodies to SFTSV. We do not know the characteristics of the neutralizing antibodies against SFTSV, which need to be further investigated. In general, neutralizing antigens of bunyavirus are located on the viral glycoproteins (*11–13*). A neutralizing monoclonal antibody to SFTSV is found to bind a linear epitope in the ectodomain of glycoprotein Gn of SFTSV. Its neutralizing activity is attributed to blockage of the interactions between the Gn protein and the cellular receptor (*14*). The limitation of this study is that we obtained SFTS patients' serum samples for only up to 4 years after diagnosis; we do not know how long the neutralizing antibodies in patients will last.

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Increased Community-Associated Infections Caused by Panton-Valentine Leukocidin–Negative MRSA, Shanghai, 2005–2014

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During 2005–2014, community-associated methicillin-resistant *Staphylococcus aureus* infections increased in Shanghai, China. Most infections were caused by sequence type 59 *S. aureus* that lacked Panton-Valentine leukocidin. This finding challenges the notion that Panton-Valentine leukocidin is necessary for epidemiologic success of community-associated methicillin-resistant *S. aureus*.

In the United States, community-associated (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) infections in otherwise healthy persons in the community, first reported in the late 1990s (*1*), have reached epidemic dimensions (*2*). Despite considerable research efforts, the molecular underpinnings of the epidemiologic success of CA-MRSA are still not completely understood. Most typically connected with CA-MRSA is Panton-Valentine leukocidin (PVL). However, the role of PVL in CA-MRSA infection is controversial, primarily because of contradictory results from studies of animal infection models (*3*). A common belief is that if a clone from a patient with CA–*S. aureus* infection is positive for PVL, the *S. aureus* is probably a more dangerous clone and the patient would require specific care (*4*).

In the United States, virtually all CA-MRSA infections are caused by a PVL-positive clone of pulsed-field type USA300 (*5*). CA-MRSA infections with USA300 have also occasionally occurred outside the United States and adjacent regions. However, according to a recent study, they are derived from multiple importation events, suggesting that further spread in those locations is unlikely (*6*). Rather, global CA-MRSA infections are caused by geographically divergent clones that are unrelated to USA300. Like USA300, most of them contain PVL genes (*2*), although PVL is extremely rare in hospital-associated MRSA clones. This epidemiologic correlation is the predominant basis of the notion that PVL is causally associated with the enhanced virulence potential of CA-MRSA clones (*7*). Of note, despite generally enhanced virulence in animal models at levels similar to that of USA300 (*8*), the number of infections caused by global CA-MRSA clones remains limited (*9*,*10*). This situation raises the question of whether non-USA300 global CA-MRSA lineages have the potential to further intensify infection frequency and severity, and if so, whether PVL would be a necessary factor in such a scenario.

The CA-MRSA lineage that predominates in China and many other parts of Asia, thus threatening the largest global population, is sequence type (ST) 59 (*10*). Recent studies performed in Taiwan and northern Vietnam found a correlation between a PVL-positive subset of ST59 (Taiwan clone) and infection, but PVL-negative ST59 (Asia–Pacific clone) was found to be a largely noninfectious colonizer (*11*,*12*). Therefore, a causal relationship between PVL and infection has also been proposed for that CA-MRSA lineage.

The Study

We studied *S. aureus* isolates collected over 10 years (2005–2014) at Shanghai Renji Hospital, Shanghai, China, a large teaching hospital at which ≈10,000 patients from the entire Shanghai metropolitan area are admitted each day. We obtained 2,048 infectious *S. aureus* isolates and characterized them by multilocus sequence and *spa* typing, antibiotic resistance profiling, determination of the staphylococcal cassette chromosome (SCC) *mec* type (encoding methicillin resistance), and analytical PCR to determine presence of the *lukSF* genes encoding PVL. For isolates obtained during 2005–2010, we investigated randomly selected subsets (100 isolates/year); for isolates obtained during 2011, 2012, and 2014, we investigated all isolates. No isolates were collected in 2013. CA–*S. aureus* was defined as an isolate obtained from either an outpatient or an inpatient (including from general and urgent care and emergency rooms) ≤24 h after hospital admission, who lacked risk factors (contact with the hospital environment in the 6 months preceding the culture, *S. aureus* infection history, residence in a long-term care facility in the 12 months before culture, presence of a central vascular catheter at the

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Table. Characteristics of *Staphylococcus aureus* isolated at Shanghai Renji Hospital, Shangai, China, 2005–2014*

		No. (%)											
Year	Total†	HA-MSSA	HA-MRSA	CA-MSSA	CA-MRSA	Invasive among CA-MRSA	CA	HA	MSSA	MRSA	ST59	ST59 CA-MRSA	Invasive among ST59 CA-MRSA
2005–2010	500	105 (21)	340 (68)	43 (8.6)	12 (2.4)	5 (42)	55 (11)	445 (89)	148 (30)	352 (70)	15 (3.0)	4 (0.8)	2 (50)
2011	600	124 (21)	398 (66)	57 (9.5)	21 (3.5)	14 (67)	78 (13)	522 (87)	181 (30)	419 (70)	24 (4.0)	9 (1.5)	6 (67)
2012	478	126 (26)	287 (60)	38 (7.9)	27 (5.6)	18 (67)	65 (14)	413 (86)	164 (34)	314 (66)	39 (8.2)	18 (3.8)	14 (78)
2014	470	114 (24)	275 (59)	46 (9.8)	35 (7.4)	18 (51)	81 (17)	389 (83)	160 (34)	310 (66)	45 (9.6)	25 (5.3)	13 (52)

*CA, community-associated; HA, hospital-associated; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; ST, sequence type.
†For 2005–2010, of a total of 2,681 isolates collected, 100 randomly selected isolates/year selected by using a random sample of data (SPSS, Chicago, IL, USA). For 2011, 2012, and 2014, all isolates obtained at the hospital were tested.

time of infection, or recent use of antimicrobial drugs). These data were obtained by a review of medical records. The study was approved by the ethics committee of Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai (protocol RJ-H-2015–0221).

The percentage of methicillin resistance in the *S. aureus* infectious isolates was high, as is generally reported for China (*13*), and remained stable (at ≈70%) over the past 10 years (Table; Figure, panel A). In contrast, during the same time, resistance among CA–*S. aureus* infections

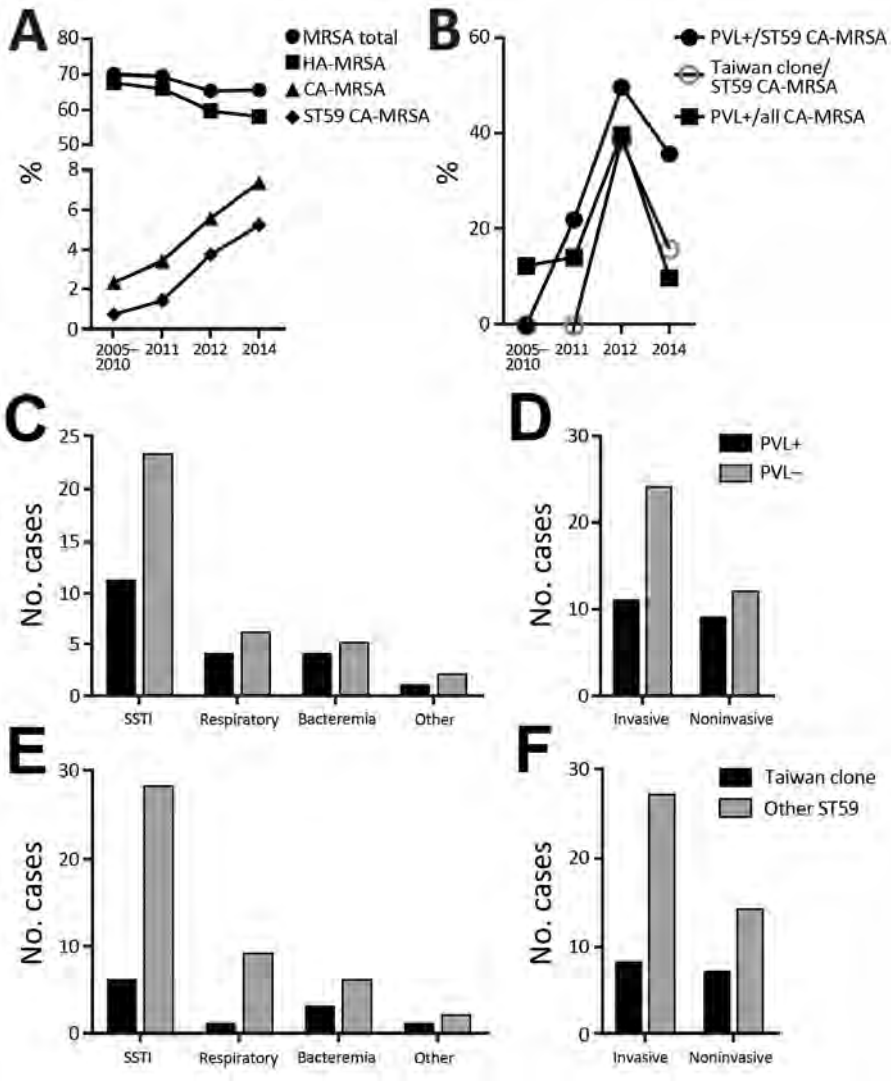

Figure. Epidemiology of MRSA in Shanghai, 2005–2014. Of infectious *Staphylococcus aureus* isolates obtained during 2005–2010, a random selection of 100 from each year were analyzed; of those obtained during 2001, 2012, and 2014, all isolates were analyzed. A) Percentages of MRSA (methicillin-resistant *S. aureus*) isolates among all obtained *S. aureus* isolates. B) Percentages of Panton-Valentine leukocidin (PVL)–positive clones among all or sequence type (ST) 59 community-associated (CA)–MRSA and of the Taiwan clone among ST59 CA-MRSA. C) Infection types from which ST59 CA-MRSA clones were obtained, differentiated by presence of PVL genes. D) Invasiveness of infections, differentiated by presence of PVL genes. E) Infection types from which ST59 CA-MRSA clones were obtained, differentiated by Taiwan clone versus other ST59 types. F) Invasiveness of infections, differentiated by Taiwan clone versus other ST59 types. HA, hospital acquired; SSTI, skin and soft tissue infection.

rose considerably, from 21% to 43% (p = 0.0108, Fisher exact test). While the percentage of HA-MRSA infections slightly decreased and that of CA–methicillin-sensitive *S. aureus* infections remained stable, CA-MRSA infections increased significantly, from 2.4% (12/500 total *S. aureus* infections) during 2005–2010 to 7.4% (35/470) in 2014 (p = 0.0003, Fisher exact test) (Table; Figure, panel A). ST59 dominated among CA-MRSA infections; increasing relative frequency reached a level of 71.4% in 2014 (Table; Figure, panel A). This finding links the observed surge in the CA-MRSA infection rate nearly exclusively to the ST59 lineage.

Of the 56 ST59 CA-MRSA infections, most (34 [61%]) were skin and soft tissue infections (i.e., spontaneous pyogenic skin abscesses), but a considerable number were respiratory (10 [18%]) or blood (9 [16%]) infections. The percentage of invasive infections (as defined by isolation from an otherwise sterile site, such as primary skin and soft tissue infection, with subsequent isolation from the blood, lung, or other otherwise sterile body fluids) among ST59 CA-MRSA patients was high at 62.5% (35/56). Fatality rate was 14% (5/35) among patients with invasive infections. Multidrug resistance was frequent. In addition to being resistant to β-lactams, most of the recently (2014) isolated ST59 CA-MRSA was also resistant to erythromycin (23/25; 92%), clindamycin (23/25; 92%), gentamicin (2/25; 8%), levofloxacin (3/25; 12%), trimethoprim/sulfamethoxazole (4/25; 16%), fosfomycin (3/25; 12%), or rifampin (5/25; 20%). None was resistant to tetracycline, linezolid, or vancomycin.

The ST59 CA-MRSA isolates were genetically heterogeneous and belonged to 7 *spa* types, predominantly t437 (31/56; 55%), t216 (12/56; 21%), and t441 (6/56; 11%). This finding is in contrast to the scenario described for USA300 CA-MRSA isolates, which are closely related (*14*), and suggests independent acquisition of SCC*mec* elements by genetically divergent parental ST59 methicillin-sensitive *S. aureus* strains.

Of note, only 20 (36%) of the 56 ST59 CA-MRSA isolates that we obtained contained the *lukSF* genes encoding PVL (Figure, panel B), and presence of the PVL genes was not correlated with more severe (i.e., invasive) infection (Figure, panel C). The PVL-positive Taiwan clone (*spa* types t437/t441, *lukSF*$^+$, SCC*mec* V) was responsible for only 20% of cases (Figure, panel D). Moreover, while the percentage of PVL-positive ST59 CA-MRSA isolates and those belonging to the Taiwan clone increased in 2012, probably because of dissemination of the Taiwan clone into China, those numbers recently declined, indicating that PVL and the Taiwan clone are not main driving forces explaining the increase and current high percentage of CA-MRSA infections in Shanghai (Figure, panel B). Also, these subsets were not correlated with a specific infection type (Figure, panels C,D). Last, the Taiwan clone was not more frequently involved with invasive infections than were other ST59 CA-MRSA isolates (Figure, panel D).

Conclusions

CA-MRSA infections caused by a non-USA300 clone increased significantly in a highly populated area in China. Whether our findings are representative of all of China and adjacent countries remains to be addressed. Our findings do not support the previously indicated correlation of the PVL-positive ST59 subset (Taiwan clone) with infection (*11*,*12*). Thus, our study provides epidemiologic evidence challenging the widespread notion about a significant role of PVL in CA-MRSA dissemination in the ST59 lineage and in general. Inasmuch as our findings underscore the idea that the development of CA-MRSA clones is less limited to specific genetic backgrounds than previously thought, they underscore that novel successful CA-MRSA clones will probably continue to emerge.

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Severe Fever with Thrombocytopenia Syndrome in Patients Suspected of Having Scrub Typhus

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To determine prevalence of severe fever with thrombocytopenia syndrome in South Korea, we examined serum samples from patients with fever and insect bite history in scrub typhus–endemic areas. During the 2013 scrub typhus season, prevalence of this syndrome among patients suspected of having scrub typhus was high (23.0%), suggesting possible co-infection.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by a novel phlebovirus in the family *Bunyaviridae* (*1*,*2*). The disease is characterized by fever, gastrointestinal signs and symptoms, leukopenia, and thrombocytopenia (*1*,*2*). Exposure to ticks, weeds, and shrubs have been found to be risk factors (*2*,*3*).

In South Korea, areas in which SFTS and scrub typhus are endemic overlap (*4*,*5*). Scrub typhus is caused by *Orientia tsutsugamushi*, which are bacteria transmitted to humans by chigger mite bites. Scrub typhus is a major public health problem during harvest season in South Korea (October and November); 10,485 cases were reported in 2013 (*5*). The clinical presentations of scrub typhus and SFTS are similar; signs and symptoms typically develop within 1–2 weeks of infection and include fever, headache, malaise, and gastrointestinal upset.

In South Korea, febrile patients with a history of bug bites are generally suspected of having scrub typhus and are prescribed antimicrobial drugs (e.g., doxycycline or azithromycin) in the early phases of the disease. We investigated the prevalence of SFTS in patients with fever and a history of insect bites in scrub typhus–endemic areas.

The Study

During scrub typhus season (September–December) in 2013, we collected serum samples from 74 patients at Samsung Changwon Hospital (Sungkyunkwan University, Changwon-si, South Korea) who had fever and a history of bug bites. At the time of patient admission, we tested for antibodies against *O. tsutsugamushi* by using a commercial immunochromatography kit (SD Bioline Tsutsugamushi Assay; Standard Diagnostics, Yongin, South Korea). For molecular diagnosis of SFTS virus (SFTSV), we performed reverse transcription PCR of partial small RNA segments as previously described (*6*). We then performed sequencing by using a BigDye Terminator Cycle Sequencing Kit (PerkinElmer Applied Biosystems, Warrington, UK).

Characteristics of SFTSV-positive and -negative populations were compared by using the χ^2, Fisher exact, 2-sample *t*, or Mann–Whitney U tests, as appropriate. Logistic regression was used to identify predictors of SFTS virus infection. Variables for which p value was <0.05 in univariate analysis were candidates for multivariate analysis. All analyses were conducted with SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

Among the 74 patients who had fever and a history of bug bites during scrub typhus season, the overall prevalence of SFTS infection was 23.0% (17/74). Detected SFTSV sequences showed 97.0%–99.0% identity with the partial sequence of the small RNA segment from SFTSV strains from South Korea (GenBank accession nos. KR612072–KR612088). No significant differences were found between the 2 patient groups (with and without SFTS) with regard to farming (Table). Patients infected with SFTSV were much older than those not infected. Among patients infected with SFTSV, clinical presentations of anorexia, nausea/vomiting, and a decreased level of consciousness were more prevalent; lactate dehydrogenase and C-reactive protein levels were remarkably higher; and albumin levels were lower than among patients without SFTSV infection. Incidence of lymphocytopenia was lower among patients with than without SFTSV.

Multivariate analysis revealed that low albumin level at admission (odds ratio 0.19, 95% CI 0.04–0.99; p = 0.049) and anorexia (odds ratio 13.1, 95% CI 2.2–78.7; p = 0.005) were independent predictors of SFTS in patients suspected of having scrub typhus. The goodness of fit of the final logistic regression model seemed to be satisfactory (Hosmer-Lemeshow statistic, χ^2 = 7.321; p = 0.396). Of the 17 SFTS patients, 4 had an *O. tsutsugamushi* antibody titer of 1:2,560, determined by immunochromatography at

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Table. Demographic and laboratory characteristics of SFTS patients, South Korea, 2013*

Characteristics	SFTS PCR+, n = 17	SFTS PCR–, n = 57	p value
Male sex, no (%)	7 (41.2)	24 (42.1)	0.946
Age, mean ± SD	64.2 ± 15.5	54.5 ± 16.4	0.033
Farming, no (%)	12 (70.6)	29 (50.9)	0.151
Coexisting condition, no. (%)			
Chronic lung disease†	3 (17.6)	3 (5.3)	0.130
Chronic heart disease‡	6 (35.3)	11 (19.3)	0.197
Chronic renal disease	1 (5.9)	1 (1.8)	0.409
Diabetes	4 (23.5)	5 (8.8)	0.197
Chronic liver disease	1 (5.9)	5 (8.8)	0.580
Corticosteroid use	0	2 (3.5)	0.591
Cancer	0	3 (5.3)	0.451
Cerebrovascular disease	1 (5.9)	3 (5.3)	0.657
Clinical presentation			
Fever (temperature ≥38.3°C)	13 (76.5)	48 (84.2)	0.480
Headache	5 (29.4)	18 (31.6)	0.865
Myalgia	8 (47.1)	31 (54.4)	0.595
Anorexia	10 (58.8)	5 (8.8)	<0.001
Nausea/vomiting	8 (47.1)	10 (17.5)	0.022
Abdominal pain	2 (11.8)	5 (8.8)	0.657
Diarrhea	1 (5.9)	1 (1.8)	0.409
Cough	1 (5.9)	5 (8.8)	0.580
Dyspnea	1 (5.9)	2 (3.5)	0.549
Decreased consciousness	3 (17.6)	-	0.010
Rash	12 (70.6)	34 (59.6)	0.414
Laboratory findings at admission			
Leukopenia (<4,000 cells/mm^3) no. (%)	2 (11.8)	18 (31.6)	0.131
Lymphocytopenia (<1,500 cells/mm^3) no. (%)	6 (35.5)	43 (75.4)	0.002
Anemia (hematocrit <30%), no. (%)	3 (17.6)	4 (7.0)	0.341
Thrombocytopenia (<10^{6}cells/mm^3) no. (%)	7 (41.2)	13 (22.8)	0.221
CPK, IU/L, median (IQR)	67 (33–132)	76 (45–128)	0.512
LDH, IU/L, mean ± SD	533 ± 202	402 ± 151	0.021
AST, IU/L, median (IQR)	104 (48–194)	69 (54–112)	0.210
ALT, IU/L, median (IQR)	70 (30–119)	53 (35–83)	0.616
PT (INR), median (IQR)	1.08 (1.03–1.15)	1.03 (0.97–1.09)	0.057
CRP, mg/L, median (IQR)	71.6 (46.4–110.4)	42.9 (23.2–80.3)	0.034
BUN, mg/dL, median (IQR)	12.7 (9.3–18.2)	12.7 (9.4–15.3)	0.634
Creatinine, mg/dL, median (IQR)	0.9 (0.8–1.3)	0.8 (0.6–1.0)	0.510
Albumin, g/dL, mean ± SD	2.9 ± 0.7	3.3 ± 0.5	0.005
Hematuria, no. (%)	3 (20.0)	20 (35.7)	0.356
Outcome			
Intensive respiratory or vasopressor support	3 (17.6)	1 (1.8)	0.036
Time from symptom onset to admission, d, median (IQR)	3 (3–6.5)	4 (3–6)	0.599
Hospital stay, d, median (IQR)	0 (0–3)	1 (0–4)	0.432
Death, no (%)	1 (5.9)	0	0.230

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CPK, creatinine phosphokinase; CRP, C-reactive protein; INR, international normalized ratio; IQR, interquartile range; LDH, lactate dehydrogenase; PT, prothrombin time; SFTS, severe fever with thrombocytopenia syndrome.
†Asthma, chronic obstructive pulmonary disease, bronchiectasis.
‡Underlying coronary heart disease, chronic heart failure.

admission, and 3 showed seroconversion on paired serum samples. Those infected with SFTSV were more likely to require intensive respiratory or vasopressor support than those not infected. During hospitalization, 1 (5.9%) SFTS patient died. Length of hospital stay was similar between patients infected and not infected with SFTSV.

Conclusions

In scrub typhus–endemic areas of South Korea, prevalence of SFTS among patients with suspected scrub typhus is quite high (23.0%). Independent predictors of SFTS in patients with suspected scrub typhus are low albumin level at admission and anorexia. Co-infection with SFTSV and *O. tsutsugamushi* was suspected for 7 patients. Patients with SFTS experienced a more severe clinical illness; however, outcomes such as death and length of hospital stay did not vary significantly between groups of patients with and without SFTSV.

As an emerging infectious disease, SFTS is an increasing public health threat because of its wide distribution

and high mortality rate (*1*,*2*). In South Korea during April–December 2013, a total of 35 cases of SFTS were reported (*4*). The major signs and symptoms of these 35 patients were fever (100%), gastrointestinal upset (74%), fatigue (74%), thrombocytopenia (100%), and leukocytopenia (100%) (*4*). During 2013, the reported mortality rate among patients with SFTS in South Korea was 45.7% (16 deaths/35 patients), which is higher than that reported in China (6%–30%) (*1*,*2*). However, our study found a mortality rate of 5.9%, and illness was severe in only 3 (17.6%) patients. A large-scale serologic survey of 2,547 farmers living in rural areas of Jiangsu Province in China (*7*) found an overall SFTSV antibody prevalence rate of 1.30%, and the farmers seropositive for SFTS did not report having typical symptoms of SFTS. This finding suggests occurrence of asymptomatic or mild cases of SFTS.

In our study, all SFTS patients initially received a diagnosis of scrub typhus because of its seasonality and the similar presentation of the 2 diseases. The Korea Center for Disease Control and Prevention and the National Notifiable Disease Surveillance System reported that during 2013, SFTS occurred mainly from April to September (*4*). However, we showed that SFTS was also prevalent in October and November (Figure).

SFTS is mainly transmitted to humans by SFTSV-infected ticks; however, person-to-person transmission by direct contact with infected blood or body fluids has also been reported (*8*,*9*). Therefore, standard precautions are necessary for healthcare workers in contact with patients with fever and a history of bug bites.

Previous studies have suggested that *Haemaphysalis longicornis* and *Rhipicephalus microplus* ticks are the most likely vectors of SFTSV transmission to humans (*10*,*11*). *H. longicornis* ticks are widespread in South Korea and their density is high during May–August, when temperatures are usually warm (*10*). In contrast, *Leptotrombidium scutellare* mites are major scrub typhus vectors with high density during autumn (scrub typhus season) (*12*). Therefore, for patients with fever and a history of bug bites, physicians in South Korea tend to suspect SFTS during summer and scrub typhus during autumn. In our study, the antibody titer of 1:2,560 in 7 patients suggests the possibility of SFTSV and *O. tsutsugamushi* co-infection. In China, SFTSV has also been detected by reverse transcription PCR in *L. scutellare* mites (*13*). Therefore, further research is needed to confirm the mite–SFTSV association in addition to the prevalence of SFTSV and *O. tsutsugamushi* co-infection.

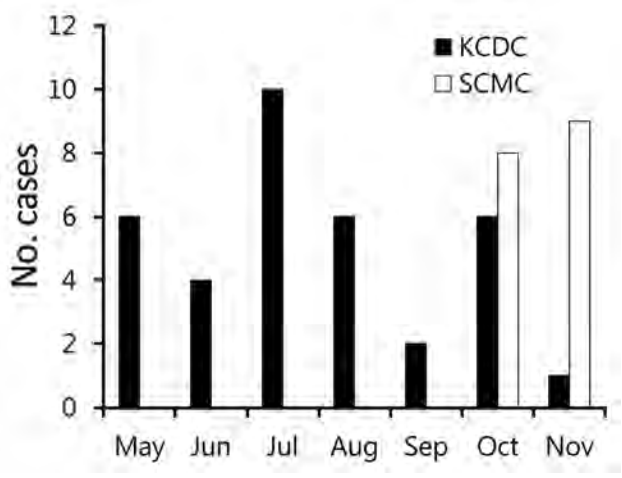

Figure. Seasonal distribution of severe fever with thrombocytopenia syndrome, South Korea, 2013. KCDC, Korea Center for Disease Control and Prevention; SCMC, Samsung Changwon Hospital.

The results of this study suggest that in South Korea, prevalence of SFTS is quite high among patients suspected of having scrub typhus. Signs and symptoms of SFTS can be atypical. Therefore, healthcare workers in contact with patients suspected of having scrub typhus should take standard precautions. Further epidemiologic research is needed to improve ability to accurately differentiate SFTS from other diseases and to confirm the vector of SFTS.

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Nasopharyngeal Pneumococcal Density and Evolution of Acute Respiratory Illnesses in Young Children, Peru, 2009–2011

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We examined nasopharyngeal pneumococcal colonization density patterns surrounding acute respiratory illnesses (ARI) in young children in Peru. Pneumococcal densities were dynamic, gradually increasing leading up to an ARI, peaking during the ARI, and decreasing after the ARI. Rhinovirus coinfection was associated with higher pneumococcal densities.

Streptococcus pneumoniae commonly colonizes the nasopharynx of young children (*1*). Nasopharyngeal colonization density is relevant for transmission of bacteria and pathogenesis of pneumococcal diseases (*2*). Few studies have evaluated the longitudinal relationship between nasopharyngeal pneumococcal density and acute respiratory illnesses (ARIs). We examined the evolution of nasopharyngeal pneumococcal density surrounding ARIs in young children.

The Study

We performed sequential cross-sectional assessments from a prospective cohort study of Andean children in Peru (*3*). During 2009–2011, children <3 years of age from the District of San Marcos, Cajamarca, Peru, were assessed for ARIs during weekly household visits. The population was rural and had low incomes and limited access to healthcare (*3*,*4*). Use of 7-valent pneumococcal conjugate vaccine (PCV7) started in late 2009. Institutional review boards of Vanderbilt University (Nashville, TN, USA) and the Instituto de Investigacion Nutricional (Lima, Peru) approved the study.

An ARI episode was defined as the length of time a child had cough or fever (*5*,*6*). If a child was ill during a household visit, we assessed for pneumonia or lower respiratory tract infection using IMCI-WHO (Integrated Management of Childhood Illness–World Health Organization) criteria (*5*,*7*). If the child had an ARI during the preceding 7 days, we collected a nasal swab sample and tested it for respiratory viruses by reverse transcription PCR at Vanderbilt University (*6*,*8*–*11*). Nasopharyngeal swab samples were collected monthly without regard to ARI and tested at Emory University (Atlanta, GA, USA) by using quantitative PCR for pneumococcal density determinations. For this study, we used samples collected in 2009 and 2011, representing periods before and after routine PCV7 use (*12*) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-0902-Techapp1.pdf).

Nasopharyngeal samples were classified according to their collection time surrounding ARIs: peri-ARI periods included pre-ARI (8–14 or 1–7 days before an ARI) and post-ARI (1–7 or 8–14 days after an ARI). Samples outside these periods were considered non-ARI samples. We compared log-transformed pneumococcal nasopharyngeal densities of samples from ARI, peri-ARI, and non-ARI periods by using multivariable quantile regression with robust SEs and adjusting for relevant covariates.

In secondary analyses, we assessed the role of respiratory viruses on pneumococcal density in children with ARIs. Because detection of nonrhinovirus respiratory viruses in nasal swabs was infrequent, we grouped samples into 4 distinct groups: rhinovirus only, rhinovirus and other viruses, other viruses only, and negative for any viruses.

We examined the role of pneumococcal acquisition on pneumococcal density using pneumococci-positive nasopharyngeal samples from children who had a sample collected within the preceding 60 days. Samples were categorized as 1) new colonization if the prior sample was negative, 2) serotype persistence if the prior sample was the same serotype, and 3) serotype replacement if the prior sample was a different serotype. If either serotype was nontypeable or unknown, the pattern was considered undetermined.

We assessed 3,579 nasopharyngeal samples from 833 children: 450 (12.6%) were collected during ARIs, 956 (26.7%) during peri-ARI periods, and 2,173 (57.8%) during non-ARI periods. The median age was 1.39 years. The median duration for ARIs was 8 days (interquartile range [IQR] 5–13 days). According to IMCI-WHO criteria, 33 samples were associated with pneumonia or severe pneumonia (*13*) (Table).

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Table. Demographic characteristics for children from whom nasopharyngeal swab samples were collected during different periods surrounding ARIs, Peru, 2009–2011*

Characteristic	Period of sample collection						
		Pre-ARI			Post-ARI		
	Non-ARI, n = 2,173	8–14 days, n = 211	1–7 days, n = 222	Current ARI, n = 450	1–7 days, n = 332	8–14 days, n = 191	Total, N = 3,579
No. children†	765	186	189	320	262	172	833
Demographics							
Median age, y, at sample collection	1.45	1.36	1.37	1.23	1.25	1.37	1.39
Male	50.5	56.4	52.3	52.9	49.4	48.2	51.1
Attend daycare equivalent	7.8	7.1	8.6	4.2	6.6	5.2	7.1
Patient's home							
Traditional stoves for cooking	63.1	73.5	63.5	61.1	68.7	62.8	64.0
Running water	24.7	17.5	17.6	24.0	18.7	27.2	23.3
Sewer or septic tank	21.1	18.5	18.5	20.4	22.3	22.5	20.9
Electricity	42.3	34.6	37.4	38.7	40.7	41.9	40.9
Season and year at sample collection							
Fall 2009	4.4	8.1	6.8	9.8	6.0	6.8	5.7
Winter 2009	19.3	25.1	27.5	30.0	23.2	25.7	22.2
Spring 2009	23.0	19.0	17.1	19.3	23.2	19.9	21.8
Fall 2011	24.6	25.1	24.3	21.6	22.6	25.1	24.1
Winter 2011	28.7	22.8	24.3	19.3	25.0	22.5	26.2
Altitude, m, of residence							
1,976–2,321, quartile 1	25.4	26.5	23.9	23.8	24.7	26.7	25.1
2,322–2,644, quartile 2	25.1	23.2	24.3	23.8	25.6	26.7	24.9
2,645–2,861, quartile 3	24.6	19.9	25.7	29.3	24.7	25.7	25.0
2,862–3,803, quartile 4	24.9	30.3	26.1	23.1	25.0	20.9	24.9

*Data are %, except for no. children. ARIs, acute respiratory illnesses; non-ARI, a period outside the pre-ARI, current ARI, and post-ARI periods.
†No. children who contributed samples during each period.

Overall, 36.7% of nasopharyngeal samples were from children who had received ≥2 PCV7 doses and were considered vaccinated. Approximately 5.0% of samples were from children who had received aminopenicillins, cotrimoxazole, chloramphenicol, or furazolidone within the 7 days preceding sample collection.

Quantitative PCR detected *S. pneumoniae* in 68.9% of nasopharyngeal samples; 78.9% of ARI and 65.3% of non-ARI samples were positive (p = 0.06). Unadjusted log-transformed pneumococcal densities varied by ARI periods (online Technical Appendix).

Adjusted analyses showed that densities peaked during ARIs. In post hoc adjusted comparisons, densities were higher during the 1–7 days pre-ARI (p<0.0001), ARI (p<0.0001), 1–7 days post-ARI (p<0.0001), and 8–14 days post-ARI (p = 0.007) than during the non-ARI period (Figure 1).

Of 450 ARI nasopharyngeal samples, 435 (97%) had corresponding nasal swab samples available for identification of respiratory viruses; 299 (68.7%) tested positive for at least 1 virus. Rhinovirus, which was detected in 44.6% (194/435) of samples, was the most common virus (online Technical Appendix). The median log-transformed pneumococcal densities of 299 virus-positive samples and 136 virus-negative samples were not significantly different (4.73 vs 3.94, respectively; adjusted p = 0.06).

During ARI, the median log-transformed pneumococcal densities varied among virus groups: virus-negative (3.94, IQR 0.00–5.67; n = 136), nonrhinovirus (4.49, IQR 3.12–5.48; n = 105), rhinovirus-only (4.91, IQR 3.43–6.23; n = 147), and rhinovirus detected with other viruses samples (5.03, IQR 3.28–6.53; n = 47). In multivariable analyses, the only significant difference was between rhinovirus-only and virus-negative samples (p = 0.02) (Figure 2).

For the colonization patterns assessment, 2,479 (69.3%) nasopharyngeal samples had another sample collected ≤60 days before the current sample; the median time between samples was 28 days. The median log-transformed pneumococcal densities among samples that represented new colonizations (5.14, IQR 3.56–6.24; n = 411), serotype replacement (5.49, IQR 4.53–6.44; n = 322), and serotype persistence (5.79, IQR 4.82–6.47; n = 489) were compared. In multivariable analysis, serotype-replacement (p = 0.005) and serotype-persistence (p = 0.0003) samples had higher density than new colonization samples. The difference between serotype replacement and serotype persistence was not significant (p = 0.2).

Conclusions

Our findings demonstrate a dynamic evolution of pneumococcal densities before, during, and after ARI episodes among young children. We observed a gradual increase in pneumococcal density leading up to an ARI episode, peak density during symptomatic ARI, and a decrease in density post-ARI to levels similar to those in baseline non-ARI periods.

Our observations of higher densities during ARI than non-ARI episodes align with those in studies from Vietnam

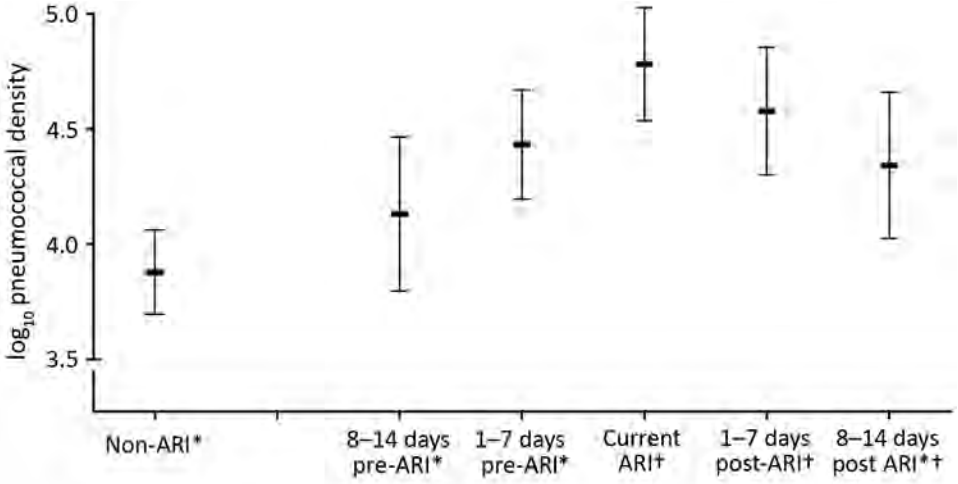

Figure 1. Estimated median pneumococcal densities with 95% CIs (vertical bars) by acute respiratory illness (ARI) period. Estimates derived from a quantile regression model that accounted for sex, age, daycare attendance, electricity, water supply, housing materials, kitchen type, smokers at home, vaccination, antimicrobial drug use, season, and altitude of residence. Asterisk indicates significantly different from ARI samples; dagger indicates significantly different from non-ARI samples.

and South Africa (*14*,*15*) and complement those assessments by illustrating the dynamic evolution of pneumococcal densities and the role of virus co-infections and pneumococcal colonization patterns. Unlike other studies that focused on hospitalized children, our community-based study showed relatively modest variations in nasopharyngeal pneumococcal density.

Rhinovirus detection was associated with increased pneumococcal density during ARI. Although we observed an even higher median pneumococcal density in samples co-infected with rhinovirus and other respiratory viruses, the number of observations was small and statistical power to demonstrate significant differences was limited.

Compared with new colonization in our study, serotype persistence and replacement were associated with higher pneumococcal density. Because many new colonizations might ultimately succumb to host mechanisms and fail to establish stable colonization (*2*), the observed lower densities might reflect a decline of pneumococcal populations as clearance evolved. Nevertheless, although statistically significant, the differences in density were relatively modest, and we cannot establish the precise time of colonization or clearance in our samples.

Our study has several limitations. ARI identification depended on the presence of cough or fever, which are subjective but widely used for routine ARI surveillance (*5*–*7*). Because our study used household-based rather than health facility–based surveillance, severe disease was infrequent, precluding detailed assessments of disease severity. Due to small numbers, we could not study serotype-specific pneumococcal densities. In addition, because the study was conducted in rural communities of Peru, caution is warranted when extrapolating our findings to other settings.

Our findings demonstrated that, among young children, nasopharyngeal pneumococcal density started increasing before the onset of ARI symptoms, peaked during symptomatic ARI, and decreased after symptoms subsided.

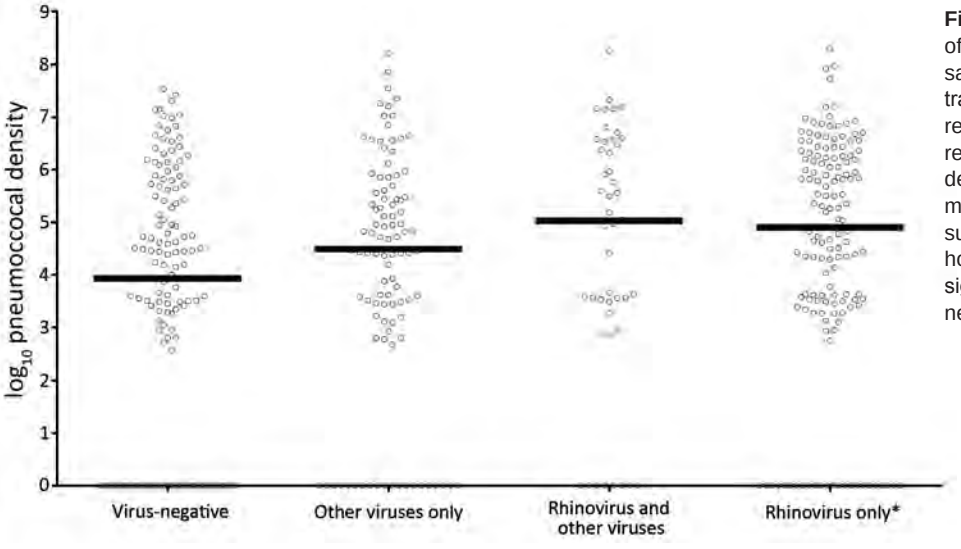

Figure 2. Pneumococcal densities of current acute respiratory illness samples subdivided by reverse transcription PCR detection of respiratory viruses. Each circle represents a single bacterial density measurement. The median for the samples of each subgroup is represented by a gray horizontal line. Asterisk indicates significantly different from virus-negative samples.

Rhinovirus co-infection, serotype persistence, and serotype replacement were associated with increased nasopharyngeal pneumococcal density. Nasopharyngeal pneumococcal density is dynamic surrounding ARI episodes and likely driven by complex virus–bacteria–host interactions.

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Mayaro Virus in Child with Acute Febrile Illness, Haiti, 2015

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Mayaro virus has been associated with small outbreaks in northern South America. We isolated this virus from a child with acute febrile illness in rural Haiti, confirming its role as a cause of mosquitoborne illness in the Caribbean region. The clinical presentation can mimic that of chikungunya, dengue, and Zika virus infections.

Mayaro virus (MAYV; genus *Alphavirus*, family *Togaviridae*) is a single-stranded positive RNA virus that was first isolated in Trinidad in 1954 (*1*) and is one of the viruses that comprise the Semliki Forest virus complex (*2*). Its transmission cycle is thought to occur mainly through mosquito vectors, especially those of genus *Haemagogus* (*3*), but *Aedes* spp. mosquitoes may also be competent vectors (*4*,*5*). The natural reservoirs of MAYV have been reported to be sylvatic vertebrates, mainly nonhuman primates but also birds and reptiles (*3*).

Since May 2014, when chikungunya virus (CHIKV) swept across the island of Hispaniola, researchers at the University of Florida (Gainesville, FL, USA) have studied alphavirus and flavivirus transmission in Haiti in collaboration with the Christianville Foundation. This foundation operates 4 schools in the Gressier/Leogane region of Haiti (≈20 miles west of Port-au-Prince) that serve ≈1,250 students from prekindergarten to grade 12 (*6*). The University of Florida has protocols in place for the collection of diagnostic blood samples from children seen at the school clinic with acute undifferentiated febrile illness (i.e., febrile illness with no localizing signs, such as would be expected with pneumonia, urinary tract infections, etc.).

From May 2014 through February 2015, blood samples were obtained from 177 children who met the criteria for acute undifferentiated febrile illness. The protocol for sample collection was approved by the University of Florida Institutional Review Board and the Haitian National Institutional Review Board. Written parental informed consent was obtained from parents or guardians of all study participants. Plasma samples were screened by reverse transcription PCR (RT-PCR) for CHIKV and dengue virus (DENV); samples that were negative for CHIKV were cultured by using cell lines and conditions as previously described (*7*). Zika virus and enterovirus D68 have been previously isolated from members of this school cohort (*7*,*8*). We report detection of MAYV in a child as part of this screening process.

The Case

On January 8, 2015, an 8-year-old boy was examined at the school clinic because of fever and abdominal pain. His temperature was 100.4°F, lung sounds were clear, and his abdomen was soft and not tender. He had no rash and no conjunctivitis. On the basis of this clinical presentation, the clinic physician empirically diagnosed typhoid and administered co-trimoxazole.

A blood sample was collected, and RNA was extracted from plasma by using RT-PCR primers and the procedure described by Santiago et al. (*9*). The sample was negative for CHIKV but positive for DENV-1 (cycle threshold 26). In Vero E6 cells, which had been inoculated with the specimen, diffuse cytopathic effects typical for DENV-1 developed but at a much later time than for DENV-1–positive plasma specimens from other patients; this finding raised the possibility that DENV-1 had either mutated to reduced replication fitness or that the cells were co-infected with ≥2 incompatible viruses that were interfering with the replication of each other. DENV-1 viral RNA was detected by RT-PCR in the spent cell media of the plasma-inoculated cells but not in the spent media from noninoculated cells (negative control; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-1015-Techapp1.pdf). Furthermore, no CHIKV- or Zika virus–specific amplicons were amplified from the spent media. However, apart from DENV-1, an alphavirus amplicon corresponding in size to that expected for MAYV was detected in viral RNA extracted from infected Vero cells. Sequencing confirmed that the amplicon corresponded to MAYV (GenBank accession no. KX496990).

The MAYV genome from Haiti was aligned with all MAYV strains available in GenBank, and a neighbor-joining

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tree was inferred from pairwise genetic distances estimated with the best fitting nucleotide substitution model (general time reversible plus gamma), as previously described (*7*). The phylogeny clearly shows 2 major and well-supported (bootstrap >90%) clades (Figure). The first clade includes strains sampled over the past 60 years from several South American countries (Peru, Bolivia, Venezuela, Trinidad and Tobago, and French Guiana); the second clade clusters the new Haiti strain with isolates from Brazil sampled during 1955–2014.

Conclusions

Although MAYV was originally isolated in Trinidad in 1954, subsequent reports of illness associated with this virus have tended to be associated with small, occasional outbreaks (30–100 cases) in northern South America (*10*,*11*), within and close to the Amazon forest. Signs and symptoms reported in association with MAYV infection include arthralgias, eye pain, fever, headache, myalgias, rash, and occasionally nausea and vomiting, photophobia, abdominal pain, cough, diarrhea, sore throat, and bleeding gums (*12*). A fatal infection associated with hemorrhagic fever has been reported (*13*). MAYV infections are probably underdiagnosed because of confusion with other mosquitoborne virus infections, especially dengue fever, which is endemic to the same areas. The emergence of CHIKV has further added to this confusion, especially because prolonged arthralgia is reportedly associated with CHIKV and MAYV infections (*3*).

Our findings suggest that MAYV is actively circulating in the Caribbean region and that there may be a link between the strain circulating in Haiti and the strains that have been circulating in Brazil since isolation of the virus in the 1950s. The patient from whom we isolated the organism had fever and abdominal pain but no rash or arthralgia. However, given that the patient was co-infected with DENV-1, it is difficult to separate out symptoms that are specific for MAYV infection. Of note, the clinic physician empirically diagnosed typhoid and treated the patient accordingly. The patient was from a rural/semi-rural area of Haiti, reflecting an ecologic setting that differs greatly from sylvan Amazon regions where many of the other reported MAYV infections have occurred. Little is known about vectors for MAYV in Haiti; potential animal reservoirs, if any, remain to be identified.

The recent emergence of Zika virus infection in the Caribbean region, and its identification as a major cause of birth defects, has brought a great deal of attention to arboviruses. Our findings highlight the multiplicity of arbovirus species in Haiti and the evolutionary relatedness among the viruses in Haiti and those circulating in Brazil, in keeping with prior work on Zika virus (*7*). Findings also underscore the complexity of the interactions among different species and the apparent proclivity for Zika virus/DENV (*7*) and MAYV/DENV co-infections. Although a better understanding of Zika virus infection is clearly needed, careful studies of other arboviruses

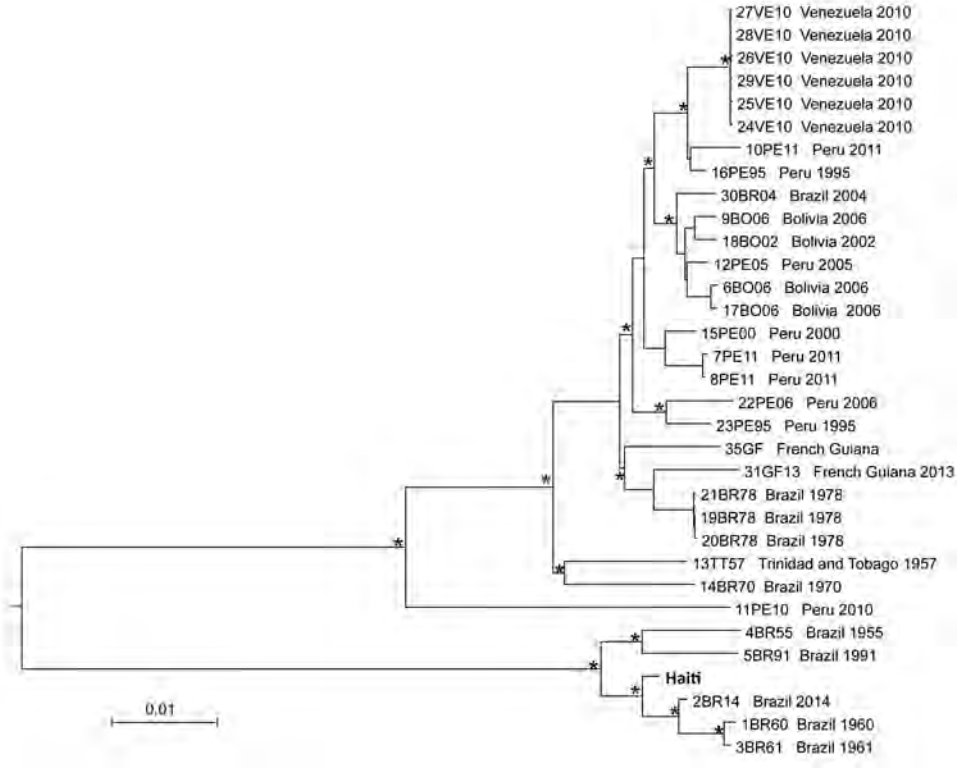

Figure. Neighbor-joining tree of full-genome Mayaro virus sequences. The tree was inferred from pairwise distances estimated with the best fitting nucleotide substitution model (general time reversible plus gamma). The tree includes the isolate from Haiti identified in this study (in boldface) and all full-genome sequences with known country of origin and sampling date downloaded from GenBank. Branches are drawn according to the scale bar at the bottom, which indicates nucleotide substitutions per site. An asterisk along a branch indicates bootstrap support >90% for the subtending clade.

(and their vectors and possible reservoirs) in these same geographic regions are correspondingly needed. We do not know if MAYV has epidemic potential; however, in light of recent observations with CHIKV, DENV, and Zika virus and the potential for transmission of MAYV by *Aedes* and *Haemagogus* spp. mosquitoes, inclusion of MAYV in studies of arbovirus transmission seems to be indicated.

This study was conducted with use of internal funds from the Emerging Pathogens Institute, University of Florida.

Dr. Lednicky is an associate professor of Environmental and Global Health in the College of Public Health and Health Professions at the University of Florida. His research interests are virus isolation and identification from human and environmental sources.

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Co-infections with Chikungunya and Dengue Viruses, Guatemala, 2015

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We screened serum samples referred to the national reference laboratory in Guatemala that were positive for chikungunya or dengue viruses in June 2015. Co-infection with both viruses was detected by reverse transcription PCR in 46 (32%) of 144 samples. Specimens should be tested for both arboviruses to detect co-infections.

Chikungunya virus (CHIKV) and dengue virus (DENV) are arboviruses currently circulating in Southeast Asia, Central and West Africa, the Pacific islands, and the Americas, and their transmission can occur simultaneously (*1*). DENV and CHIKV co-infections have been reported from 13 of 98 countries/territories to which the viruses are endemic and are more likely to occur in areas with high transmission intensity. Co-infection rates reported have ranged from 2% in Gabon to 34% in Nigeria (*1*–*3*).

The Americas are currently experiencing an unprecedented number of DENV infections that coincided with emergence of CHIKV infections. A total of 2.3 million DENV infections and 635,000 CHIKV infections were reported in this region in 2015 (*4*,*5*). However, details of the frequency of co-infection are lacking (*1*), although a recent study involving 173 samples from Nicaragua that were positive for either virus found a co-infection rate of 22% (*6*).

Co-infections might be frequently missed by surveillance systems because Pan American Health Organization (PAHO) diagnostic algorithms indicate that DENV-positive samples do not need to be tested for CHIKV or other viruses and vice versa (*7*). The clinical role of co-infection is somewhat disputed because some (*8*) but not all (*1*,*9*) studies reported an association between co-infection and disease severity for symptoms such as diarrhea (*9*) or hemorrhage (*10*).

Although Guatemala has had constant dengue transmission for ≥20 years (*11*), the first cases of CHIKV infection were reported in September 2014. We report the proportion of patients co-infected with DENV and CHIKV and the association of co-infection with disease severity among patients referred for diagnosis to the National Health Laboratory (NHL) in Guatemala City, Guatemala, in June 2015. Because Zika virus was not introduced until November 2015, we did not test for this virus in this study.

The Study

Serum samples from febrile persons suspected of having arboviral infections are referred to the NHL in Guatemala for surveillance and confirmation purposes. For logistical reasons, most samples are received from 12 of the 22 districts in Guatemala, and most samples originate from Quetzaltenango, Guatemala, and Escuintla Districts, the largest urban centers in Guatemala.

We selected for screening a convenience set of consecutive samples received in June 2015, which is the peak transmission season for DENV, and reported as having positive results by reverse transcription PCR (RT-PCR) for either virus. Clinical data for patients were obtained from surveillance databases to compare patients with monoinfections or coinfections with DENV or CHIKV. All samples were obtained from patients ≤5 days of symptom onset. Samples were obtained after patients provided consent and donated to laboratories for further testing. All samples were anonymous. Results of the study were not used for clinical management or surveillance purposes.

During June 2015, a total of 523 samples were tested for CHIKV at the NHL; 328 (63%) were positive for CHIKV RNA by RT-PCR. A total of 514 samples were also tested by RT-PCR for DENV; 75 (9%) were positive (Figure). Seventy-four of samples reported as positive for DENV RNA and 70 samples reported as positive for CHIKV RNA were available for further screening for the other virus.

Samples selected were consecutive positive samples from the beginning of the month; there were no additional exclusion or inclusion criteria. We included as many samples as possible within the time available. RNA was extracted by using a Viral RNA Mini Kit (QIAGEN, Manchester, UK) and tested by using the US Centers for Disease Control (CDC; (Atlanta, GA, USA) multiplex DENV RT-PCR and the CDC CHIKV 6856F/6981c/6919-FAM RT-PCR (*12*).

Twenty-five (33.8%) of 74 DENV-positive samples were positive for CHIKV, and 21 (30%) of 70 CHIKV-

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

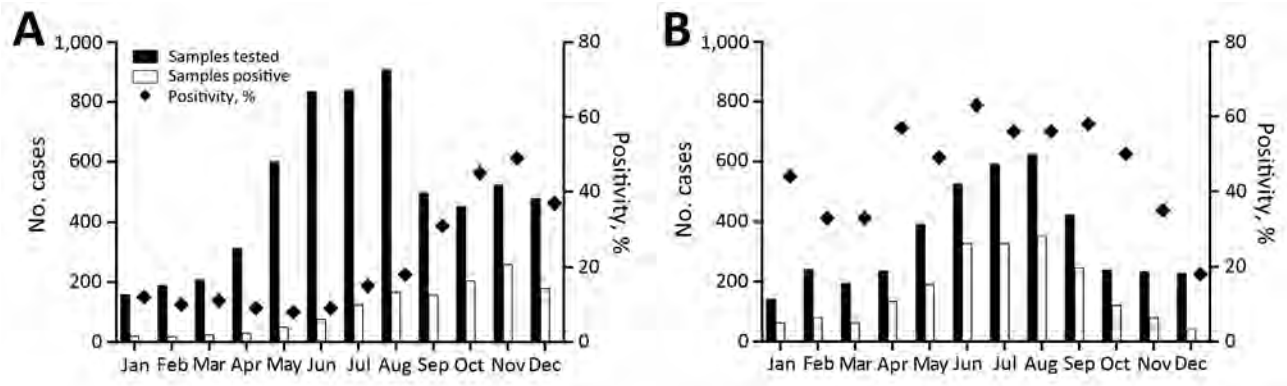

Figure. Positivity of samples tested for A) dengue virus and B) chikungunya virus, Guatemala, 2015.

positive samples were positive for DENV RNA. Co-infection with CHIKV was detected in 4/9 samples containing DENV-1, 40/76 samples containing DENV-2, 0/3 samples containing DENV-3 and 2/7 samples containing DENV-4.

Clinical characteristics of patients with CHIKV and DENV monoinfections and co-infections were similar (Table). Patients co-infected with CHIKV and DENV were more likely to have a rash than those with DENV monoinfections (p = 0.02) and were more likely to be hospitalized than those with CHIKV monoinfections (p = 0.002). All other associations were not statistically significant.

Conclusions

Our findings must be viewed with caution because of the limitations of this study. The study was not powered to analyze clinical associations with a high robustness because of the small number (n = 46) of co-infected samples tested. Also, because samples were selected at a reference laboratory and only symptomatic cases were tested, the prevalence of co-infection might be different in a less selected population.

The PAHO algorithm recommends screening clinical specimens by using consecutive assays and ending screening once a pathogen is identified. CDC in turn recommends simultaneously conducting RT-PCRs for DENV, CHIKV, and Zika virus. Emergence of Zika virus in Latin America has further complicated diagnosis of arboviral infections, and simultaneous co-infections with all 3 arboviruses have been reported (*13*). Although the PAHO approach misses co-infections, the CDC approach is costlier.

Guatemala used the PAHO algorithm because of a shortage of consumable supplies. On further testing, 25 (33.8%) of 74 DENV-positive samples were positive for CHIKV, and 21 (30%) of 70 CHIKV-positive samples were positive for DENV. Because Guatemala reported 49,043 cases of DENV and CHIKV infections in 2015, it is likely that the algorithm missed a large number of co-infections.

Table. Characteristics of patients infected with chikungunya virus, dengue virus, or both, Guatemala, 2015*

Characteristic	CHIKV moninfection	DENV monoinfection	DENV/CHIKV co-infection	p value	
				CHIKV vs. co-infection	DENV vs. co-infection
No. samples tested	49	49	46		
Sex					
M	15/48 (31)	23/45 (51)	21/46 (46)	0.2	0.6
F	33/48 (69)	22/45 (49)	25/46 (54)	0.2	0.6
Age, y					
≤1	2/48 (4.2)	3/45 (7)	5/46 (11)	0.2	0.7
2–16	15/48 (31)	26/45 (58)	19/46 (41)	0.4	0.1
17–30	13/48 (27)	6/45 (13)	9/46 (20)	0.4	0.5
31–60	14/48 (29)	9/45 (20)	10/46 (22)	0.5	1
≥60	4/48 (8.3)	1/45 (2)	3/46 (7)	1	0.6
Sign/symptom					
Hemorrhage	7/49 (14)	11/44 (25)	8/44 (18)	0.8	0.6
Arthralgia/myalgia	41/44 (93)	36/42 (86)	35/45 (77)	0.07	0.4
Fever	43/48 (90)	41/42 (98)	46/46 (100)	0.06	0.5
Rash	31/48 (65)	11/43 (26)	23/44 (52)	0.3	0.02
Nausea	26/46 (57)	31/44 (70)	22/44 (50)	0.7	0.08
Vomiting	13/46 (28)	21/43 (49)	16/45 (36)	0.5	0.3
Diarrhea	3/18 (17)	10/34 (29)	6/25 (24)	0.7	0.8
Headache	40/47 (85)	35/42 (83)	40/44 (91)	0.5	0.3
Hospitalization	0/48 (0)	16/45 (36)	8/46 (17)	0.002	0.09
Death	0/48 (0)	4/45 (9)	2/46 (4)	0.2	0.4

*Values are no. positive/no. tested (%) unless otherwise indicated. CHIKV, chikungunya virus; DENV, dengue virus.

Patients with single and dual infections had similar clinical manifestations in this limited study.

The frequency of arbovirus coinfections in Guatemala is high. Simultaneous screening for DENV, CHIKV, and Zika virus in disease-endemic areas would improve the quality of arboviral surveillance and potentially aid in clinical management of the disease.

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Dr. Edwards is a postdoctoral research associate at the Research Centre for Drugs and Diagnostics in the Liverpool School of Tropical Medicine, Liverpool, UK. His primary research interests are design and implementation of molecular tools for diagnosing arboviral infections and antimicrobial drug resistance.

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Early Mention of the Term Epidemiology

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To the Editor: The excellent article on measures for controlling plague in Alghero, Sardinia, describes the procedures introduced by the Calabrian doctor Quinto Tiberio Angelerio (1532–1617) to combat an outbreak during 1582–1583 (*1*). The authors cite 2 works published by Angelerio relating to these events, Ectypa (1588) (*2*) and Epidemiologìa (1598) (*3*). To say that Epidemiologìa was written only in Spanish is a small error, however, because both works were written in Latin. Ectypa contains an appendix written in Catalan with the measures to take during an epidemic, whereas in Epidemiologìa, this appendix was written in Spanish.

A third and posthumous edition, not cited in the article, was found recently in the Bibliothèque Nationale de France (*4*). Epydem (*5*) was published in Naples in 1651 by Angelerio's nephew. This work was written in Latin and did not contain appendices but did include a brief biography of Angelerio. The terms epidemic (Greek) and plague (Latin) were used ambiguously to refer to "maladies that came from abroad or afflicted us collectively."

The major aspect of Angelerio's texts, especially Epidemiologìa (*3*), is that the term epidemiology was used here for the first time in a "treatise on the plague" in the sense of "how to protect yourself from it when it erupts." The term was adopted by the Spanish physician Joaquín de Villalba (1752–1807) who, citing Angelerio, used it as the title for his work Epidemiología Española (*6*). This treatise gained wide circulation, and the term was espoused by various authors from the beginning of the 19th century onward. Villalba used it to compose a historical chronology of the epidemics in Spain, noting the type of disease and the place and year in which it had occurred; this was an initial approach to the concept of epidemiology, which coincided with the development of medical topographies and statistics applied to infectious diseases.

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Travel-Associated *Vibrio cholerae* O1 El Tor, Russia

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To the Editor: Cholera—a severe, waterborne, virulent enteric infection caused by toxigenic strains of *Vibrio cholera*—frequently causes epidemics in developing countries and sporadic cases or local outbreaks in developed countries. The geographic features of Russia and intensive globalization have established favorable conditions for travel-associated cholera from regions to which it is endemic. During 2005–2012, six such cases occurred in Russia; these cases were related to travel from India. Three of the cases were registered in 2010, three months before the cholera outbreak in Haiti, one of the most extensive outbreaks in recent history (*1*). We genetically analyzed 4 isolates collected in 2010 and 2012 using whole-genome sequencing (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/11/15-1727-Techapp1.pdf) and compared the results with a public data-

base of representative *V. cholerae* strains (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/22/11/15-1727-Techapp2.xlsx) to identify whether these isolates were linked to cholera in Haiti and Nepal.

Isolate RND6878 was isolated on July 7, 2012 from a 28-year-old male Russian citizen. The infection was most likely caused by the patient drinking fountain water and coming into contact with river water while living in Srinagar, India. Isolate RND19191 originated from a 25-year-old female flight attendant operating a Moscow–Delhi–Moscow flight. Her infection was suspected to have occurred in Delhi during June 26–28, 2010, from ingestion of contaminated fruit. Isolate RND19187 was obtained on June 9, 2010, from a 29-year-old woman with severe cholera. Microbiological testing also confirmed the presence of *V. cholerae* in a fecal specimen from her 10-month-

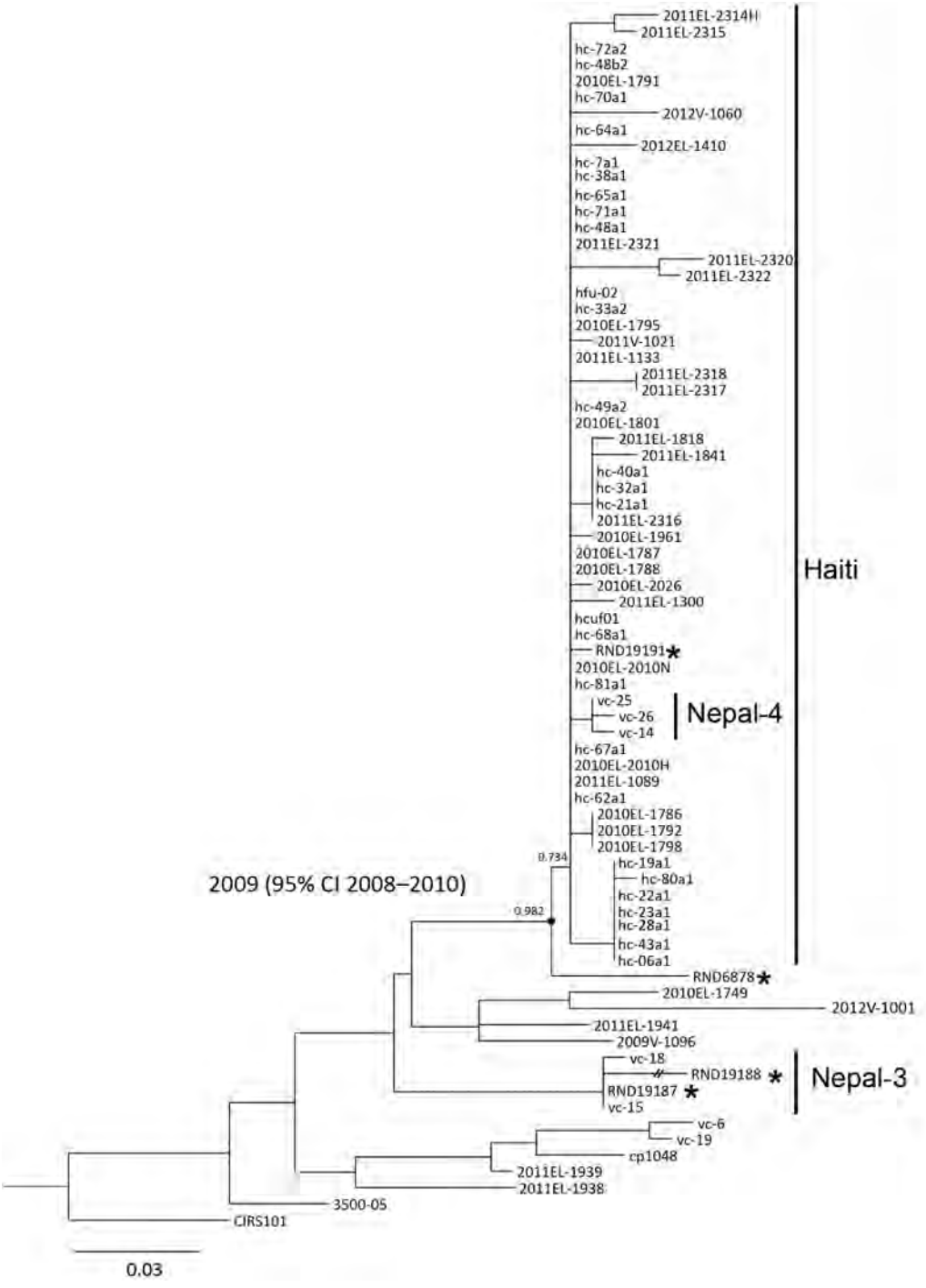

Figure. Maximum-likelihood tree based on an orthologous 193-nt–long high-quality orthologous single-nucleotide polymorphism (hqSNP) matrix of 75 *Vibrio cholerae* O1 El Tor genomes using the general time-reversible model, with estimation of invariant sites. The phylogenetic tree shows clustering of strains isolated from travel-associated cases of *V. cholerae* O1 El Tor (asterisks) with isolates collected worldwide. The CIRS101 genome was used as the outgroup. The numbers above nodes represent a statistical branch supports calculated using PhyML (black circle). The internal node between the RND6878 isolate and the Haiti/Nepal-4 clade is labeled with the estimated most recent common ancestor date, which was predicted using BEAST (http://beast.bio.ed.ac.uk). The date range provided represents the 95% CI of the estimate. Scale bar indicates substitutions per variable site.

old daughter (isolate RND19188), even though she had no distinct symptoms of cholera. The source of infection for the woman and child was unclear but was assumed to be related to eating fruit rinsed in tap water while in the city of Vrindavan in India.

Maximum-likelihood phylogenetic analysis based on high-quality orthologous single-nucleotide polymorphisms (hqSNPs) among 75 *V. cholerae* genomes showed that all the isolates from the travel-associated cases clustered with cholera cases that occurred in 2010. The isolates from the 29-year-old woman and her daughter (RND19187 and RND19188) accurately clustered with isolates from the Nepal-3 clade (*2*) (Figure). Isolate RND19187 exhibited no hqSNP differences from VC-15 and differed from VC-18 by only 1 hqSNP. Isolate RND19191 was located in the Haiti\Nepal-4 clade and differed by only 1 hqSNP (132291G>A), located in the integrative and conjugative element encodes resistance to sulfametoxazol and trimethoprim (SXT-ICE) gene Vch1786-I0110 (Figure). RND19191 and 2010EL-1786 showed high genetic similarity and nucleotide identity to *Vibrio* pathogenic islands (VPI-1, VPI-2), *Vibrio* seventh pandemic islands (VSP-I, VSP-II), and SXT-ICE (online Technical Appendix 3, http://wwwnc.cdc.gov/EID/article/22/11/15-1727-Techapp3.xlsx). Notably, isolate RND19191 has intact SXT-ICE, whereas all 3 Nepal-4 genomes have an SXT-ICE 13-gene deletion (Vch1786_I0089-I0102) (*3*). This genome also carries a *ctxB7* variant of the *ctxB* gene and five 7-mer tandem repeats (TTTTGAT). Finally, isolate RND6878 and the Haiti/Nepal-4 clade formed a well-supported monophyletic group with an estimated most recent common ancestor date of 2009 (95% CI 2008–2010) (Figure). In addition, the RND6878 genome harbored virulence-associated mobile genomic elements similar to 2010EL-1786 and contained a *ctxB7* allele and an intact SXT-ICE, but only four 7-mer tandem repeats (TTTTGAT).

The phylogenetic relatedness between the India and Nepal strains shows that the strains similar to the latter were first found in northern India not far from the frontier of Nepal. Collectively, these data support previously established assumptions that *V. cholerae* strains similar to those from Nepal can be detected in countries other than Nepal and Haiti (*2*). Moreover, isolate RND6878, which is phylogenetically related to the Haiti/Nepal-4 clade and was isolated in 2012, might have a common genetic lineage with the Haiti-like strains found in Nepal and northern India since 2009 (Figure). However, sequencing of representative strains isolated from different geographic regions and varying time frames is needed to reconstruct this lineage.

Remarkably, an India isolate (RND19191) from 3 months before the first cholera cases occurred in Haiti showed higher genetic similarity to the Haiti strain than Nepal isolate VC-25. This finding should be interpreted with caution because this study was limited to the analysis of only 1 isolate, with no epidemiologic context to link the isolate to the Haiti or Nepal outbreaks. Thus, India could not be validated as a primary source of Haiti strains, and the existence of a direct transmission route from India to Haiti that does not involve Nepal could not be substantiated. It is generally accepted on the basis of epidemiologic data and molecular phylogenetics that the Haiti strain was introduced from Nepal (*2*,*4*). Thus, epidemiologic studies remain critical for defining an outbreak's origin, especially when a pathogen is rapidly disseminated by its host. This is true even when modern molecular subtyping methods, such as whole-genome sequencing, offer highly resolved phylogenetic insights.

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Marseillevirus in the Pharynx of a Patient with Neurologic Disorders

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To the Editor: *Marseilleviridae* is a recently described family of giant amebal viruses (*1*). Although Marseillevirus, its founding member, and subsequently discovered representatives were isolated primarily from environmental water, marseilleviruses have been recovered from

humans (*2*,*3*). Senegalvirus, a close Marseillevirus relative, was serendipitously isolated from a healthy man's feces (*2*). Metagenomics then unexpectedly identified Marseillevirus-related sequences in blood of healthy donors (*3*), which was confirmed by PCR, fluorescence in situ hybridization, and serologic testing. Further PCR and serologic studies suggested substantial exposure of humans to marseilleviruses (*4*,*5*).

During assessment of Marseillevirus serology at Institut Hospitalo-Universitaire (IHU) Méditerranée Infection (Marseille, France), we found serum from an 11-month-old boy with lymphadenitis that exhibited a high Marseillevirus IgG titer; the virus was detected by PCR in serum and by fluorescence in situ hybridization and immunohistochemistry in the lymph node (*6*). Subsequently, the hospital implemented systematic Marseillevirus PCR in cases of gastroenteritis or pharyngitis, which led to detection of Marseillevirus DNA in pharyngeal and blood samples from a 20-year-old man. He had sought treatment in November 2013 for a 2-day febrile gastroenteritis that was treated with amoxicillin and acetaminophen; however, several hours later, his fever reached 40°C, and intense headache and stiff neck led to his hospitalization. No adenopathy was palpable. Laboratory analyses showed elevated C-reactive protein (194 mg/L), elevated bilirubin (44 μmol/L), low platelet count (120 G/L), and elevated polynuclear cell count (9 G/L). Cerebrospinal fluid (CSF) was clear and acellular; the CSF to blood glucose ratio was normal, but the protein level was elevated (0.73 g/L).

We tested CSF and feces by culture, PCR, or immunoenzyme assay for common infectious agents of meningitis, encephalitis, and gastroenteritis, including enteroviruses, herpesviruses, *Neisseria meningitidis*, *Streptococcus pneumoniae*, caliciviruses, rotavirus, adenoviruses, and *Clostridium difficile*. All results were negative. Feces were also negative for Marseillevirus DNA. Serologic test results were negative for HIV and cytomegalovirus. However, a pharyngeal sample was positive for Marseillevirus in routine diagnosis using the PCR system ORF152 (*3*); sequencing showed 100% nucleotide identity with the Marseillevirus genome (http://www.mediterranee-infection.com/article.php?laref=495&titre=marseillevirus-pharynx). Retrospective testing of CSF for Marseillevirus DNA yielded negative results.

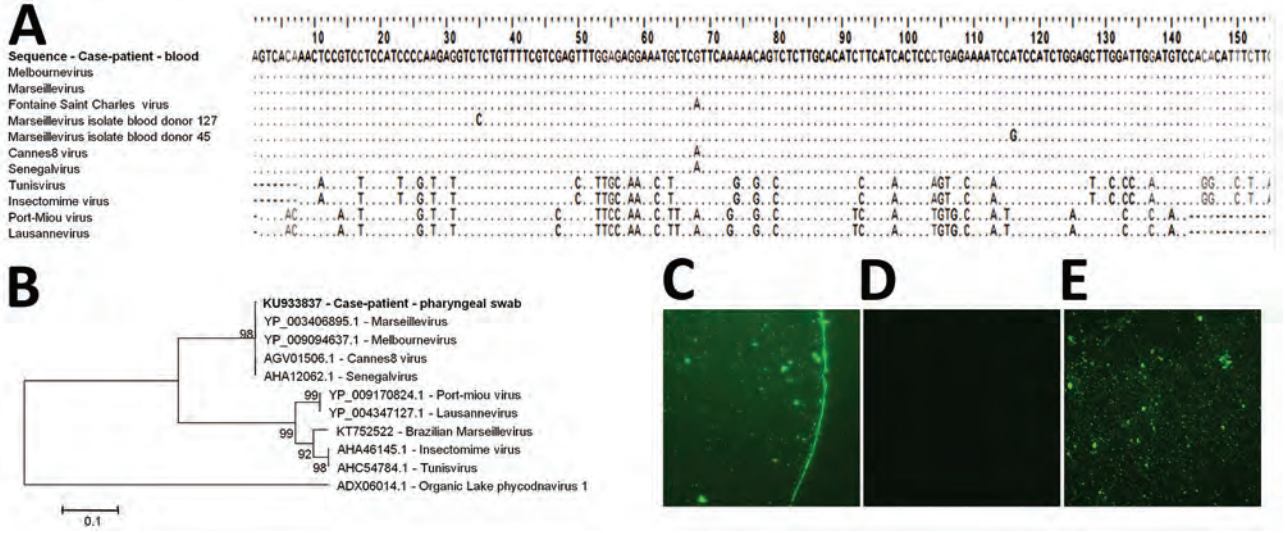

Figure. Marseillevirus sequences and serologic analysis for a 20-year-old man in Marseille, France, who initially sought treatment in November 2013 for a 2-day febrile gastroenteritis. A) Alignment of the sequence obtained in November 2014 from the blood of the case-patient with sequences from Marseillevirus and other related viruses. GenBank accession nos.: Marseillevirus, GU071086.1; Melbournevirus, KM275475.1; Fontaine Saint-Charles virus, KF582416.1; Senegalvirus, KF582412.1; Marseillevirus isolate blood donor 127, KF233993.1; Marseillevirus isolate blood donor 45, KF233992.1; Cannes 8 virus, KF261120.1; Tunisvirus, KF483846.1; Insectomime virus, KF527888.1; Port-Miou virus, KT428292.1; Lausannevirus, HQ113105.1. B) Phylogenetic reconstruction based on an amino acid alignment of the translated sequence obtained in November 2014 from a pharyngeal swab specimen from the case-patient (GenBank accession no. KU933837; indicated in bold) and homologous sequences from Marseilleviruses. Sequence from Organic Lake phycodnavirus 1 was used as an outgroup. The evolutionary history was inferred in MEGA6 software (http://www.megasoftware.net/) by the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The evolutionary distances were computed by using the Kimura 2-parameter method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Scale bar indicates nucleotide substitutions per site. C–E) Marseillevirus IgG detection by immunofluorescence in a serum sample from the case-patient. C) Serum sample from the case-patient at dilution 1:50; D) negative control (serum sample from a rabbit not exposed to Marseillevirus) at dilution 1:50; E) positive control (serum sample from a rabbit exposed to Marseillevirus) at dilution 1:50.

The patient recovered after receiving ciprofloxacin and was discharged after 72 hours. One year later, he exhibited vertigo and a 7-kg weight loss over 4 months, although no additional episode of gastroenteritis or fever had occurred. He reported a slight impairment of cognitive functions (i.e., memory, attention), but clinical examination and cerebral positron emission and computed tomographic scan results were normal. Vertigo was attributed to vestibular deficiency and treated with betahistine. CSF testing still showed an isolated high protein level (0.68 G/L) without hypercellularity but negative results for bacteria and viruses. However, Marseillevirus DNA was detected by 2 PCR systems that target a helicase gene: blood testing using the ORF152 PCR and pharyngeal swab specimen testing using the HelF6R6 PCR system (primers: 5′-GAGGATGTACGGAAGGTC-3′ [forward]; 5′-GTCCTTCACCTGTTCTTCC-3′ [reverse]). Sequence identities were 99% and 100%, respectively, with Marseillevirus (GenBank accession no. KU933837; http://www.mediterranee-infection.com/article.php?laref=495&titre=marseillevirus-pharynx). In addition, Marseillevirus IgG was detected by indirect immunofluorescence assay; serum samples that were negative or positive for Marseillevirus IgG in previous experiments were used as negative and positive controls, respectively (*4*). After 2 months, the patient's general condition had improved, and neurocognitive and vestibular symptoms resolved.

Marseillevirus presence in the case-patient is indisputable, as supported by specific molecular detection and sequencing of 2 sequential pharyngeal swab specimens and of blood, with concurrent IgG positivity (Figure, http://wwwnc.cdc.gov/EID/article/22/11/16-0189-F1.htm). The presence of the virus in 2 samples collected at a 1-year interval suggests chronic carriage.

Several reports showed that giant viruses may be common in humans, but association with pathogenicity was documented differently, depending on the viruses. Thus, many serologic, virologic, and clinical findings argued for a causative role of mimiviruses in pneumonia, which was strengthened in 2013 by the culture isolation of mimiviruses from 2 pneumonia patients (*7*,*8*). In addition, *Acanthocystis turfacea* chlorella virus-1, a phycodnavirus that infects algae, was detected by metagenomics in human oropharyngeal samples, and this association was further confirmed by PCR in 92 samples, with a prevalence of 44% (*9*). Unexpectedly, DNA detection of this virus was associated with a decrease in cognitive performance in these patients; such cognitive disorders were also observed in mice inoculated with this virus.

The presence of Marseillevirus in healthy humans was described by high-throughput sequencing and subsequent culture isolation from feces (*2*), then by metagenomics in blood donors' blood (*3*). Unexpectedly, seroprevalence studies conducted in the general population showed high (up to 13%) positivity rates of Marseillevirus IgG, which suggested a common human exposure (*3*–*5*). Presence of Marseillevirus in a symptomatic human was reported in 2013 in an 11-month-old boy with lymphadenitis and possibly corresponded to a primary infection (*6*). Marseillevirus was then detected in the lymph node of a 30-year-old woman with Hodgkin's lymphoma (*10*). In the case we describe, Marseillevirus was detected in the human oropharynx in association with cognitive impairment and possible chronic carriage with concurrent persistence of clinical signs. The involvement of Marseillevirus in these symptoms cannot be established here, but these findings warrant further investigation.

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Lack of Mimivirus Detection in Patients with Respiratory Disease, China

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To the Editor: Mimivirus (*Acanthamoeba polyphaga* mimivirus), which was initially identified as a gram-positive parasitic bacterium, is the first member of the virus family Mimiviridae (*1*,*2*). Although mimivirus was initially isolated in the context of a human pneumonia outbreak, its pathogenicity to humans remains uncertain.

Mimivirus DNA was detected in a bronchoalveolar lavage specimen from a 60-year-old comatose patient with hospital-acquired pneumonia (*3*) and isolated from a 72-year-old woman with pneumonia (*4*). However, many studies that used a PCR detection method reported that mimivirus is a negligible cause of respiratory infections in humans (*5*–*9*). Furthermore, serologic evidence for this new virus was suggested as being caused by cross-reactivity to *Francisella tularensis* (*10*). To estimate the prevalence of mimivirus and its potential role in causing respiratory infections, we conducted a retrospective study by screening 2 cohorts of patients in China for this virus.

Cohort 1 was composed of 2,304 children with acute lower respiratory tract infections who were hospitalized in Children's Hospital of Chongqing Medical University (Chongqing, China), from whom nasopharyngeal aspirates were obtained during June 2011–July 2015. Patients ranged in age from 1 month to 16 years (median 15.0 months), and 2,034 (88.3%) had pneumonia.

Cohort 2 was composed of 768 children (43 hospitalized patients and 725 outpatients) and 624 adults (440 hospitalized patients and 184 outpatients) with acute lower or upper respiratory tract infections at 307 Hospital (Beijing, China), from whom throat swab specimens were obtained during January 2013–December 2015. Children ranged in age from 1 to 192 months (median 48.0 months), and 62 (8.1%) had pneumonia. Adults ranged in age from 17 to 95 years (median 52.9 years) and 401 (55.3%) had pneumonia.

Virus nucleic acids were extracted by using a QIAamp MinElute Virus Spin Kit (QIAGEN, Hilden, Germany). Mimivirus was detected by real-time reverse transcription PCR (RT-PCR) as described (*4*).

Of the 3,696 patients, only 1, a 6-month-old boy, had a positive real-time RT-PCR result (cycle threshold [C_t] 31) for mimivirus. This positive result was verified by using 2 other RT-PCRs specific for the helicase and thiol oxidoreductase genes and a nested RT-PCR (*3*,*6*). The PCR specific for thiol oxidoreductase showed a positive result (C_t 36). An independent retesting that was performed on this positive sample in the laboratory affiliated to PLA 307 hospital was also positive. However, we could not amplify mimivirus sequences by using nested RT-PCR.

Samples from 3,696 patients were simultaneously screened for influenza virus; respiratory syncytial virus; parainfluenza virus types 1, 2, 3, and 4; metapneumovirus; human rhinovirus; human adenovirus; coronavirus, and human bocavirus by using PCR. All 11 of these respiratory viruses were detected at prevalences ranging from 0.35% to 21.98% (Table). Co-infections with 2 other respiratory pathogens, parainfluenza virus type 3 (C_t 30) and bocavirus (C_t 29), were detected in the mimivirus-positive patient. A sputum smear from this patient was negative for *Mycobacteria tuberculosis*, and other bacteria were not detected.

The mimivirus-positive patient had neonatal respiratory distress syndrome at birth and had been hospitalized 6 times because of reoccurring respiratory tract infections before the episode during which mimivirus was detected. On July 24, 2013, he had a sudden onset of a slight fever (37.8°C), cough, and diarrhea (6–7 bowel movements/day). After he was given supportive treatment, diarrhea improved, while fever and cough were aggravated; onset of larynx asthma was also recorded. He was admitted to the Respiratory Department of Children's Hospital of Chongqing Medical University on July 28. Physical examination at admission showed lip cyanosis and 3 depression signs. Pulmonary computed tomography after hospitalization showed inflammation of the left upper lung and right lung. Laboratory investigations at admission showed a platelet count of 484 × 10^9/L, an erythrocyte count of

Table. Prevalence of 11 other viruses in 3,696 patients with respiratory diseases tested for infection with mimivirus, China

Virus	No. (%) patients
Influenza virus	307 (8.31)
Respiratory syncytial virus	812 (21.97)
Parainfluenza virus type 1	275 (7.44)
Parainfluenza virus type 2	13 (0.35)
Parainfluenza virus type 3	253 (6.85)
Parainfluenza virus type 4	38 (1.03)
Metapneumovirus	85 (2.30)
Human rhinovirus	543 (14.69)
Human adenoviruses	123 (3.33)
Coronavirus	79 (2.14)
Human bocavirus	179 (4.84)

[1]These authors contributed equally to this article.

4.28 × 10^9 cells/L, a hemoglobin level of 86 g/L, and a leukocyte count of 8.28 × 10^9 cells/L with 17% neutrophils and 78% lymphocytes.

The patient was given symptomatic supportive treatment, methylprednisolone sodium succinate, and 5 g of γ-globulin. He was not given any antimicrobial drugs. On August 4, he was discharged from hospital after symptoms had resolved.

In conclusion, our results confirm that mimivirus is an unlikely cause of human respiratory infections in China, as reported in other countries (*5*–*9*). Sporadic detection of mimivirus in 1 child who was born with a compromised respiratory system and had numerous hospitalizations was most likely caused by colonization of the child with this virus during numerous hospitalizations and critical care stays. In addition, parainfluenza virus 3 and bocavirus were detected in the mimivirus-positive child. Because parainfluenza virus 3 causes pneumonia and bocavirus causes infections with respiratory symptoms, particularly in children of his age, these 2 pathogens probably caused the illness in the child.

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Severe Pneumonia Associated with Adenovirus Type 55 Infection, France, 2014

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To the Editor: Human adenoviruses (HAdVs) comprise 70 recognized genotypes (as of February 15, 2016; http://hadvwg.gmu.edu/) and are frequently associated with mild and acute upper respiratory tract infections, depending on virus type and host immune status (*1*). HAdV type 55 (HAdV-55) has recently reemerged as a highly virulent pathogen, causing severe and sometimes fatal pneumonia among immunocompetent adults, particularly in Asia (*2*–*4*). Formerly known as HAdV-11a, HAdV-55 is a genotype resulting from recombination between HAdV-11 and HAdV-14 (*5*). We report 2 cases of severe pneumonia associated with HAdV-55 infection in France.

In November 2014, two immunocompetent women, 71 (patient A) and 36 (patient B) years of age, sought care 4 days apart at the emergency unit of the University

Hospital of Clermont-Ferrand, France, for an influenza-like syndrome characterized by fever, cough, and dyspnea. Laboratory investigations at admission revealed thrombocytopenia (98 and 88 × 10^9 thrombocytes/L for patients A and B, respectively; reference range 150–450 × 10^9 thrombocytes/L) and elevated C-reactive protein concentrations (71.6 and 45.8 mg/L, respectively; reference range <3 mg/L). Chest radiographs and thoracic tomodensitometry images showed acute left lobar pneumonia in each patient. Therapy with intravenous antimicrobial drugs (cefepime and levofloxacin) and oxygen was initiated. Patient A was transferred to the intensive care unit 4 days after admission because of unimproved respiratory function; patient B was transferred 5 days after admission because of acute respiratory distress syndrome.

Results for all bacteriologic analyses were negative (blood cultures, bronchoalveolar lavage fluid cultures, PCR for *Mycobacterium tuberculosis* [Xpert MTB/RIF; Cepheid, Sunnyvale, CA, USA] of bronchoalveolar lavage fluid, and urinary antigen testing [BinaxNOW *Legionella* and *Streptococcus pneumoniae*; Alere, Scarborough, ME, USA]). No specific antibodies were detected against *Chlamydia pneumoniae* (Anti-*C. pneumoniae*; Euroimmun, Lübeck, Germany) and *Mycoplasma pneumoniae* (Platelia *M. pneumoniae* IgM; Bio-Rad, Hercules, CA, USA). For each patient, HAdV was the only pathogen detected in nasopharyngeal secretions collected at admission and in bronchoalveolar lavage fluids collected while in the intensive care unit (molecular multiplex assay [FilmArray Respiratory Panel; bioMérieux, Durham, NC, USA]). HAdV DNA was also detected in whole blood (Adenovirus R-gene; bioMérieux); viral load was 280,524 copies/mL for patient A 9 days after hospital admission and 951,146 copies/mL for patient B 4 days after admission. During hospitalization, transient hepatitis developed in each patient; serum aspartate aminotransferase levels were elevated up to 6–10 times reference range, and leukocyte counts indicated leukopenia (2.17 and 1.28 × 10^9/L for patients A and B, respectively; reference range 4–10 × 10^9/L). Patient B had acute pancreatitis and hyperlipasemia (lipase 1,697 UI/L; reference range 73–393 UI/L). Healthy respiratory function was restored for both patients, who were discharged 26 (patient A) and 19 (patient B) days after admission.

A partial region of the hexon gene was amplified and sequenced from DNA extracts of respiratory and blood

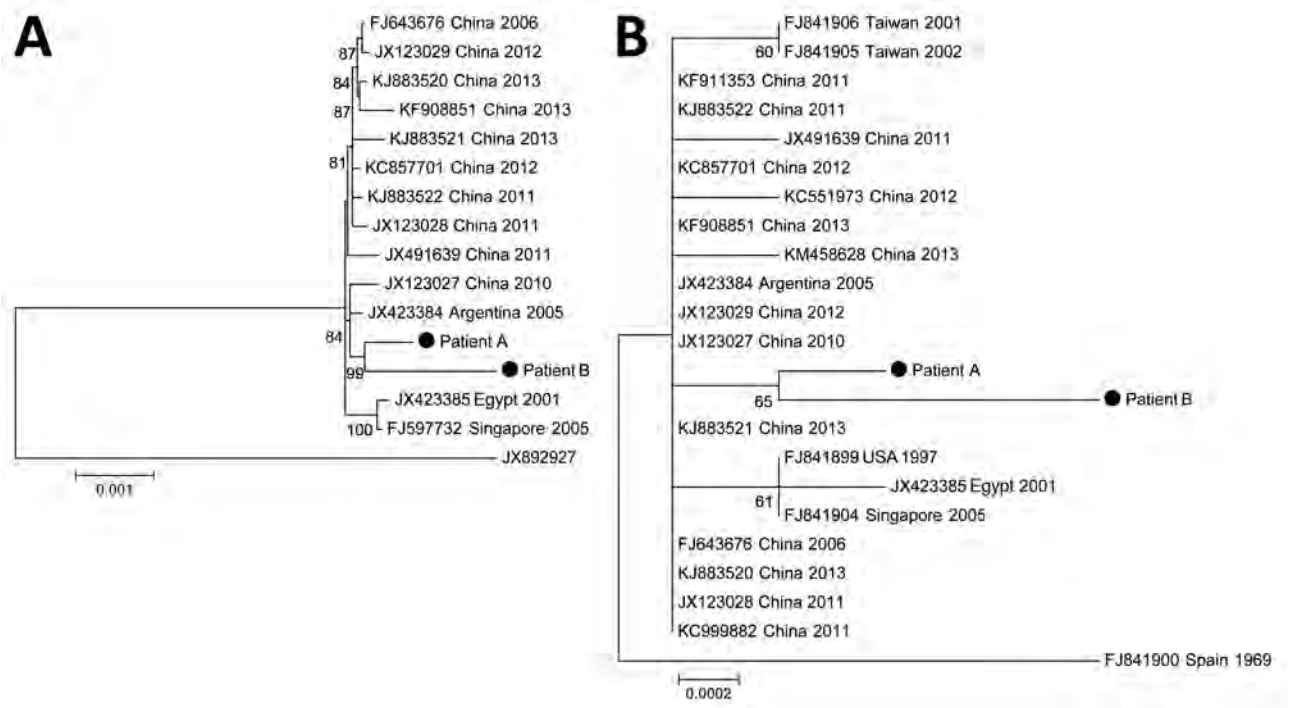

Figure. Phylogeny of 13 complete-genome sequences of human adenovirus type 55 (A) and of 21 sequences of hexon genes (B). The complete genome tree (A) is rooted to a human adenovirus type 14 isolate (GenBank accession no. JX892927). The strains from patients A and B (immunocompetent women with human adenovirus infection) reported in this study are indicated. The phylogenetic tree was calculated by using the maximum-likelihood method in MEGA6 (http://www.megasoftware.net). The best algorithm was chosen by the criterion score of the Bayesian information criteria. The statistical robustness of branches was estimated by 1,000 bootstraps. Only bootstrap values >70% are indicated. The tree is drawn to scale; branch lengths are measured in number of substitutions per site (scale bar). All positions containing gaps and missing data were eliminated. The sequence of the hexon gene from patient B was partially complete (2,821/2,841, 99.3%).

samples, as previously described (*6*). Phylogenetic analysis with strains representing all HAdV genotypes identified the viruses as HAdV-55 (data not shown). We performed complete-genome sequencing, which is now recommended for confirmation of HAdV type, by using next-generation sequencing from blood samples (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-0728-Techapp1.pdf). Genome coverage (34,755 nt) was 99.1% (patient A) and 96.1% (patient B). Phylogenetic analysis showed that the sequences of the isolates from the 2 patients clustered together (bootstrap 99%) and were genetically more closely related to the sequences of the CQ-814 strain isolated in China in 2010 and the strain from Argentina (GenBank accession no. JX423384) (Figure, panel A). To investigate genetic relationships with more strains from distant geographic areas, we performed phylogenetic analyses with all available sequences of the hexon gene of HAdV-55 strains. However, because diversity of this gene between strains was low, we could not determine the geographic origin of the strains from France, which were genetically distant from the strain isolated in Spain in 1969 (Figure, panel B).

Over the past 10 years, reports of HAdV-55 have been increasing in Asia during outbreaks of respiratory diseases that in some cases led to severe pneumonia and deaths in immunocompetent adults and children (*2*–*4*,*7*,*8*). Of the 969 cases of community-acquired pneumonia in adults, 48 (5%) were associated with HAdVs; HAdV-55 was identified in 21 (43.8%) of these patients (*7*). For the 2 patients we report, clinical features were similar to those described elsewhere (*4*,*8*). Neither patient had traveled recently, and the 2 patients had not had contact with each other. Analysis of complete genomic sequences showed that the viruses infecting the patients were distinguishable from strains previously isolated in other countries. HAdV-55 could thus have been circulating in France for several years. Since its first detection in Spain in 1969 (*9*), HAdV-55 has been reported only 1 time in Europe, in Germany in 2004 (*10*).

Because most HAdV infections are asymptomatic and respiratory virus screening in routine practice does not systematically include HAdV detection, the true prevalence and clinical effect of HAdV-55 infection has probably been underestimated. The involvement of virus of this genotype in severe pneumonia emphasizes the need to reinforce HAdV surveillance by including HAdV genome detection and genotyping (if positive) in the documentation of severe respiratory infections.

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Spotted Fever Group *Rickettsia* in the Pampa Biome, Brazil, 2015–2016

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To the Editor: Several cases of tickborne rickettsiosis have been reported in South America in recent years (*1*,*2*). In Brazil, 2 spotted fever group (SFG) *Rickettsia* species, *R. rickettsii* and *Rickettsia* sp. strain Atlantic Rainforest, have been identified as causes of human disease. Rio Grande do Sul is the southernmost state of Brazil and the only part of the country located in the Pampa biome. Despite confirmed cases of human spotted fever in that state since 2005, little information is available regarding *Rickettsia* species. We report an eco-epidemiologic investigation of *R. parkeri* in *Amblyomma tigrinum* ticks on dogs from a household (and neighborhood) where a case of human spotted fever was diagnosed.

In 2011, a 44-year-old woman from the municipality of Rosário do Sul in Rio Grande do Sul (Figure) sought medical attention at the municipal health center. On examination, she had a cutaneous eschar, fever, malaise, lymphadenopathy, myalgia, headache, and rash; she reported receiving a tick bite a few days before. The diagnosis of spotted fever was confirmed at the Brazil National Reference Laboratory (Instituto Adolfo Lutz) in São Paulo after paired serologic testing (21-day interval) against *R. rickettsii* (first antibody titration 1:64; second 1:256) because the official diagnosis of human spotted fever in Brazil is based on serologic testing using only the *R. rickettsii* antigen. After doxycycline treatment (2×/d for 7 d), the patient had a complete recovery.

During September 2015–March 2016, we performed tick collections at the patient's house, in the surrounding neighborhood (i.e., 7 other homes located within a radius of 1 km), and in the venues used by the patient for hunting. The patient and 11 relatives lived in a small house under extremely poor economic and sanitary conditions. They survived exclusively by government social programs and illegal hunting. The patient usually hunted several wild animals, including capybaras (*Hydrochoerus hydrochaeris*), armadillos (*Dasypus* spp.), the pampas fox (*Lycalopex gymnocercus*), and the crab-eating fox (*Cerdocyon thous*). We collected 251 *Amblyomma dubitatum* ticks from capybaras carcasses (74 adults and 173 nymphs) and from vegetation by dragging/flagging (2 adults and 2 nymphs); 60 *Amblyomma* sp. larvae were obtained by dragging/flagging. We obtained 47 adult *A. tigrinum* ticks and 2 adult *Rhipicephalus sanguineus* ticks from 14 owned free-roaming dogs with permanent access to wild habitats. We obtained ticks from the patient's 8 dogs and from 6 other dogs from among 3 other households.

We taxonomically identified the ticks by morphology (*3*), processed whole ticks individually to obtain genomic DNA (*4*), and used PCR amplication of the rickettsial citrate synthase gene (*gltA*) as a screening procedure (*5*). We further tested tick samples that were positive for *Rickettsia* spp. by *gltA* PCR by using a second PCR, which amplified a fragment of the *ompA* gene from SFG *Rickettsia* spp. (*6*). We then tested positive samples a third time by using PCR amplification of a *htrA* gene fragment (*5*,*7*). PCR products of the *ompA* and *htrA* genes were purified and sequenced and then compared with sequences available in GenBank. All samples of *A. dubitatum* ticks were negative. Of the ticks collected from dogs, 13 *A. tigrinum* (28%) and 1 *R.*

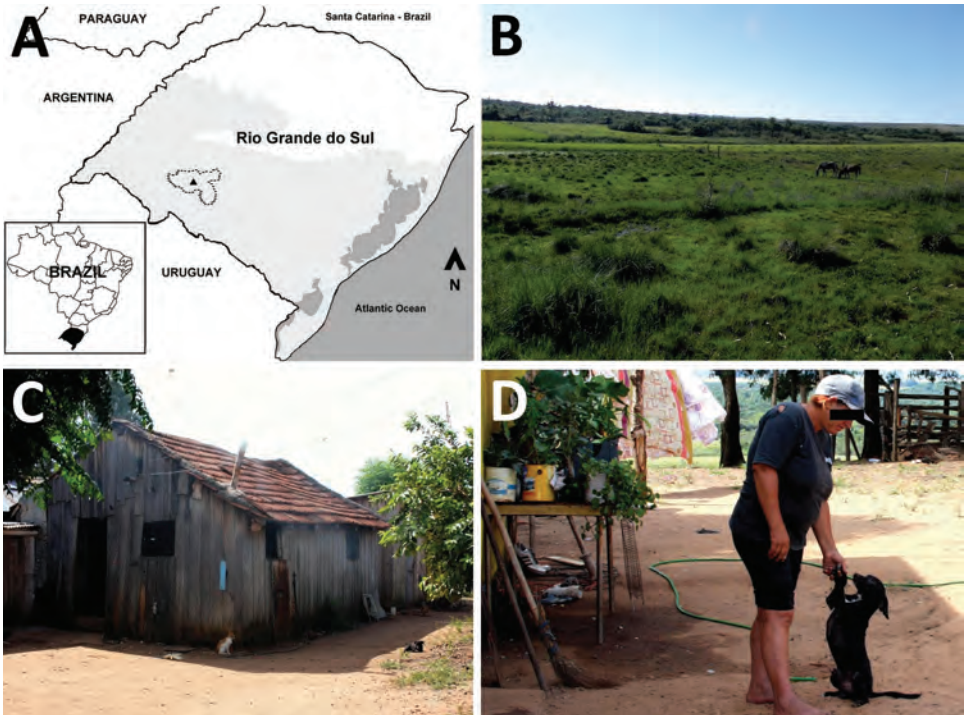

Figure. Setting for investigation of human infection with spotted fever group *Rickettsia* in the Pampa biome, Brazil, 2015–2016. A) Rio Grande do Sul state, Brazil, and neighboring countries. Light gray shading indicates the Pampa biome; dark gray shading indicates bodies of water; dotted line indicates Rosário do Sul municipality; black triangle indicates patient's household. B) Typical view of Pampa vegetation (and area used for hunting by patient). C) Patient's home. D) The patient and 1 of her dogs in the patient's backyard.

sanguineus (1/2) were positive in all PCR analyses (*gltA*, *ompA, and htrA*); 11 of these ticks were from the patient's dogs. In all properties where ticks were collected, at least 1 was PCR positive. Thus, we detected *R. parkeri* in half (4/8) of investigated households.

All the sequences generated for the *ompA* and *htrA* genes showed 100% identity to sequences from the *Rickettsia parkeri* strain Portsmouth (GenBank accession no. CP003341.1). We deposited into GenBank the sequences of the *ompA* gene (KX196265) and *htrA* gene (KX196266) from samples analyzed in this study. The *ompA* sequence we obtained for *R. parkeri* showed 98% identity with *Rickettsia* sp. strain Atlantic Rainforest (GenBank accession no. GQ855237.1).

Although *Rickettsia* sp. strain Atlantic Rainforest had previously been considered the only SFG *Rickettsia* in southern Brazil, we demonstrate here the presence of *R. parkeri* in Rio Grande do Sul in the Pampa biome. We detected *R. parkeri* infection in *A. tigrinum* ticks collected at the probable site of infection (the patient's home) of a confirmed case of human spotted fever. Considering the *A. tigrinum* tick abundance in southern Brazil and its remarkable ability to parasitize domestic and wild animals (*8*), in addition to the high *R. parkeri* infection rate observed (28%), further epidemiologic studies are needed to address the role of *A. tigrinum* ticks as vector of spotted fever in the Pampa biome. Finally, our results show that, in addition to *R. rickettsii* and *Rickettsia* sp. strain Atlantic Rainforest, *R. parkeri* occurs and might be associated with cases of spotted fever in Brazil. Additional surveys are needed to assess the infection prevalence of *R. parkeri* in *A. tigrinum* ticks in other areas of Pampa and in other regions of Brazil.

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Shigella flexneri with Ciprofloxacin Resistance and Reduced Azithromycin Susceptibility, Canada, 2015

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To the Editor: In 2015, a locally acquired, multidrug-resistant *Shigella flexneri* infection was identified in Montreal, Quebec, Canada, in an HIV-positive man who had sex with men (MSM). In September, the 53-year-old man consulted his physician at an outpatient clinic after experiencing abdominal pain, fatigue, and diarrhea without blood in stools or fever. The week before the symptom onset, although he had not traveled, he had unprotected oral and anal sexual contact in a Montreal bathhouse with a man visiting Canada from an unknown country. The patient did not work in daycare centers or healthcare facilities, and he was not a food handler. He did not have sex during illness.

He was HIV positive and was receiving antiretroviral treatment; recent CD4 cell count was 480 × 10^6/L, and HIV viral load was <40 copies/mL. *S. flexneri* was isolated from his culture of a fecal sample, and *Neisseria gonorrhoeae*, diagnosed by PCR, was found in a throat specimen. The patient did not have a medical record of other past sexually transmitted infections.

Phenotypic identification of the *S. flexneri* was confirmed at Laboratoire de Santé Publique du Québec (*1*). Serologic identification, pulsed-field gel electrophoresis (PFGE), and antimicrobial susceptibility testing were performed as described (*1*). This *S. flexneri,* serotype 2a pulsovar 21 (a new PFGE combination pattern in the province of Quebec), was resistant to ampicillin, trimethoprim/sulfamethoxazole (TMP/SMX), nalidixic acid, ciprofloxacin, tetracycline, and chloramphenicol. The isolate was also nonsusceptible to azithromycin and amoxicillin/-clavulanic acid, and susceptible to ceftriaxone, cefixime, ertapenem, and gentamicin (Table). The *mph*(A) gene, which codes for the macrolide 2′-phosphotransferase, tested positive by PCR (*1*).

On day 10 of diarrhea, the patient was treated with ceftriaxone, 250-mg dose, intramuscularly, followed by cefixime, 800 mg/day, for 5 days; the patient's condition showed progressive improvement. Two control cultures of fecal specimens were negative 7 and 16 days, respectively, after completion of a regimen of cefixime.

Shigella spp. are transmitted from person-to-person through low inocula of the bacteria, directly or indirectly (*1*,*3*–*5*). In MSM, *Shigella* spp. are mostly transmitted sexually, with clusters documented in many countries (*1*,*3*–*5*). In Canada and the United States, *Shigella* isolates have high levels of resistance to ampicillin and TMP/SMX (*1*,*3*–*6*). In adult patients, when antimicrobial drug treatment is indicated, ciprofloxacin and azithromycin are, respectively, the agents of first and second choices for treating *Shigella* infections (*1*,*3*–*5*).

In the United States, *Shigella* spp. resistant to at least nalidixic acid and azithromycin have been found in surveillance isolates: 1/293 in 2011 (*Shigella* spp.), 1/353 in 2012 (*S. sonnei*), and 1 of 344 in 2013 (*S. flexneri*) (*6*). In Illinois and Montana, during September 2014–April 2015, 3 of 5 patients infected with multidrug-resistant *S. sonnei* (resistant to ampicillin, TMP/SMX, ciprofloxacin, and nalidixic acid and nonsusceptible to azithromycin), identified themselves as MSM, and 2 of these patients had diarrhea for >14 days (*3*).

Clinical treatment failure has been reported in patients infected with azithromycin-nonsusceptible *Shigella* isolates treated with this drug (*7*,*8*), including 1 of our patients (unpub. data). In a previous study, the *mph*(A) gene was acquired by 4 of 7 locally acquired *Shigella* pulse types infecting MSM. This raises concern that reduced *Shigella* susceptibility to azithromycin is developing rapidly (*1*). Azithromycin epidemiologic cutoff values for wild- and non–wild-types of *S. flexneri* and *S. sonnei* are newly reported by CLSI (*8*). In recent years, ciprofloxacin-resistant and/or azithromycin-nonsusceptible *Shigella* spp. acquired during international travel or acquired locally were reported in the United States and in our hospital center (*1*,*3*–*6*; unpub. data). *S. flexneri* that is resistant to ceftriaxone and ciprofloxacin has been reported in the United States (*9*). Infections with multidrug-resistant *Shigella* spp. may be of longer duration and have higher costs (*3*).

When evaluating patients with diarrhea, physicians should identify risk factors and request bacterial cultures of fecal specimens. Antimicrobial drug susceptibility testing of *Shigella* isolates is essential for effective antimicrobial drug treatment. Serologic identification and PFGE are essential for epidemiologic purposes for ascertaining clusters or multidrug-resistant *Shigella* isolates (*1*,*3*–*5*). Patients with *Shigella* infection should be advised about preventive practices such as frequent handwashing and precautions when handling food and water (*3*). MSM should use barriers during oral, anal, and genital sex and wash their genitals, anus, and hands before and after sex (*1*,*3*–*5*).

We suggest obtaining 2 control cultures of fecal specimens on days 2 and 3 after the patient completes

Table. Antimicrobial susceptibility of the *Shigella flexneri*,serotype 2a pulsovar 21, isolated in Montreal, Quebec, Canada, 2015*

Antimicrobial agent	Disk diffusion, mm	MIC, mg/L	Interpretation
Ampicillin	6	≥32	R
TMP/SMX	6	≥320	R
Ciprofloxacin	12	≥4 and 8	R
Nalidixic acid	6	NA	R
Ceftriaxone	33	≤0.25	S
Cefixime	26	0.25	S
Azithromycin†	6	>256	NS
Tetracycline	6	32	R
Chloramphenicol	NA	>256	R
Amoxicillin-clavulanic acid	14	16	I
Ertapenem	NA	≤0.5	S
Gentamicin	21	≤1	S

*I, intermediate; NA, not available; NS, nonsusceptible; R, resistant; S, susceptible; TMP/SMX, trimethoprim/sulfamethoxazole.
†Azithromycin epidemiologic cutoff values for wild-type (MIC ≤8 mg/L) and non–wild-type (MIC ≥16 mg/L) *Shigella flexneri* (*2*) and the susceptibility and resistance breakpoints for the other 11 antimicrobial agents were CLSI *Enterobacteriaceae* breakpoints (*2*).

antimicrobial treatment for infection with multidrug-resistant *Shigella* spp. Patients should avoid sex during symptomatic infections and wait for 2 negative stool cultures. Montreal public health officials investigated and counselled this patient as they do for every patient with *Shigella* infections. In Quebec, physicians and microbiology laboratories are notified of *Shigella* clusters and multidrug-resistant *Shigella* infections.

To our knowledge, no other ciprofloxacin-resistant and azithromycin-nonsusceptible *Shigella flexneri* isolates have been documented in the province of Quebec. No PFGE matches to *S. flexneri* serotype 2a pulsovar 21 have been identified in Canada. Multidrug-resistant *Shigella* isolates, including those with both resistance to ciprofloxacin and nonsusceptibility to azithromycin, may be underestimated and incidence may be increasing (*1*,*3*–*5*).

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HIV/Hepatitis C Virus Co-infection among Adults Beginning Antiretroviral Therapy, Malawi

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To the Editor: Throughout the world, ≈115 million persons have hepatitis C virus (HCV) antibodies, ≈37 million are infected with HIV type 1, and an estimated 2.3 million persons are infected with both viruses (*1*). The estimated prevalence of HIV infection among adults in Malawi is 9.1% (*2*). Data concerning HCV seroprevalence in Malawi are conflicting and range from 0.0% to 18.0%, depending on the studied population and the chosen methods for HCV infection diagnosis (*3*–*6*). In a recent study, researchers used stored blood samples (without HCV confirmatory assays) from studies in rural and urban Malawian populations (1989–2008); an HCV seroprevalence of 6.8% was found in HIV-positive patients (*7*). In contrast, in a cohort of HIV-negative mothers (2006–2010), only 0.5% were found to be HCV positive with confirmatory HCV testing by immunoblot (*8*). These studies were not included in a 2015 metaanalysis that estimated the seroprevalence of HCV infection and HIV/HCV co-infection in Malawi to be 7.7% and 2.0%, respectively (*9*). Liver disease progresses more rapidly in HIV/HCV co-infected patients than in HCV monoinfected patients (*10*), and the highly effective second-generation direct-acting antiviral therapies are less toxic than interferon-based treatment regimens. It is crucial to gather accurate epidemiologic information on the burden of HIV/HCV co-infection to support the design and implementation of HCV treatment initiatives in resource-limited settings such as sub-Saharan Africa.

The aim of our study was to evaluate the prevalence of HIV/HCV co-infection in HIV-positive adults. We used baseline data obtained during the first 4 months of the ongoing observational, prospective Lighthouse Tenofovir Cohort (LighTen) study (Clinicaltrials.gov identifier: NCT02381275), which is conducted at the Lighthouse Clinic in Lilongwe, Malawi, in cooperation with the Institute of Public Health, University of Heidelberg, Germany, together with the Division of Infectious Diseases and the Clinic for Gastroenterology and Hepatology, University Hospital of Cologne, Germany. Ethical approvals by the respective committees of the Research Commission of the Ministry of Health, Malawi, and the participating German universities were granted before the study was initiated. A patient was included if he or she had confirmed HIV infection, was ≥18 years of age, and had given written informed consent for study participation. We included patient demographic information, medical history, concomitant diseases, bodyweight and height, as well hepatic panel results, platelet count, estimated glomerular filtration rate (determined by the CKD Epi formula), hemoglobin level, leukocyte count, CD4 cell count, and quantitative HIV RNA (Roche COBAS TaqMan HIV-1 v2.0, Risch, Switzerland) recorded at patient enrollment from the Kamuzu Central Hospital, Lilongwe, Malawi.

Aliquots of blood samples were stored at -80°C and kept on dry ice during airfreight to Cologne, then stored at -80°C until testing. Samples were thawed and tested for HCV IgG by a chemiluminescent micro-particle immunoassay (Abbott ARCHITECT, Wiesbaden, Germany) at the Institute of Virology, University of Cologne, Germany. Specimens that reacted to this immunoassay underwent supplemental testing with PCR for HCV RNA by a quantitative assay (Abbott RealTime HCV). We analyzed baseline characteristics of the study population (Table) using descriptive statistics (SPSS software version 22; IBM Inc., Chicago, IL, USA).

All 227 patients (137 female, median age 36.1 years) were HIV positive, with a median quantitative HIV RNA of 44,389 copies/mL and median CD4 cell counts of 284 cells/μL. Twenty-two patients (9.7%) had a history of blood transfusion, and 0.9% had a history of jaundice. Results for alanine aminotransferase level, aspartate aminotransferase level, total bilirubin level, and platelet counts were within the reference range in almost all patients, and no patient was jaundiced. Five patients (2.2%) had HCV IgG. However, none of these patients had detectable HCV RNA. Thus, the prevalence of active HIV/HCV co-infection was 0% in the studied cohort. One of the 5 patients who were positive for IgG against HCV also had a history of blood transfusion; none had a history of jaundice, and all but 1 seropositive patient had liver function tests within the reference range.

Three studies in Malawi have used PCR to test for HCV: 2 studies in HIV-positive pregnant women (included in the aforementioned metaanalysis) (*9*) and 1 study in blood donors (*5*), of whom 10.7% were HIV infected. PCR results for HCV RNA were positive in 0/2,041 (0%), 1/309 (0.3%), and 1/140 (0.7%) of these cases. Our findings confirm this low prevalence of PCR-positive active HCV infection also among HIV-infected adults from the general population of urban Lilongwe. Together, these studies indicate an overestimation of HCV prevalence on the basis of screening assays (*7*).

From a public health point of view, HCV infection as a cause of liver-related illness seems of minor importance in Malawi. The fact that HCV prevalence in antenatal care cohorts with already established serum sampling for routine HIV testing is similar to that in this mixed cohort of urban Malawi residents suggests that samples from these

Table. Characteristics of HIV-positive adults with HIV/HCV co-infection, Malawi*

Characteristic	HIV-positive and HCV antibody–negative patients, n = 222	HIV-positive and HCV-antibody–positive patients, n = 5
Age, y	36.1 (30.5–41.5)	34.5 (24.8–50.2)
Sex, M/F	88/134 (40/60)	2/3 (40/60)
Weight, kg, n = 167	59.1 (52.7–66.7)	59.6 (55.9–68.7)
Height, cm, n = 170	157.0 (151.0–165.0)	159.5 (151.0–167.0)
Quantitative HIV RNA [<40] × 10^3, copies/mL, n = 205	44.43 (12.09–158.96)	30.87 (21.14–95.38)
History of blood transfusion	21 (9)	1(20)
History of jaundice	2 (1)	0
eGFR mL/min/1,73 qm, n = 194	96.0 (79.0–110.0)	91.0 (88.0–94.0)
ALT [7–35], IU/L, n = 183	23.3 (14.9–33.4)	15.6 (13.2–22.5)
AST [≤38], IU/L, n = 183	31.7 (23.0–42.4)	31.9 (23.2–43.7)
Total bilirubin level [≤1.3], mg/dL, n = 185	0.30 (0.20–0.45)	0.26 (0.20–0.48)
Platelet count [122–330] × 10^9/L, n = 214	226 (181–293)	283 (160–320)
Hemoglobin level [10.9–17.3], g/dL, n = 215	12.4 (10.8–13.7)	13.3 (11.8–15.1)
Leukocyte count [2,800–8,400], cells/μL, n = 213	4,400 (3,500–5,400)	3,900 (2,700–6,600)
CD4 cell count, cells/μL, n = 139	284 (101–421)	319 (189–449)

*Values are given as total no. (%) or as median with interquartile range for the HCV antibody–negative study population and median with range for the HCV antibody–positive study population, respectively. Reference values are given in brackets. Number of missing values for the HCV-antibody negative patients ranged from 88 to 3, indicated by differing patient numbers in stub column. ALT, alaninine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus.

programs might represent a cost-efficient opportunity for monitoring trends of HCV infection in the population.

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Exposures among MERS Case-Patients, Saudi Arabia, January–February 2016

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To the Editor: Risk factors for primary acquisition of Middle East respiratory syndrome (MERS) coronavirus (CoV) include recent direct contact with dromedary camels (*1*), but secondary transmission, associated with healthcare settings (*2–4*) or household contact (*5*), accounts for most reported cases. Because persons with MERS often do not report any of these risk factors, we investigated MERS cases in Saudi Arabia during an apparent period of limited hospital transmission. Through telephone interviews of case-patients and information from routine investigations, we aimed to characterize exposures and to explore additional factors potentially important in disease transmission. We also genetically sequenced MERS-CoV from respiratory specimens to identify circulating strains.

For confirmed MERS cases (*6*) reported in Saudi Arabia during January–February 2016, we assessed exposures during the 2 weeks before illness onset (exposure period), including direct (*1*) and indirect camel contact; indirect contact was defined as 1) having visited settings where camels were kept but without having direct contact or 2) exposure to friends or household members who themselves had direct camel exposure (*1*). We assessed whether case-patients had worked at, visited, or been admitted to a healthcare setting or had contact with a person known to have MERS during the case-patient's exposure period. We also asked about recent travel and if any household members were healthcare personnel. For persons too ill to participate or deceased, we interviewed relatives or close friends.

We classified as secondary any case identified through routine case-contact tracing and testing. We considered persons whose cases were identified through routine testing

of occupational contacts of MERS-CoV–positive camels to have had direct camel contact. For the remaining cases, we used interview responses to characterize exposures.

All MERS cases reported during January–February 2016 were confirmed in Saudi Arabia by testing of respiratory specimens with real-time reverse transcription PCR assays targeting the MERS-CoV upstream envelope protein gene and the open reading frame (ORF) 1a gene (*7*,*8*). Available specimens (or RNA extracts) were shipped to the US Centers for Disease Control and Prevention (Atlanta, GA, USA) for full genome sequencing. Methods for sequencing and phylogenetic analysis have been described previously (*9*).

During January–February 2016, a total of 27 MERS case-patients were reported by public health officials from 6 of the 13 administrative regions of Saudi Arabia (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/11/16-1042-Techapp1.xlsx). Case-patients were evaluated at 20 different hospitals, 3 of which reported >1 case during the investigation period. Among the 27 case-patients, 4 (15%) were identified through routine contact tracing and testing as having secondary cases. Two case-patients (7%) were identified as occupational contacts of MERS-CoV–positive camels. Of the remaining 21 case-patients, 17 (81%) were interviewed during March 13–16, 2016; three were unavailable for interview, and 1 provided incomplete data. Ten (59%) of the 17 interviews were completed by proxy.

Among the 17 case-patients interviewed, 5 (29%) reported direct camel contact (1 of these also reported visiting a healthcare facility), and 4 (24%) reported indirect camel contact (2 of these also reported visiting a healthcare facility) during the exposure period (online Technical Appendix 1). Three case-patients reported having close acquaintances who regularly interacted with camels but reported they had not seen these acquaintances during the exposure period. One case-patient was the spouse of a healthcare worker employed in a facility with a reported MERS patient during the putative exposure period; the spouse was found to be MERS-CoV–negative by real-time reverse transcription PCR of a respiratory specimen. The 4 remaining cases could not be further characterized.

Viruses from 13 of the 27 case-patients were sequenced, and all belonged to the MERS-CoV recombinant subclade NRC-2015 (*9*), first detected in humans in January 2015; these 13 case-patients were from the Riyadh and Makkah regions (online Technical Appendix 1). Full genome sequences were obtained from the specimens of 11 case-patients (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/22/11/16-1042-Techapp2.pdf). Continued and predominant circulation of NRC-2015 supports increased epidemiologic fitness compared with other clades, as postulated previously (*9*).

A novel nucleotide substitution was identified in the MERS-CoV sequence from 1 case-patient (online Technical Appendix 1) at position 337 located in the stop codon of ORF8b (TAA [Stop] >CAA [Gln] = Stop113Q), predicted to yield a 143aa protein versus the 112aa wild-type. ORF8b is an internal ORF overlapped by the nucleocapsid protein gene (*10*); the corresponding substitution in the nucleocapsid protein gene predicts a conserved amino acid change (V178A). The virologic and clinical significance of these findings is unknown.

Since the emergence of MERS-CoV in 2012, virus acquisition has been associated with direct exposure to camels (*1*) and with person-to-person transmission in households and healthcare settings (*2*–*5*); other sources of infection are less clear. Among the patients in our study whose cases were successfully characterized (23/27), 4 had contact with other known case-patients, and 7 reported direct camel contact. Among the remaining 12 case-patients without these risk factors, 7 were identified as having at least some exposure to persons with direct camel contact. Our findings suggest that community and household exposure to persons with direct camel contact might play a role in MERS-CoV acquisition. Further investigation is needed to determine any specific roles of these interactions in MERS-CoV transmission.

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Leishmania major Cutaneous Leishmaniasis in 3 Travelers Returning from Israel to the Netherlands

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To the Editor: Cutaneous leishmaniasis (CL) is a protozoan disease transmitted by sand flies that usually runs a relatively mild course. Classic CL starts as a red papule at the place of the insect bite; it gradually enlarges into a painless nodule or plaque-like lesion, which eventually becomes encrusted. When the crust falls off, a typical ulcer with raised and indurated border becomes apparent. CL can cause considerable illness and may leave disfiguring and disabling scars after healing. The interplay between *Leishmania* species and host immune response is complex, and, as a result, disease manifestations may vary substantially among species as well as among infected persons (*1*,*2*). An estimated 0.7–1.2 million new CL cases occur annually in tropical and subtropical regions of the world. CL is currently endemic in >98 countries worldwide; Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru together account for up to 75% of global estimated CL incidence (*3*).

We report 3 travel companions from the Netherlands who all acquired CL after they participated in a short-term study course in Israel during September–October 2015. The travelers visited several places in the Negev Desert in southern Israel. All cases were confirmed by PCR with additional sequence analysis of the mini-exon locus and the 3′ untranslated region of the HSP70 locus, demonstrating *L. major* as the causative species (*4*).

The first case-patient was a 55-year-old man who observed red papules on his head and shoulder 1 month after he returned to the Netherlands. Gradually, these papules increased in size, and number and showed a tendency to ulcerate. On examination at the Institute for Tropical Diseases in Rotterdam, 12 painless, hyperkeratotic, plaquelike, sharply demarcated lesions were identified, some partially ulcerated, located on the head, shoulders, arms, legs, and across the chest. Histopathologic examination on skin biopsy specimens acquired from 1 lesion revealed *Leishmania* amastigotes, consistent with a diagnosis of CL. The patient was treated with miltefosine (50 mg orally 3×/d for 28 d). Clinical recovery followed gradually.

The second case-patient, a 52-year-old woman, noticed some red papules on both legs that gradually increased in size and ulcerated in the 2 months after return to the Netherlands. She was initially treated by a general practitioner for a presumed bacterial skin infection but did not show a clinical response to antibiotic treatment. On examination, also at the Institute for Tropical Diseases, 6 painless ulcers were seen on her legs. CL was suspected after taking into account the clinical manifestations and the recent diagnosis of CL in her travel companion. She was successfully treated with miltefosine after the diagnosis was confirmed.

A third case-patient, a 52-year-old woman, was diagnosed with CL after she sought treatment for a single small, sharply demarcated, painless pretibial plaquelike skin lesion on her arm that had been present for 2 months after her return to the Netherlands. Repeated PCRs of skin biopsy specimens confirmed the diagnosis of *L. major* CL. She preferred a "wait and see" policy over treatment.

The 3 patients with CL, a cluster of travel companions, were conceivably infected in the Negev Desert. Only 1 previous report has documented a traveler returning from Israel who was diagnosed with CL at the Institute for Tropical Diseases during 2007–2016 (Table). Most cases originated from the New World, in particular from South America, followed by Central America (Table). Few of these cases

Table. Characteristics of 3 travel companions to Israel and a cohort of patients with imported cutaneous leishmaniasis previously diagnosed at the Institute for Tropical Diseases, Harbour Hospital, Rotterdam, the Netherlands, 2007–2016

Characteristics	Case-patient 1	Case-patient 2	Case-patient 3	Cohort, n = 27*
Age, y (SD)	55.2	52.6	52.3	45.4 (15.2)
Sex	M	F	F	M: 19 (70.4); F: 8 (29.6)
New World/Old World	Old World	Old World	Old World	New World: 18 (66.7); Old World: 9 (33.3)
Country of acquisition	Israel	Israel	Israel	Argentina: 1 (3.7); Brazil; 2 (7.4); Costa Rica: 2 (7.4); Ecuador: 3 (11.1); Ethiopia: 1 (3.7); French-Guyana: 1 (3.7); Israel: 1 (3.7); Mexico: 1 (3.7); Morocco: 4 (14.8); Peru: 1 (3.7); Spain: 2 (7.4); Suriname: 6 (22.2); Trinidad and Tobago: 1 (3.7); Turkey: 1 (3.7)
Reason for visit	Tourism	Tourism	Tourism	Tourism: 18 (66.7); work-related visit: 2 (7.4); family visit: 6 (22.2); unknown: 1 (3.4)
Clinical manifestation	Ulceration/plaque-like lesion	Ulceration	Plaque-like lesion	Erythematous papule: 1 (3.7); nodule/plaquelike lesion: 9 (33.3); ulceration: 17 (63.0)
Mean no. skin lesions	12	6	1	1.6 (median 1.0, range 1.0–5.0)
Localization	≥1 localization	≥1 localization	Lower leg	Face: 4 (14.8); upper arm: 3 (11.1); lower arm: 10 (37.0); upper leg: 0; lower leg: 6 (22.2); trunk or neck: 1 (3.7); ≥1 localization: 3 (11.1)
Diagnostic method	Histology/PCR	Histology/PCR	Histology/PCR	Histology: 0; PCR: 19 (70.3); histology/PCR: 8 (29.7)
Leishmania spp.	*L. major*	*L. major*	*L. major*	*L. major*: 2 (7.4); *L. tropica*: 1 (3.7); *L. infantum*: 4 (14.8); *L. donovani*: 1 (3.7); *L. mexicana*: 1 (3.7); *L .braziliensis*: 7 (25.9); *L. guyanensis*: *4* (14.8); *L. panamensis*: 1 (3.7); *L. lainsoni*: 1 (3.7); Unknown: 5 (18.5)
Treatment	Miltefosine	Miltefosine	Wait and see	Cryotherapy: 2 (7.4); topical paromomycin/methylbenzethoniumchloride: 1 (3.7); theurapeutic excision: 1 (3.7); fluconazole: 1 (3.7); pentamidineisethionate: 2 (7.4); systemic stibogluconate: 3 (11.1); miltefosine: 17 (63.0)

*Values are no. (%) except as indicated.

originated from the Old World, where most of the global effects of CL occur. CL is more frequently diagnosed among long-term travelers such as military personnel, adventure travelers, photographers, and researchers, rather than among short-term travelers (*5*).

Recent reports have described a 7-fold increase in *L. major* CL cases among inhabitants of the Negev Desert (*6*), with urban expansion into CL-endemic foci and changes in land use currently regarded as the most probable causes for this increase in incidence (*6*,*7*). A concurrent increase among travelers has not occurred so far, although the aforementioned cases might indicate that the increasing risk of contracting CL in the Negev Desert is not only restricted to its inhabitants but may also pose a risk to short-term travelers.

The spectrum of disease of CL is highly variable, even among persons infected with the same *Leishmania* species (*1*,*2*,*8*), as illustrated by this cluster of *L. major* CL cases among persons who traveled together to Israel. Because *L. major* CL might mimic other infectious and inflammatory diseases, physicians assessing travelers with painless and persistent skin ulcers after their return from CL-endemic countries should therefore consider CL in their differential diagnosis. In conclusion, awareness should be raised among physicians and healthcare workers that CL is not exclusively limited to tropical countries but may also be acquired by short-term travelers to more temperate regions, such as southern Europe and the Levant (Syria, Lebanon, Israel, and Jordan) (*3*,*9*).

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Molecular Evidence of Oysters as Vehicle of Norovirus GII.P17-GII.17

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To the Editor: Norovirus is the world's leading cause of nonbacterial acute gastroenteritis (*1*). Since their emergence, GII.P17-GII.17 noroviruses have replaced the GII.4 Sydney 2012 variant as the dominating norovirus genotype in parts of Asia (*2*), although they have been detected only sporadically, in a limited number, on other continents (*3*).

The major reservoir(s) of GII.17 that contribute to transmission are unknown, but it has been suggested that oysters and other bivalve shellfish are common vehicles for transmission of the emerging GII.17 viruses (*2*). In this study, we demonstrate the link between oysters and human disease by presenting molecular evidence of norovirus GII.P17-GII.17 in Denmark causing acute gastroenteritis, characterized by the sudden onset of vomiting with or without diarrhea after consumption of oysters. We further document molecular evidence providing linkage between norovirus detected in fecal samples from patients and food samples from imported oysters.

During January 23–February 4, 2016, acute gastroenteritis developed in 58 of 67 persons who consumed oysters served on 18 separate occasions at 8 different restaurants and a private party, with onset of symptoms within 24–40 hours after the patients ate oysters. All oysters originated from 2 distinct oyster lots provided by 1 wholesaler in France and distributed by 1 wholesaler in Denmark. Oysters from both lots were harvested off the coast of La Rochelle, France.

In Denmark, submitting fecal samples in connection with foodborne outbreaks is voluntary. A total of 5 samples from 3 cases representing 2 different parties were submitted to the National Virus Surveillance Laboratory at Statens Serum Institut (Copenhagen, Denmark) for norovirus analysis. In addition, 4 samples of oysters from the same producer in France were sent to the National Food Institute, Technical University of Denmark (Kongens Lyngby, Denmark), for norovirus analysis. Two samples, an opened (sample A) box and an unopened (sample B) box collected at one of the restaurants involved in the outbreak, contained oysters from 1 batch (I), and another 2 samples (C and D) contained oysters from a separate batch (II) collected at the wholesale level.

Norovirus in fecal samples was detected and polymerase (open reading frame [ORF]1) and capsid (ORF2) gene regions were amplified as described elsewhere (*4*). Sequencing was performed by using an ABI 3500 genetic analyzer (Thermo Fisher Scientific, Nærum, Denmark). A fragment of 1,111 nt spanning the ORF1–ORF2 junction was amplified and sequenced by using forward ORF1 primer JV12 and reverse ORF2 primer G2SKR (*4*). Genotyping was performed by using the Web-based norovirus typing tool NoroNet (http://www.rivm.nl/mpf/norovirus/typingtool) (*5*).

We found that all fecal samples contained norovirus belonging to genogroup II (GII). Sequencing of the polymerase region was successful in 4 of the 5 samples; all 5 samples were sequenced in the capsid region. Typing of the sequences indicated GII.P17 and GII.17, respectively. Furthermore, PCR and sequencing of the long fragment covering the ORF1–ORF2 junction were successful in 2 samples, both genotyping as GII.P17-GII.17. Sequences had high homology (99.91%) to several Asian strains, such as Hu/GII.P17_GII.17/KR/2014/CAU-265 (*6*).

Oysters were analyzed for norovirus (*7*), and the capsid region of the detected strains was sequenced (*8*). Samples A, B, and C contained norovirus GI, whereas all 4 samples (A–D) contained norovirus GII. GI.2 was identified in 1 sample (B), and GII.17 was identified in all samples (A–D). The GII.17 sequences obtained from samples A–C showed 100% homology, whereas the sequence identified in sample D varied at 3 positions.

Comparisons of the capsid sequences obtained from 4 human samples and oyster samples A–C showed 100%

homology. The last of the human sequences differed at 2 positions owing to mixed bases. The chromatogram showed equal intensities at both positions C and T (Figure, http://wwwnc.cdc.gov/EID/article/22/11/16-1171-F1.htm). This finding indicates that quasispecies might be present. These positions in the human sequence coincided perfectly with 2 of the 3 positions found to vary in the sequence obtained from oyster sample D. All 3 base variations were a replacement of a C with a T (Figure), which further supports the presence of quasispecies. However, in this setup, it was not possible to prove the origin of these closely related species. Whole-genome sequencing using next-generation sequencing would be a way to prove the simultaneous presence of all quasispecies in relevant samples.

Since the emerging of GII.P17-GII.17 in Asia, sporadic cases have been reported worldwide (*3*,*9*). In this study, we established a direct molecular link between a common food source and a series of acute gastroenteritis outbreaks. Even though these represent European outbreaks, our results show that oysters act as vehicles for the rapid spread of emerging noroviruses to distant geographic areas. Furthermore, we document that quasispecies of GII.P17-GII.17 might occur simultaneously in a host. This finding should be considered in future molecular-epidemiologic outbreak investigations.

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Recent Chikungunya Virus Infection in 2 Travelers Returning from Mogadishu, Somalia, to Italy, 2016

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To the Editor: Since chikungunya virus (CHIKV) was first isolated in 1952 (in Tanzania), outbreaks have occurred every 7–20 years in countries in Africa and Asia, and since 2013, it has been identified in the Americas (*1*,*2*). However, no cases have been reported from the Horn of Africa (*3*,*4*). We confirmed CHIKV infection acquired in 2016 by 2 travelers to Somalia who returned to Italy.

In June 2016, a Somali woman (patient 1) was referred to the Infectious and Tropical Diseases Unit, Careggi University Hospital, in Florence, Italy, because of severe diffuse

bilateral arthralgia and edema in hands, wrists, ankles, and feet. Five days earlier, she had returned to Italy from Mogadishu, Somalia, where she had spent 45 days visiting relatives. The woman had been living in Italy since the 1990s and returned to Somalia each year for ≈2 months; she denied travel to other countries. She reported that symptoms started abruptly in May, 17 days after arriving in Somalia (28 days before returning to Italy). At symptom onset, arthralgia was associated with fever and skin rash, which lasted a few days.

In early July 2016, another Somali woman (patient 2) with bilateral arthralgia in her hands, wrists, ankles, and feet associated with foot edema sought medical care at the same hospital 7 days after returning from a 65-day trip to Mogadishu, where she visited relatives. The woman had been living in Italy ≈20 years; the only other travel she reported was to Kenya in 2012. Her symptoms started in June, 20 days after arriving in Somalia (45 days before returning to Italy). At symptom onset, she also had skin rash and fever, which lasted a few days.

Both patients reported that, during the same period, some of their relatives in Mogadishu had similar symptoms and were clinically diagnosed as having chikungunya fever by local doctors. Both also reported that, during the same period, other cases had been reported in Mogadishu by mass and social media and, thus, the local population was aware of the disease.

Serum samples for patients 1 and 2 were positive for CHIKV antibodies (Table). Both patients were treated with nonsteroidal antiinflammatory drugs and corticosteroids and are receiving follow-up.

According to the US Centers for Disease Control and Prevention, as of April 22, 2016, CHIKV had not been reported from Somalia (*4*), and no evidence exists for CHIKV circulation in that area of the Horn of Africa (*3*). In addition, on August 3, 2016, we performed a literature search in PubMed, Embase, and ProMED-mail, and found no reports of CHIKV in Somalia. Poorly documented preliminary data on the presence of CHIKV in Somalia were recently reported in 2 documents by the United Nations Office for the Coordination of Humanitarian Affairs. One document, dated June 7, 2016, stated "There are reports of an outbreak of the deadly Chikungunya virus in Banadir Region. According to WHO [the World Health Organization], 3 of 5 blood samples have tested positive" (reference *1* in online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-1225-Techapp1.pdf). The second document, dated June 30, 2016, stated that "some 11 suspected cases of Chikungunya were confirmed... in Mogadishu" (reference *2* in online Technical Appendix). Several reports in the online press and social media have reported the current circulation of CHIKV in Somalia, including 2 Twitter posts (tweets) by the Ministry of Health of Kenya (references *3–7* in online Technical Appendix). A Somali doctor living in Italy obtained confirmation of CHIKV circulation in Somalia by contacting colleagues at the Ministry of Health in Mogadishu (Omar Abdulcadir, Careggi University Hospital, pers. comm., 2016 Jul 19).

Direct and indirect evidence exists for the presence of competent CHIKV vectors (e.g., *Aedes aegypti* mosquitoes) in Somalia. Entomologic studies conducted in 1942 and 1969 confirmed the presence of *A. aegypti* mosquitoes in several cities along Somalia's coast, including Mogadishu (*5*). Other arboviruses in which *Aedes* mosquitoes play a role as reservoir (Rift valley fever [RVF]) and vector

Table. Results of chikungunya virus testing for 2 persons who returned to Italy after traveling to Mogadishu, Somalia, 2016*

Laboratory test performed	Place where test was performed	Results	
		Patient 1†	Patient 2‡
OnSite Chikungunya IgM Combo Rapid Test-Cassette (CTK Biotech, San Diego CA, USA)§	Careggi University Hospital	Positive	Negative
Chikungunya virus IFA IgG (Euroimmun AG, Luebeck, Germany)¶	Careggi University Hospital	Titer ≥1:100#	Titer ≥1:100#
Chikungunya virus IFA IgM (Euroimmun)**	Careggi University Hospital	Positive	Positive
Anti-CHIKV IgM ELISA (Euroimmun)††	ISS, National Reference Laboratory for Arboviruses	Index 7.9‡‡	Index 3.4‡‡
PRNT for Chikungunya virus	ISS, National Reference Laboratory for Arboviruses	PRNT80 ≥1:10§§	PRNT80 ≥1:10§§

*****Testing was conducted on samples taken the day patients 1 and 2 sought care at the Infectious and Tropical Diseases Outpatient Unit at Careggi University Hospital in Florence, Italy. IFA, immunofluorescence assay; ISS, National Institute of Health in Rome, Italy; PRNT, plaque reduction neutralization test; PRNT80, 80% PRNT.
†Samples were obtained 33 d after symptom onset.
‡Samples were obtained 52 d after symptom onset.
§Sensitivity/specificity 90.3%/100% according to information reported in the kit data sheet (reference *10* in online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/11/16-1225-Techapp1.pdf) and 30%/73% according to an independent evaluation (reference *11* in online Technical Appendix).
¶Sensitivity/specificity 100%/96% according to information reported in the kit data sheet (reference *12* in online Technical Appendix).
#Cut-off for positivity ≥1:10.
**Sensitivity/specificity 95%/96% according to information reported in the kit data sheet (reference *12* in online Technical Appendix)).
††Sensitivity/specificity 98.1%/98.9% according to information reported in the kit data sheet (reference *13* in online Technical Appendix) and 85%/82% according to an independent evaluation (reference *11* in online Technical Appendix).
‡‡Cut-off for positivity ≥1.1.
§§PRNT80 titers >1:10 are considered positive.

(dengue and possibly RVF) have been reported in recent years in Somalia: RVF outbreaks occurred during 1997–1998 and 2006–2007 (*6*,*7*), and a dengue outbreak occurred during 1992–1993 (*8*).

The current outbreak in Somalia could have been triggered by several factors, including circulation of CHIKV in neighboring Kenya (references *8*,*9* in online Technical Appendix) and heavy rains that led to flooding in southern and central Somalia beginning in January 2016 (reference *1* in online Technical Appendix). CHIKV has the potential to provoke explosive outbreaks in naive populations (*9*), so the current outbreak may greatly affect the economy and public health in Somalia.

Systematic studies to understand the magnitude of the ongoing epidemic are needed. In the meantime, local public health stakeholders in Somalia and healthcare workers worldwide caring for travelers returning from Somalia should be aware that CHIKV is circulating in the country. This report confirms the importance of travel medicine services in performing early diagnosis of imported arboviral diseases, not only to thwart secondary transmission during periods of competent vector activity but also to help to detect or confirm virus circulation in previously unaffected countries.

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Meningococcal Disease in US Military Personnel before and after Adoption of Conjugate Vaccine

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To the Editor: In their recent letter (*1*), Broderick et al. provided useful information about the remarkable declines in incidence of meningococcal disease among active-duty US military personnel since the early 1970s, when meningococcal vaccination began within that population. The authors reported that the incidence of meningococcal disease from vaccine-covered serogroups was 0.183 cases/100,000 persons during 2006–2013 among persons vaccinated with quadrivalent conjugate meningococcal vaccine (MCV-4), compared with 0.307 cases/100,000 persons during 2000–2013 among persons vaccinated with quadrivalent polysaccharide meningococcal vaccine (MPSV-4). They stated that, because these rates did not differ significantly, case rates were similar in personnel vaccinated with MCV-4 and MPSV-4. Although statistically correct, this comment might mislead the unwary reader.

The absence of a significant difference does not necessarily mean that the 2 vaccines have similar effectiveness. The incidence rate of meningococcal disease was 68% higher ([0.307–0.183] × 100/0.183) during the period of MPSV-4 use than during the period of MCV-4 use. If the same findings arose in a study of sufficient size to achieve statistical significance, this difference would be considered of substantial clinical importance. A happy consequence of the long-term temporal trends in meningococcal incidence and the success of these vaccines is that the incidence of meningococcal disease is now sufficiently reduced that even the very large active-duty population is too small to provide the statistical power to declare these 2 different incidence rates as being statistically different.

The trends reported by Broderick et al. have continued. During 2006–2014, the incidence of meningococcal disease caused by vaccine-covered serogroups among US military recipients of MCV-4 fell to 0.146 per 100,000 person-years, whereas MPVS-4–related incidence did not change (M.P. Broderick, pers. comm.). Furthermore, through July 2016, the US military has not seen a case from a covered serogroup since 2011 among recipients of MCV-4. Even with these additional data, however, the difference between MCV-4 and MPSV-4 does not achieve statistical significance (M.P. Broderick, pers. comm.).

The author is an employee of a company that manufactures both conjugate and polysaccharide meningococcal vaccines.

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Correction: Vol. 22, No. 8

The name of author Natalie Witek was misspelled in *Baylisascaris procyonis*–Associated Meningoencephalitis in a Previously Healthy Adult, California, USA (C. Langelier et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/8/15-1939_article).

Corrections: Vol. 22, No. 9

Some descriptions of tickborne transmission of bacteria were unclear in Large-Scale Survey for Tickborne Bacteria, Khammouan Province, Laos (A.J. Taylor al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/9/15-1969_article).

A second affiliation for author Martie L. van der Walt was omitted in Treatment Outcomes for Patients with Extensively Drug-Resistant Tuberculosis, KwaZulu-Natal and Eastern Cape Provinces, South Africa (C.L. Kvasnovsky et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/9/16-0084_article).

Deadly River: Cholera and Cover-Up in Post-Earthquake Haiti

Ralph R. Frerichs; ILR Press, Ithaca, NY, USA, 2016

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The massive magnitude 7.0 earthquake that hit Haiti on January 12, 2010, was followed 10 months later by onset of the largest cholera epidemic in recent history, with >768,831 cases and 9,113 deaths reported through the end of April 2016 (*1*). Although initial epidemic spikes were followed by a rapid decline in case numbers, cholera remains a critical public health problem for Haiti. Of immediate concern, the number of cases in the first 4 months of 2016 (close to 14,000 reported cases) exceeds the reported case numbers for the same period in 2014 and 2015 (*1*), consistent with the hypothesis that cholera in Haiti is becoming endemic, potentially with the establishment of environmental reservoirs (*2*,*3*).

There is a consensus that cholera was introduced into Haiti by Nepalese peacekeeping troops who were part of the United Nations Stabilization Mission in Haiti. Reaching this consensus, however, was not an easy process, given the associated political implications. Along the way, unfortunately, the political issues became intertwined in longstanding scientific controversies about basic transmission pathways for cholera. This resulted in scientific infighting among French and US research groups, personified on one side by Renaud Piarroux, based in Marseille, and on the other by Rita Colwell at the University of Maryland. In 1996, in an article in Science, Colwell articulated what has been termed the "cholera paradigm," which places a strong emphasis on the role of the environment and environmental reservoirs in cholera persistence and transmission (*4*). Piarroux and colleagues, in contrast, have minimized the importance of the environment and environmental sources, both in Haiti and in Africa, and emphasized the critical role of controlling person-to-person transmission in eradicating cholera (*5*–*7*). Although the truth undoubtedly lies somewhere in the middle (*8*), the controversy has drawn in much of the cholera research community (including the Centers for Disease Control and Prevention and the World Health Organization) and has contributed to ongoing uncertainties about appropriate "on the ground" approaches to cholera control in Haiti in the face of increasing case numbers.

In this setting, Ralph Frerichs, professor emeritus of epidemiology at the University of California, Los Angeles, has published Deadly River: Cholera and Cover-Up in Post-Earthquake Haiti. In his preface, Dr. Frerichs clearly states that his goal was to highlight the work and scientific concepts put forward by Piarroux. In Dr. Frerichs' words, "As I listened to his story unfolding in real time and heard more about the role of the United Nations and reputable scientists who were espousing suspect theories about the outbreak's origins, I became convinced that the inside and ongoing story of what Piarroux had encountered in Haiti needed to be told." As reflected in the title, Dr. Frerichs saw a cover-up and is seeking to make certain that Piarroux's role and point of view (politically and scientifically) are clearly chronicled.

As long as the reader is aware that there are other, well-supported, points of view from scientists with an equal dedication to control of cholera in Haiti, the book is of value in recording the events of this massive epidemic and the factors that led to its occurrence, from the vantage point of one of the major scientific investigators in Haiti at the time. However, the tone of the book and its tendency to see all events (and science) from the viewpoint of a single investigator/investigative group is not ideal and detracts from the impact that the work might otherwise have had. Science does have its controversies and infighting, and intermingling of scientific and political issues is inevitable; however, one might hope that our goal, as scientists, would be to resolve controversies with better science, while minimizing acrimony. Unfortunately, this book, with its focus on possible cover-ups and "suspect theories," does not move us in this direction.

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Sophia Thoreau (1819–1876) Title page illustration for *Walden; or, Life in the Woods* (1854). Wood engraving, 7 1/16 in × 4 1/2 in/17.94 cm × 11.43 cm. Public domain digital image courtesy Rare Book and Special Collections Division, Library of Congress.

A Simple Sketch Symbolizing Self-Reliance

Byron Breedlove

Henry David Thoreau (1817–1862) is remembered and celebrated as an essayist, poet, philosopher, and naturalist; he was also a surveyor, historian, antislavery activist, tax resister, and teacher. Thoreau's extensive writings are collected in more than 20 volumes, including books, articles, essays, journals, and poems. In his best known book, *Walden; or, Life in the Woods*, published in 1854, Thoreau detailed his day-to-day experiences during the 2 years, 2 months, and 2 days he dwelled in a small, rustic cabin that he constructed near Walden Pond. This 64-acre lake is near the town of Concord, Massachusetts, USA, close to where Thoreau was born and lived most of his life.

Initially a modest success, the book sold 2,000 copies within 5 years but then went out of print until 1862, the year Thoreau died. Today it is among the best known works of American literature. Its chapters consist of the author's

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ruminations on human existence, society, government, economics, nature, and other topics that were inspired by his daily observations on local events or his close-up encounters with nature. In an essay about *Walden*, "A Sage for All Seasons," American writer and critic John Updike opined, "A century and a half after its publication, *Walden* has become such a totem of the back-to-nature, preservationist, anti-business, civil-disobedience mindset, and Thoreau so vivid a protester, so perfect a crank and hermit saint, that the book risks being as revered and unread as the Bible."

Thoreau died of tuberculosis in May 1862, a disease that first manifested itself in 1835 while he was a student at Harvard College. He had endured several years of declining health, starting with a bout of bronchitis. Many of his large projects were left behind in various stages of completion and would have been lost to history were it not for the industrious work of his younger sister, Sophia Thoreau.

In *Walden*, Henry Thoreau wrote, "For more than five years I maintained myself thus solely by the labor of my hands, and I found that, by working about six weeks in a year, I could meet all the expenses of living." Sophia, in contrast, managed the family's business after their father's death, and according to the Concord Museum "helped manage her brother's literary legacy in the years immediately following his death, and she is largely responsible for the preservation of his material legacy as well." She, too, is remembered as having been a teacher, naturalist, and antislavery activist. Sophia also gardened and cultivated flowers, and she served as caretaker for her brother as he succumbed to tuberculosis.

Sophia Thoreau's reputation as an artist, which is not without critics who considered her work simplistic, endures in large measure because it was her drawing of the cabin by the pond that Henry selected for the cover page of *Walden; or, Life in the Woods.* According to an article published by the Thoreau Society, this simple sketch "has become a symbol of individuality and self-reliance throughout the world." In her sketch, Sophia depicted the cabin nestled among the thrusting evergreens and lacy deciduous trees. A path from the door shows the starting—and ending—point for Henry's long rambles in the countryside or nearly daily walks to Concord. This path would also take visitors to the rustic cabin where Henry enjoyed his largely solitary life: "I had three chairs in my house; one for solitude, two for friendship, three for society."

Perhaps Henry Thoreau's active lifestyle and love of the outdoors helped him live with tuberculosis—the leading cause of death during his lifetime—for decades. This illness also claimed his grandfather, his father, and his older sister. His brother John, who died from tetanus, was also living with tuberculosis. That so many members of the Thoreau family died of tuberculosis is not remarkable: in the 1800s, living conditions in the United States contributed to outbreaks of infectious diseases such as tuberculosis, dysentery, cholera, malaria, pneumonia, typhoid fever, and whooping cough.

Since the initial publication of *Walden; or, Life in the Woods*, ensuing advances in sanitation, the advent of immunizations, the development of antibiotics, and improvements in care and diagnosis have greatly reduced deaths from diseases caused by many of the respiratory pathogens that were so devastating during the 1800s. However, during the 21st century, myriad outbreaks of novel respiratory tract infections that could potentially cause epidemics or pandemics, including Middle East respiratory syndrome, severe acute respiratory syndrome, H1N1 influenza, and invasive pneumococcal disease, underscore the value of continuously advancing and assessing public surveillance, diagnosis, prevention, and treatment strategies and responses.

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Upcoming Issue

- Assessing the Epidemic Potential of RNA and DNA Viruses
- Investigation and Response to 2 Plague Cases, Yosemite National Park, California, USA, 2015
- African Horse Sickness Caused by Genome Reassortment and Reversion to Virulence of Live, Attenuated Vaccine Viruses, South Africa, 2004–2014
- Anomalous High Rainfall and Soil Saturation as Combined Risk Indicator of Rift Valley Fever Outbreaks, South Africa, 2008–2011
- Whole-Genome Characterization and Strain Comparison of VT2f-Producing *Escherichia coli* Causing Hemolytic Uremic Syndrome
- Cutaneous Granulomas in Dolphins Caused by Novel Uncultivated *Paracoccidioides brasiliensis*
- Vertebrate Host Susceptibility to Heartland Virus
- Time Course of MERS-CoV Infection and Immunity in Dromedary Camels
- Hepatitis E Virus in 3 Types of Laboratory Animals, China, 2012–2015
- Genetically Different Highly Pathogenic Avian Influenza A(H5N1) Viruses in West Africa, 2015
- Highly Pathogenic Reassortant Avian Influenza A(H5N1) Virus Clade 2.3.2.1a in Poultry, Bhutan
- Highly Divergent Dengue Virus Type 2 in Traveler Returning from Indonesia to Australia
- *Baylisascaris procyonis* Roundworm Seroprevalence among Wildlife Rehabilitators, United States and Canada, 2012–2015
- Detection of Vaccinia Virus in Dairy Cattle Serum Samples from 2009, Uruguay
- Detection and Genotyping of *Coxiella burnetii* in Pigs, South Korea, 2014–2015
- Human Brucellosis in Febrile Patients Seeking Treatment at Remote Hospitals, Northeastern Kenya, 2014–2015
- Human Infection with Novel Spotted Fever Group *Rickettsia* species, China, 2015
- Fatal Case of West Nile Neuroinvasive Disease in Bulgaria
- *Rickettsia raoultii* in *Dermacentor reticulatus* Ticks, Chernobyl Exclusion Zone, Ukraine, 2010
- Possible Foodborne Transmission of Hepatitis E Virus from Domestic Pigs and Wild Boars from Corsica

Complete list of articles in the December issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

2016

November 4–7, 2016
IMED
International Meeting on Emerging Diseases and Surveillance
Vienna, Austria
http://imed.isid.org/

November 13–17, 2016
ASTMH
American Society of Tropical Medicine and Hygiene
Atlanta, GA, USA
https://www.astmh.org/

November 29–December 2, 2016
Institut Pasteur International Network Scientific Symposium
Paris, France
http://www.pasteur-network-meeting2016.org/

December 3–8, 2016
ASLM
African Society for Laboratory Medicine
Cape Town, South Africa
http://aslm2016.org/

2017

March 29–31, 2017
SHEA
Society for Healthcare Epidiemiology of America
St Louis, MO, USA
http://www.shea-online.org/

April 22–27, 2017
ECCMID
European Congress of Clinical Microbiology and Infectious Diseases
Vienna, Austria
http://www.eccmid.org/

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

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Article Title

Transmission of *Babesia microti* Parasites by Solid Organ Transplantation

CME Questions

1. Your patient is a 52-year-old man with end-stage diabetic nephropathy who experiences fever and anemia after kidney transplantation. According to the case reports by Brennan and colleagues, which of the following statements about presentation and likely mode of transmission of babesiosis after organ transplantation is correct?

A. The kidney recipients were diagnosed with babesiosis 1 month after transplantation
B. The kidney recipients had *Babesia microti* antibodies detected by serologic testing of archived pretransplant specimens
C. The organ donor came from a *Babesia*-endemic region and had a history of tick bite
D. The organ donor likely served as a conduit of *Babesia* parasites from a seropositive blood donor to both kidney recipients

2. According to the case reports by Brennan and colleagues, which of the following statements about treatment of babesiosis in these patients is correct?

A. Typical duration of antimicrobial therapy is 2 to 3 weeks in immunocompetent persons
B. In immunosuppressed patients, cure is more likely with combination antimicrobials for at least 6 weeks, including 2 weeks after parasites clear from blood smear
C. The recipients were treated with clindamycin plus quinine
D. One patient experienced clinical resistance during antibiotic therapy and had a relapse of babesiosis

3. According to the case reports by Brennan and colleagues, which of the following statements about the clinical implications of findings from these cases of babesiosis is correct?

A. Clinicians should consider babesiosis in the differential diagnosis of unexplained fever and hemolytic anemia after transfusion or transplantation only in regions where babesiosis is endemic
B. Suspected cases of transmission after transfusion or transplantation should be reported only to state and local public health authorities
C. The US Food and Drug Administration's (FDA's) Blood Products Advisory Committee supports the concept of year-round *B. microti* serologic testing of all US blood donors and nucleic acid–based testing of donors in certain states
D. The FDA has licensed *Babesia* tests for screening US blood donors

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title

Multidrug-Resistant *Corynebacterium striatum* Associated with Increased Use of Parenteral Antimicrobial Drugs

CME Questions

1. You are called to evaluate a female patient with a complicated medical history who is admitted to the hospital with fever and bacteremia. She has a history of diabetes mellitus and chronic kidney disease. She was treated with antibiotics for osteomyelitis of the left leg 1 year ago, and she underwent a right total knee arthroplasty last month. A preliminary result for her blood culture demonstrates a *Corynebacterium* species. What should you consider regarding infections with corynebacteria?

A. Corynebacteria are part of the normal skin flora
B. *Corynebacterium striatum* is the most commonly isolated corynebacteria in infections
C. *C. striatum* is invariably pathogenic in promoting infection
D. *C. striatum* has a long history of resistance to multiple antibiotics

2. You consider potential sources for this patient's infection. Which of the following infection sites with *Corynebacterium striatum* was most commonly judged as clinically meaningful in the current study?

A. Blood
B. Bone
C. Bronchoalveolar lavage
D. Surgical site

3. You need to recommend antibiotic therapy for this patient. What should you consider regarding resistance patterns of *Corynebacterium striatum* in the current study?

A. Approximately one-third of patients treated with vancomycin experienced clinical failure
B. Most isolates were from outpatient settings
C. Nearly three-quarters of isolates were resistant to all antibiotics tested
D. Rates of resistance were much higher among isolates from inpatient vs outpatient settings

4. Which of the following statements comparing patients with *Corynebacterium striatum* infections vs patients with coagulase-negative staphylococci in the current study is most accurate?

A. Patients with *C. striatum* infections received a longer average course of parenteral antibiotics
B. Patients with *C. striatum* infections were more likely to be treated with multiple antibiotics
C. Patients with *C. striatum* infections were less likely to experience complications related to infections
D. Patients with *C. striatum* experienced fewer complications related to the use of vancomycin specifically

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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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.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

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Research. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Research Letters Reporting Cases, Outbreaks, or Original Research. Starting with the January 2017 volume, EID will publish letters that report cases, outbreaks, or original research as Research Letters. Authors should provide a short abstract (50-word maximum), references (not to exceed 10), and a short biographical sketch. These letters should contain no more than 850 words (including the abstract) and may include either 1 figure or 1 table. Do not divide Research Letters into sections.

Letters Commenting on Articles. Letters commenting on articles should contain a maximum of 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication.

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Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

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Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

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