

Probable Importation of Dengue Virus Type 4 to Angola from Brazil

To the Editor: The prevalence of dengue virus (DENV) infection in Africa may have been grossly underestimated for many years (1). Even though published reports are scarce, dengue has been documented in at least 34 countries in Africa (2). The role of travelers as sentinels of infectious disease has proven to be invaluable in this regard; dengue in returning travelers was the only evidence for local DENV transmission for 12 of these countries.

During April 2013, simultaneous reports of travelers returning from Luanda, Angola, with dengue fever emerged from Israel, several countries in Europe, Canada, and South Africa; PCR and sequencing of viral RNA confirmed the causative species to be DENV type 1 (DENV-1) (3). Concomitantly, a large outbreak of dengue was confirmed by local authorities in Luanda, and DENV-1 was isolated in samples from local residents (4). The origin of the outbreak strain was not ascertained, but phylogenetic analysis suggested that it had close affinity with isolates from West Africa (4,5). These reports involved almost 100 travelers and >500 residents of Luanda who became within a short time. Active DENV transmission and occurrence of new cases in Luanda were still ongoing during early July 2013 (6), but since then, to our knowledge, no additional local data on DENV activity in Luanda have been published.

We report 3 new cases of dengue fever acquired in Luanda during December 2013–February 2014. Two cases occurred in travelers who returned to Israel, and 1 occurred in a traveler who returned to Germany. All 3 cases occurred in middle-aged businessmen who had traveled to

Angola and who were hospitalized within days of returning to their home countries because of signs and symptoms of dengue fever. None of the case-patients met criteria for severe dengue, and all recovered uneventfully. Acute DENV infection was confirmed by serologic testing in all 3 patients and by positive results for a nonstructural protein 1 serum antigen test in 2 travelers.

In a serum sample taken from 1 of the travelers from Israel on the second day of fever, DENV RNA was detected by using reverse transcription PCR. This strain was found to belong to DENV type 4 (DENV-4). Phylogenetic analysis was performed, and multiple sequence alignment of this DENV-4 sequence, in comparison to other DENV-4 sequences retrieved from GenBank, was performed by using the Sequencher 5.0 program (Gencodes Corporation, Ann Arbor, MI, USA). A maximum-likelihood phylogenetic tree was inferred from the sequence alignment by using ClustalX (<http://www.clustal.org>), and the robustness of the tree was assessed by 1,000 bootstrap replications. The tree was visualized and produced by using NJ plot software

(<http://doua.prabi.fr/software/njplot>). Results showed that RNA from this isolate was most closely related to that of a DENV-4 strain identified in 2010 in Boa Vista, the capital of Roraima State in the Amazon Region of Brazil (7) (Figure).

DENV-4 infection was previously reported in Africa in 1986, when 2 cases were identified in travelers returning from Senegal (8), but to our knowledge, no other cases have been reported in western or southwestern Africa. During the April 2013 dengue outbreak in Luanda, only DENV-1 was isolated (3,4). After that outbreak, a study conducted on the basis of modeling of international commercial flight data to and from Angola suggested that DENV would most likely have been imported from Latin America (5). Our finding, a year later, of DENV-4 in Angola that was closely related to strains from Brazil appears to vindicate this modeling system. During the past decade, Brazil had been experiencing a consistent increase in dengue epidemics and in severity of disease (9). Brazil's large and growing economy, its increasingly prominent place in world trade, and its growing tourism industry high-

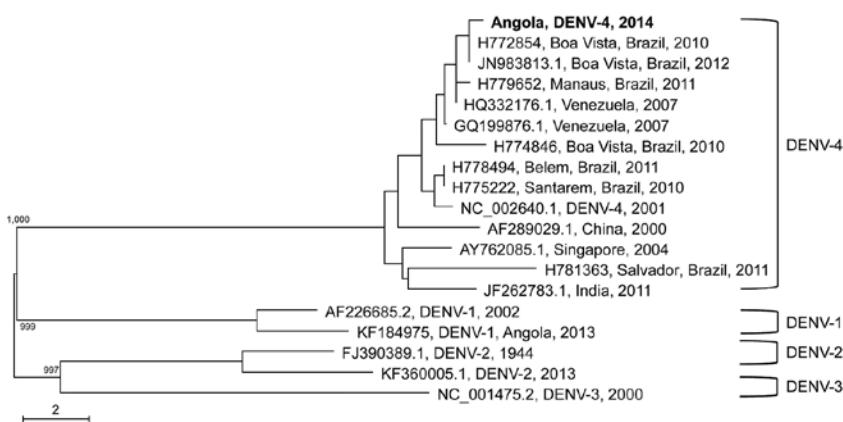


Figure. Phylogenetic analysis of a dengue virus (DENV) type 4 strain isolate (boldface) from a patient in Israel who apparently acquired DENV in Angola in 2014, showing close relationship with isolates from Brazil. The DENV isolate was aligned with representative DENV sequences from around the world, representing serotypes 1–4. Reference strains, downloaded from public databases (<http://www.ncbi.nlm.nih.gov/nucleotide>), are identified by accession number, place, and year of isolation (DENV-4 isolates) or by serotype, accession number, and year of isolation (DENV-1–3 isolates). Specific branches indicate bootstrap values. Scale bar indicates percentage identity difference.

light the country's potential role in the global circulation of DENV.

Our findings are corroborated by a recently reported case of dengue in a traveler from Portugal that was acquired in Luanda concomitantly with our cases and also found to be caused by DENV-4 (10). In light of the apparent introduction of DENV-4 to Angola, probably from Brazil, health authorities should be encouraged to enhance surveillance and vector control efforts. In addition, health practitioners treating travelers returning from Angola should be aware of the risk for DENV infection.

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***Borrelia garinii* and *Rickettsia monacensis* in *Ixodes ricinus* Ticks, Algeria**

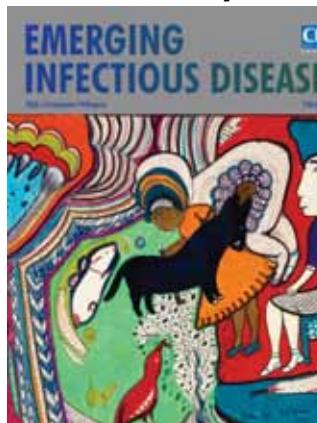
To the Editor: Lyme disease (Lyme borreliosis) is caused by a group of related spirochetes (*Borrelia burgdorferi* sensu lato) that include ≥ 11 species (1). In northern Africa, the main vector of Lyme disease in Europe (*Ixodes ricinus* ticks) is also present, and this disease has been suspected to be present in this region of Africa (2). Twenty-one cases of Lyme disease were reported in Algiers, Algeria, during 1996–1999 (3). However, these cases were diagnosed by detection of only serum antibodies against *B. burgdorferi* by ELISA without confirmation by Western blotting.

I. ricinus ticks are also known to harbor spotted fever group rickettsiae, including *Rickettsia monacensis*, which was detected in Algeria in 2009 (4). This rickettsia has been recently identified as a human pathogen in Spain and Italy (5).

To investigate Lyme disease and tickborne rickettsioses transmitted by *I. ricinus* ticks in northeastern Algeria, we collected ticks by using the flag method in El Ghora (Bougous, El Tarf) (36°39'34"N, 8°22'10"E). Ectoparasites were collected in March 2012 and identified to genus and species by using taxonomic morphologic keys (6).

Total genomic DNA was isolated by using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) and BioRobot EZ1 (QIAGEN) as described by the manufacturer. DNA was used as template for quantitative real-time PCR. We used the RKND03 system, which is specific for the *gltA* gene of *Rickettsia* spp. (7), and the Bor16S system, which is specific for the *rrs* gene of *Borrelia* spp. (8). Real-time PCRs were performed by using the CFX96 Real Time System C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Singapore).

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