

Asian lineage and had 99% identity with sequences of ZIKV isolated in French Polynesia in 2013 (10).

Serum specimens from both patients were cultured on Vero cells, and supernatants were evaluated by RT-PCR. Each specimen was positive only for DENV, which was probably caused by low viral loads for ZIKV.

We report co-infection of 2 patients with DENV and ZIKV; each patient was infected with a different DENV serotype. No synergistic effects of the 2 viral infections were observed because both patients were not hospitalized and recovered after a mild clinical course.

During this outbreak, patients in New Caledonia were tested for DENV, chikungunya virus, and ZIKV within the framework of the arboviruses sentinel network, which enabled detection of co-infections. Thus, clinicians should be aware of infections with multiple pathogens in the differential diagnosis of dengue-like illness, especially in patients who returned from tropical regions. This diagnostic procedure could be improved by using multiplex RT-PCR for travelers, given the frequent co-circulation of multiple arboviruses in tropical regions.

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## Fatal Meningoencephalitis in Child and Isolation of *Naegleria fowleri* from Hot Springs in Costa Rica

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**To the Editor:** Primary amebic meningoencephalitis (PAM) is an acute and fulminant disease caused by *Naegleria fowleri*, an amphizoic amoeba belonging to the family *Vahlkampfiidae*. About 235 PAM cases have been described worldwide, most in children and immunocompetent young adults (1,2). The infection occurs through the nose; the amoeba enters through the nasal passages and ascends the olfactory nerve until it reaches the olfactory bulb of the central nervous system. The incubation period for PAM ranges from 5 to 7 days, and infection leads to death within a week. Symptom onset is abrupt, with bifrontal or bitemporal headaches, fever, and stiff neck, followed by nausea, vomiting, irritability, and fatigue. The mortality rate is as high as 95%; few cases of survival have been reported (2,3).

The epidemiology of most reported cases of PAM indicates an association between aquatic activity and infection. Swimming, free diving, and immersion in hot springs, spas, and warm, freshwater bodies have been related to the acquisition of *N. fowleri* amebae (3). To date, most cases have been reported from subtropical or temperate zones, and underreporting in tropical regions has been cited (2); in the Americas, PAM has been reported in Venezuela, Brazil, Cuba, Mexico, and the United States. Most infections occur after swimming in water naturally heated by the sun or geothermal water (2).

On July 29, 2014, an 11-year old boy, a resident of Florida, USA, was admitted to a hospital for an illness that began after he returned from vacation in La Fortuna, San Carlos, Costa Rica. The infection was fulminant, and the boy died <72 hours after admission. Tests conducted by the hospital confirmed PAM (4). The background of the case indicates that the boy spent 1 week in Costa Rica and stayed for 4 days in La Fortuna area. The onset of symptoms occurred 3 days after he left Costa Rica, which is consistent with the incubation period for PAM. Furthermore, the boy's family stated that in Florida they did not allow him to swim in lakes or rivers because of the known risk of amebal infection (4,5), which further suggests that the infection may have occurred in Costa Rica.

The Florida Department of Health was alerted about the case, and personnel from the Centers for Disease Control and Prevention contacted the Costa Rica Ministry of Health to identify the potential source of infection. Water samples from a swimming pool, a river pond, and a hot spring from the resort visited by the boy in La Fortuna were collected and analyzed within 12 hours. The samples were filtered through nitrocellulose membranes with 0.45- $\mu$ m pore diameter, and the filters were placed over 1.5% non-nutritive agar plates, supplemented with *Escherichia coli* (6). Plates were incubated at 35°C for 7 days and observed daily. After 3–4 days of incubation, cysts and trophozoites with morphologic characteristics compatible to *Naegleria* spp. were observed in the samples from the hot spring and the river pond. Cysts were round and 10–12  $\mu$ m in size, with a *Limax*-type nucleus. Trophozoites showed very active movement, with wide pseudopods of rapid formation. Results of an exflagellation test were positive, and a thermotolerance test showed organism growth at 44°C–45°C (7).

To molecularly characterize the isolate at the species level, we extracted DNA from the culture using the method described by Reyes-Battle et al. (8). A specific PCR for *N. fowleri* was performed, and the complete internal transcribed spacer region was amplified as previously described (1). The 18S rDNA gene of this free-living ameba was also amplified by using the universal eukaryotic P2 and P3r primer pair (9). *N. fowleri* Lee ATCC 30894 DNA

was used as a positive control in the PCR reactions. The obtained PCR products were purified and sequenced by using a MEGABACE 1000 Automatic Sequencer (Healthcare Biosciences, Barcelona, Spain) in the University of La Laguna Sequencing Service (SEGAI, University of La Laguna). Sequences were obtained twice from both strands and aligned by using MEGA 5.0 software (10). Moreover, nucleotide similarity search was performed by BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>) of the sequenced amplicons against ameba species. These analyses revealed 97%–98% homology with other *N. fowleri* strains available in GenBank. The sequence isolated in this case has been deposited in GenBank (accession no. KM658156).

In summary, this investigation identified an *N. fowleri* ameba in water sources at a resort in Costa Rica that had been visited by a child from the United States who died of PAM as a result of *N. fowleri* infection. These amoebas pose a high risk to human health and were found in an area frequented by tourists, which should alert health authorities in Costa Rica of the need for monitoring locations such as this for possible contamination and notifying the public of the risk for infection.

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## Genome Sequence of Enterovirus D68 and Clinical Disease, Thailand

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**To the Editor:** Outbreaks of respiratory enterovirus D68 infection were particularly severe in 2014 in the United States. Wylie et al. recently analyzed the whole genomes of clinical strains from St. Louis, Missouri, USA, and the US Centers for Disease Control and Prevention (Atlanta, GA, USA) (1). Results showed that the most closely related genomes to the St. Louis strains were strains CU134 (GenBank accession no. KM361523) and CU171 (KM361524), which were identified in Thailand in 2011 (2,3).

To provide additional background regarding the origin of these strains from Thailand, including 1 additional CU70 strain (KM361525), we report clinical features of the 3 patients from which the strains were derived. This additional information might assist clinical scientists in early recognition of enterovirus D68 infections and provide insight into viral pathogenesis.

The 3 patients (1 boy and 2 girls; age range 7–24 months) were hospitalized during July–September 2011 with pneumonia. At admission, they had cough, rhinorrhea, and dyspnea. Fever, crepitation, and wheezing were observed in patients CU70 and CU134. Patients CU134 and CU171 had suprasternal and subcostal retraction, and

patient CU171 had signs of nasal flaring and inspiratory stridor (he has an underlying double aortic arch). Chest radiographs showed perihilar infiltration for patients CU70 and CU134. Hemocultures and test results for respiratory viruses for all 3 patients were negative (2).

Physicians provided respiratory support to all 3 patients by oxygen flow and nebulized bronchodilator. In addition, patient CU171 was given nebulized adrenaline, an intravenous corticosteroid, and intravenous antimicrobial drugs. Patients CU70 and CU134 were discharged after 3 and 8 days, respectively. However, patient CU171 remained hospitalized for 16 days.

Nasopharyngeal aspirates obtained from the 3 patients were subjected to next-generation sequencing and genomic analysis. From the total number of analyzed reads for isolates from patients CU70 (n = 10,482), CU134 (n = 11,504), and CU171 (n = 4,545), ≈1,100–1,600 enterovirus D68 sequence reads were identified. Anellovirus sequences (n < 60) were found in aspirates from patients CU70 and CU171. Furthermore, aspirates from patients CU134 and CU171 contained human rhinovirus B (n = 73) and human rhinovirus C (n = 15), respectively (2). Future genomic studies and surveillance of enterovirus D68 will be helpful in monitoring its spread next season.

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### Correction: Vol. 20, No. 11

The name of author Anne-Marie Roque-Afonso was listed incorrectly in the article Foodborne Transmission of Hepatitis E Virus from Raw Pork Liver Sausage, France (C. Renouet et al.). The article has been corrected online ([http://wwwnc.cdc.gov/eid/article/20/11/14-0791\\_article.htm](http://wwwnc.cdc.gov/eid/article/20/11/14-0791_article.htm)).