Co-infection with Dengue Virus and Pandemic (H1N1) 2009 Virus

To the Editor: Dengue is a mosquito-borne viral infection caused by 4 related dengue viruses. Each of these viruses is capable of causing classic dengue fever or dengue hemorrhagic fever (DHF), but may also cause non-specific febrile illnesses. As a result, dengue is often difficult to diagnose clinically, especially because peak dengue season often coincides with that of other common febrile illnesses in tropical regions (1). Concurrent outbreaks of influenza and dengue have been reported (2,3); this circumstance often leads to delayed recognition of the presence of one or other disease in the community.

In April 2009, a new strain of influenza A virus known as pandemic (H1N1) 2009 virus was first detected in the United States (4). Pandemic (H1N1) 2009 infections were first detected in Puerto Rico in June 2009, and 59 deaths caused by the virus have been confirmed to date. This influenza outbreak coincided with the typical dengue season in Puerto Rico, which led to diagnostic difficulties; both infections disproportionately affected young persons, who often had similar, nonspecific symptoms.

We describe a case of laboratory-confirmed co-infection of dengue virus and pandemic (H1N1) 2009, and discuss the difficulties in distinguishing the 2 illnesses clinically. A 33-year-old woman (healthcare worker) in Ponce, Puerto Rico, sought treatment at an emergency department of a hospital in the southern part of the island with a 3-day history of febrile illness. Her symptoms began with throat irritation and earache; subsequently, cough, fever, and headache developed. She reported palpitations and generalized malaise but no other symptoms. The patient had no notable medical his-

References
1. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet. 2005;365:1073–86.
2. Karch H, Bielaszewska M. Sorbitol-fermenting Shiga-toxin–producing Escherichia coli O157:H(–) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. J Clin Microbiol. 2001;39:2043–9. DOI: 10.1128/JCM.39.6.2043-2049.2001
3. Orth D, Grif K, Zimmerhackl LB, Wurzner R. Sorbitol-fermenting Shiga-toxin—producing Escherichia coli O157 in Austria. Wien Klin Wochenschr. 2009;121:108–12. DOI: 10.1007/s00508-008-1133-2
4. Rosser T, Dransfield T, Allison L, Hanson M, Holden N, Evans J, et al. Pathogenic potential of emergent sorbitol-fermenting Escherichia coli O157:NM. Infect Immun. 2008;76:5598–607. DOI: 10.1128/IAI.01180-08
5. Alpers K, Werber D, Frank C, Koch J, Friedrich AW, Karch H, et al. Sorbitol-fermenting enterohaemorrhagic Escherichia coli O157:H causes another outbreak of haemolytic uraemic syndrome in children. Epidemiol Infect. 2009;137:389–95. DOI: 10.1017/S0950268808001465
6. Ammon A, Petersen LK, Karch H. A large outbreak of hemolytic uremic syndrome caused by an unusual sorbitol-fermenting strain of Escherichia coli O157:H. J Infect Dis. 1999;179:1274–77.
7. Pollock KGJ, Young D, Beattie TJ, Todd WTA. Clinical surveillance of thrombotic microangiopathies in Scotland, 2003–2005. Epidemiol Infect. 2008;136:115–21. DOI: 10.1017/S0950268807008217
8. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. N Engl J Med. 2000;342:1930–6. DOI: 10.1056/NEJM200006293422601
9. Bielaszewska M, Prager R, Zhang W, Friedrich AW, Mellmann A, Tschape H, et al. Chromosomal dynamism in progeny of outbreak-related sorbitol-fermenting enterohaemorrhagic Escherichia coli O157:NM. Appl Environ Microbiol. 2006;72:1900–9. DOI: 10.1128/AEM.72.3.1900-1909.2006

Address for correspondence: Kevin G.J. Pollock, Health Protection Scotland, Clifton House, Clifton Place, Glasgow, Scotland G3 7LN, UK; email: kevin.pollock@nhs.net

 DOI: 10.3201/eid1605.091919

LETTERS
tory and denied taking any medicines apart from over-the-counter antipyretics. She reported recent exposure to influenza at work and multiple recent mosquito bites. On physical examination, she had a temperature of 37°C, a heart rate of 91 bpm, and blood pressure of 125/82 mm Hg. A tourniquet test result was positive. Her pharynx was erythematous without exudate, and she had rhinorrhea. She had no lymphadenopathy, rash, petechiae, or purpura. Several small, red papules, which the patient described as recent mosquito bites, were on her legs. The remainder of her examination showed no unusual findings.

Laboratory studies showed a leukocyte count of 5,300 cells/mm³ with a normal differential count, hematocrit 35.2%, and thrombocyte count of 239,000 cells/μL. Results of a chest radiograph was unremarkable. A nasopharyngeal swab was positive for influenza A virus by rapid test. Nasopharyngeal and serum samples were sent to the Centers for Disease Control and Prevention (Dengue Branch) for influenza and dengue testing. The patient was diagnosed with suspected pandemic (H1N1) 2009 infection and prescribed oseltamivir for 5 days. She returned for a follow-up visit 12 days after the onset of symptoms. She reported having 2 more days of fever after her initial visit, but had no rash, petechiae, bleeding, or progression of respiratory symptoms. A second serum specimen was obtained during this visit.

The initial serum specimen was positive for dengue virus by serotype-specific, singleplex, real-time reverse transcription–PCR (5). Her nasopharyngeal specimen was positive by PCR for pandemic (H1N1) 2009 influenza. The second, convalescent-phase, serum specimen was negative for dengue immunoglobulin (Ig) M by IgM antibody–capture ELISA. The acute-phase and convalescent-phase samples were positive for IgG against dengue by ELISA (6), which indicated a secondary dengue infection. IgG titers exceeded the limits of the test for acute-phase and convalescent-phase samples, showing unusually elevated levels of IgG against dengue.

Distinguishing dengue and influenza by clinical features alone can be difficult. In an investigation of simultaneous dengue and influenza A outbreaks in Puerto Rico in 1977, similar percentages of persons with confirmed dengue and confirmed influenza had classic dengue symptoms (2). Hemorrhagic manifestations, like those typically seen in DHF, have been reported with influenza A in prior outbreaks (7,8) and with pandemic (H1N1) 2009 (Centers for Disease Control and Prevention, unpub. data). Previous influenza A outbreaks were initially believed to be outbreaks of DHF until careful laboratory investigation proved otherwise (8). Our patient did not have the typical signs and symptoms of dengue (rash, eye pain, thrombocytopenia, arthralgia, petechiae, or bleeding) that would differentiate her condition from that of patients with other febrile illnesses. She did have a positive tourniquet test result and fever, which have been advocated as screening criteria for dengue infection in children, at the time of initial examination (9). Data for the specificity and sensitivity of these criteria in adults are sparse, however, and some studies have shown a high incidence of positive tourniquet test results in patients with laboratory-confirmed influenza (7,8).

Our report demonstrates that co-infection with dengue virus and pandemic (H1N1) 2009 can occur. Previous studies also have shown cases of probable co-infection with seasonal influenza and dengue (1,10), including 1 fatal case (f). Because many dengue-endemic countries are experiencing pandemic (H1N1) 2009 outbreaks, providers should consider the possibility of viral co-infection, especially in severe cases, and should consider testing for both viruses.

Eric Lopez Rodriguez, Kay M. Tomaszek, Christopher J. Gregory, Jorge Munoz, Elizabeth Hunsperger, Olga D. Lorenzi, Jorge Gutierrez Irizarry, and Carlos Garcia-Gubern

Author affiliations: Hospital San Lucas/ Ponce School of Medicine, Ponce, Puerto Rico, USA (E.L. Rodriguez, J.G. Irizarry, C. Garcia-Gubern); and Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA (K.M. Tomaszek, C.J. Gregory, J. Munoz, E. Hunsperger, O.D. Lorenzi)

DOI: 10.3201/eid1605.091920

References

1. Leelarasamee A, Chupaprawan C, Chenchittikul M, Udompanthurat S. Etiologies of acute undifferentiated febrile illness in Thailand. J Med Assoc Thai. 2004;87:464–72.
2. Morens DM, Rigau-Perez JG, Lopez-Corrales RH, Moore CG, Ruiz-Tiben ER, Sather GE, et al. Dengue in Puerto Rico 1977: public health response to characterize and control an epidemic of multiple serotypes. Am J Trop Med Hyg. 1986;35:197–211.
3. Silarug N, Foy HM, Kupradinon S, Rojasuphot S, Nisalak A, Ponguansat Y. Epidemic of fever of unknown origin in rural Thailand caused by influenza A (H1N1) and dengue fever. Southeast Asian J Trop Med Public Health. 1990;21:61–7.
4. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009;360:2605–15. DOI: 10.1056/NEJMoa0903810
5. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol. 2005;43:4977–83. DOI: 10.1128/JCM.43.10.4977-4983.2005
6. Miagostovich MP, Nogueira RM, dos Santos FB, Schatzmayr HG, Araujo ES, Vorndam V. Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis. J Clin Virol. 1999;14:183–9. DOI: 10.1016/S1386-6532(99)00059-1
7. Thaung U, Ming CK, Thein M. Dengue haemorrhagic fever in Burma. South-East Asian J Trop Med Public Health. 1975;6:580–91.
8. Thein S. Haemorrhagic manifestations of influenza A infections in children. J Trop Med Hyg. 1975;78:78–80.
9. Cao XT, Ngo TN, Wills B, Kneen R, Nguyen TT, Ta TT, et al. Evaluation of the World Health Organization standard tourniquet test and a modified tourniquet test in the diagnosis of dengue infection in Viet Nam. Trop Med Int Health. 2002;7:125–32. DOI: 10.1046/j.1365-3156.2002.00841.x

10. Suttinont C, Losuwanaluk K, Niwatayakul K, Hoontrakul S, Intaranongpi W, Silpaksorn S, et al. Causes of acute, undifferentiated, febrile illness in rural Thailand: results of a prospective observational study. Ann Trop Med Parasitol. 2006;100:363–70. DOI: 10.1179/136485906X112158

Address for correspondence: Kay M. Tomashek, Dengue Branch, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, PR 00920, USA; email: kct9@cdc.gov

---

**Bovine Tuberculosis in Buffaloes, Southern Africa**

To the Editor: Emergence of bovine tuberculosis (TB) in wildlife in southern Africa has implications not only for the conservation of the wildlife species affected (1) but also for the health of humans and livestock living at the wildlife–livestock–human interface (2). Bovine TB in South Africa’s Kruger National Park was first found in African buffaloes (*Syncerus caffer*) in 1990 (3) and likely entered the park by cattle-to-buffalo transmission (4). Bovine TB infection has been spreading northward; in 2003, infection was confirmed in a buffalo ≈60 km south of the Limpopo River. In 2005, a case was confirmed only 6 km south of the river (D. Keet, unpub. data). In 2008, we isolated *Mycobacterium bovis* from African buffaloes in Zimbabwe.

During October 9–13, 2008, a total of 38 buffaloes from 4 herds were captured in Gonarezhou National Park (south of the Mabalauta area; 22.0553°S, 31.4265°E). Blood samples were collected, sampled buffaloes were marked and released, and 3 adult females in each herd were equipped with radio collars. Buffalo tissue samples were collected, packaged, shipped, and handled at the Agricultural Research Council–Onderstepoort Veterinary Institute according to procedures recommended for controlling the spread of foot-and-mouth disease virus. Interferon-γ assay (5) results were positive for bovine TB for 4 (10.5%) buffaloes: 2 adult females and 1 young adult male from the same herd and 1 adult female from another herd.

Four months later, a radio-collared adult female and the young adult male, each of which had had positive interferon-γ assay results, were sedated and euthanized, and necropsies were performed in the field. Samples for histopathologic examination and culture were collected from lymph nodes of the head and thorax. No acid-fast organisms were detected, but the histologic findings were strongly suggestive of paucibacillary TB. *M. bovis* was isolated from the retropharyngeal lymph nodes of both buffaloes and from the bronchial and head lymph nodes of 1 of them. Both isolates were typed by analysis of variable number of tandem repeat (VNTR) sequences at 6 loci (exact tandem repeat A–F) (6) and compared with the VNTR profiles of ≈75 isolates from Kruger. All isolates showed an identical VNTR profile (7544*52.3*), which suggests an epidemiologic link between the *M. bovis* infections in the 2 parks. However, the exact tandem repeat loci had lower discriminatory power among Kruger isolates than did IS6110 restriction fragment length polymorphism typing (T. Hlokwe, unpub. data) (4). A typing regimen comprising different typing methods and markers will be useful for more accurately determining the genetic relationship between the isolates from the 2 parks, Gonarezhou and Kruger.

The confirmation of results for bovine TB–infected buffaloes in Zimbabwe (Gonarezhou National Park) raises several questions regarding the transboundary spread of animal disease and has considerable management implications for the Great Limpopo Transfrontier Conservation Area. The most likely scenario is buffalo-to-buffalo contact across the boundary because the bovine TB cases reported here were located <45 km from the unfenced northern boundary of Kruger National Park. Buffaloes, especially bulls and young heifers, frequently move from herd to herd and may contribute to the spread of *M. bovis* by mixing with unexposed herds (7).

Although transboundary movements of buffaloes between Kruger and Gonarezhou have not been specifically documented, uncontrolled movements across the Limpopo River do occur (de Garine-Wichatitsky, unpub. data). However, >12 wild species in Kruger have now been found to be infected by bovine TB (2). Most of these species are probably not effective sources of *M. bovis* infection for buffaloes, but the disease epidemiology could rely on multihost reservoirs (8). Thus, a second scenario could be a buffalo-to-unidentified wild species–to-buffalo pathway, because species like greater kudu (*Tragelaphus strepsiceros*) appear to be able to maintain, spread, and even drive a bovine TB epidemic (4,9). A third scenario involves movement of infected livestock across the boundaries of the 3 countries of the Great Limpopo Transfrontier Conservation Area, resulting in cattle-to-buffalo transmission of bovine TB. As a last scenario, we cannot rule out the possibility that bovine TB infection of buffaloes has remained silent and undetected for decades in Zimbabwe.

The management implications of bovine TB in buffaloes in Gonarezhou National Park are considerable. Once bovine TB is established in a native free-ranging maintenance host, eradication is unlikely (2,10). Evaluation...