

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.

Acknowledgments

We thank C. Goarant for providing scientific advice and critically revising the manuscript and D. Baudon for providing scientific support.

This study was supported by the Institut Pasteur and the New Caledonia Government.

References

- World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control, 2009 [cited 2014 Nov 4]. http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf
- Dupont-Rouzeyrol M, Aubry M, O Connor O, Roche C, Gourinat AC, Guigon A, et al. Epidemiological and molecular features of dengue virus type-1 in New Caledonia, South Pacific, 2001–2013. *Virology*. 2014;11:61. <http://dx.doi.org/10.1186/1743-422X-11-61>
- Direction des Affaires Sanitaires et Sociales de Nouvelle-Calédonie. Situation sanitaire de la Nouvelle-Calédonie. La dengue, 2014 [cited 2014 Nov 4]. www.dass.gouv.nc/portal/page/portal/dass/observatoire_sante/veille_sanitaire/Dengue
- Dick GW, Kitchen SF, Haddock AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg*. 1952;46: 509–20. [http://dx.doi.org/10.1016/0035-9203\(52\)90042-4](http://dx.doi.org/10.1016/0035-9203(52)90042-4)
- Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis*. 2008;14:1232–9. <http://dx.doi.org/10.3201/eid1408.080287>
- Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French Polynesia, South Pacific, 2013. *Emerg Infect Dis*. 2014;20:1085–6. <http://dx.doi.org/10.3201/eid2011.141380>
- Vinodkumar CS, Kalappannavar NK, Basavarajappa KG, Sanjay D, Gowli C, Nadig NG, et al. Episode of coexisting infections with multiple dengue virus serotypes in central Karnataka, India. *J Infect Public Health*. 2013;6:302–6. <http://dx.doi.org/10.1016/j.jiph.2013.01.004>
- Caron M, Paupy C, Grard G, Becquart P, Mombo I, Nso BB, et al. Recent introduction and rapid dissemination of chikungunya virus and dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. *Clin Infect Dis*. 2012;55:e45–53. <http://dx.doi.org/10.1093/cid/cis530>
- Cao-Lormeau VM, Roche C, Musso D, Mallet HP, Dalipanda T, Dofai A, et al. Dengue virus type 3, South Pacific Islands, 2013. *Emerg Infect Dis*. 2014;20:1034–6. <http://dx.doi.org/10.3201/eid2006.131413>
- Baronti C, Piorowski G, Charrel RN, Boubis L, Leparç-Goffart I, de Lamballerie X. Complete coding sequence of Zika virus from a French Polynesia outbreak in 2013. *Genome Announc*. 2014; 2:pil: e00500-14. <http://dx.doi.org/10.1128/genomeA.00500-14>

Address for correspondence: Myrielle Dupont-Rouzeyrol, Unité de Recherche et d'Expertise–Dengue et autres Arboviroses, Institut Pasteur de Nouvelle-Calédonie, 9–11 Ave Paul Doumer, BP 61, 98845 Noumea, New Caledonia; email: mdupont@pasteur.nc

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

Elizabeth Abrahams-Sandí,
Lissette Retana-Moreira, Alfredo Castro-Castillo,
María Reyes-Batlle, Jacob Lorenzo-Morales

Author affiliations: University of Costa Rica, San Pedro, Costa Rica (E. Abrahams-Sandí, L. Retana-Moreira, A. Castro-Castillo); University of Costa Rica Centro de Investigación en Enfermedades Tropicales, San Pedro (E. Abrahams-Sandí, L. Retana-Moreira, A. Castro-Castillo); University of La Laguna Institute of Tropical Diseases and Public Health of the Canary Islands, Tenerife, Spain (M. Reyes-Batlle, J. Lorenzo-Morales)

DOI: <http://dx.doi.org/10.3201/eid2102.141576>

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- μ 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 μ 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.

This study was supported by project 803B4050, Vicerrectoría de Investigación, University of Costa Rica. J.L.M. was supported by the Ramón y Cajal Subprogramme of the Spanish Ministry of Economy and Competitiveness RYC201108863. Microbiological Compliance Laboratories made their facilities available for sample filtration.

References

- Heggie TW. Swimming with death: *Naegleria* infections in recreational waters. *Travel Med Infect Dis*. 2010;8:201–6. <http://dx.doi.org/10.1016/j.tmaid.2010.06.001>
- De Jonckheere JF. Origin and evolution of the worldwide distributed pathogenic amoeboid flagellate *Naegleria fowleri*. *Infect Genet Evol*. 2011;11:1520–8. <http://dx.doi.org/10.1016/j.meegid.2011.07.023>
- Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunol Med Microbiol*. 2007;50:1–26. <http://dx.doi.org/10.1111/j.1574-695X.2007.00232.x>
- Arias L. Florida child dies after contracting ameba from Costa Rica hot springs [cited 2014 Aug 22]. <http://www.ticotimes.net/2014/08/19/florida-child-dies-after-contracting-ameba-from-costa-rican-hot-springs>
- Elijah R. Sandford family speaks about losing son to deadly ameba [cited 2014 Aug 22]. <http://www.myfoxorlando.com/story/26288284>
- Schuster FL. Cultivation of pathogenic and opportunistic free living amoebas. *Clin Microbiol Rev*. 2002;15:342–54. <http://dx.doi.org/10.1128/CMR.15.3.342-354.2002>
- Castro CA, Guerrero BOM. Técnicas de diagnóstico parasitológico. San Pedro (Costa Rica): Editorial de la Universidad de Costa Rica; 2004.
- Reyes-Battle M, Todd CD, Martín-Navarro CM, López-Arencibia A, Cabello-Vilchez AM, González AC, et al. Isolation and characterization of *Acanthamoeba* strains from soil samples

- in Gran Canaria, Canary Islands, Spain. *Parasitol Res.* 2014;113:1383–8. <http://dx.doi.org/10.1007/s00436-014-3778-z>
9. Mulec J, Vaupotič J, Walochnik J. Prokaryotic and eukaryotic airborne microorganisms as tracers of microclimatic changes in the underground (Postojna Cave, Slovenia). *Microb Ecol.* 2012;64:654–67. <http://dx.doi.org/10.1007/s00248-012-0059-1>
 10. Tamura K, Peterson D, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol.* 2011;28:2731–9. <http://dx.doi.org/10.1093/molbev/msr121>

Address for correspondence: Elizabeth Abrahams Sandí, Department of Parasitology, San Pedro/Mts. Oca, Costa Rica; email: elizabeth.abrahams@ucr.ac.cr

Genome Sequence of Enterovirus D68 and Clinical Disease, Thailand

Sompong Vongpunsawad,
Slinporn Prachayangprecha, Jira Chansaenroj,
Bart L. Haagmans, Saskia L. Smits,
Yong Poovorawan

Author affiliations: Chulalongkorn University, Bangkok, Thailand (S. Vongpunsawad, S. Prachayangprecha, J. Chansaenroj, Y. Poovorawan); and Erasmus Medical Center, Rotterdam, the Netherlands (B.L. Haagmans, S.L. Smits)

DOI: <http://dx.doi.org/eid2102.141742>

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.

This study was supported by Chulalongkorn University, the Commission on Higher Education, and the Thailand Research Fund.

References

1. Wylie KM, Wylie TN, Orvedahl A, Buller RS, Herter BN, Magrini V, et al. Genome sequence of enterovirus D68 from St. Louis, Missouri, USA. *Emerg Infect Dis.* 2015; 21:384.
2. Prachayangprecha S, Schapendonk CM, Koopmans MP, Osterhaus AD, Schürch AC, Pas SD, et al. Exploring the potential of next-generation sequencing in detection of respiratory viruses. *J Clin Microbiol.* 2014;52:3722–30. <http://dx.doi.org/10.1128/JCM.01641-14>
3. Linsuwanon P, Puenpa J, Suwannakarn K, Auksornkitti V, Vichiwattana P, Korkong S, et al. Molecular epidemiology and evolution of human enterovirus serotype 68 in Thailand, 2006–2011. *PLoS ONE.* 2012;7:e35190. <http://dx.doi.org/10.1371/journal.pone.0035190>

Address for correspondence: Yong Poovorawan, Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand; email: yong.p@chula.ac.th

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).