

## RAPID COMMUNICATIONS

# Zika virus infection in a traveller returning from the Maldives, June 2015

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**We report a Zika virus (ZIKV) infection in a patient with fever and rash after returning to Finland from Maldives, June 2015. The patient had dengue virus (DENV) IgG and IgM antibodies but pan-flavivirus RT-PCR and subsequent sequencing showed presence of ZIKV RNA in urine. Recent association of ZIKV with microcephaly highlights the need for laboratory differentiation of ZIKV from DENV infection and the circulation of ZIKV in areas outside its currently known distribution range.**

## Case report

A 37-year-old Finnish man returned with his family from a half-a-year work-related stay in the Maldives (in Dhiffushi island, situated in North Malé atoll as the capital Malé) to Finland 16 June 2015, without any stopovers elsewhere. Two days later he became ill with flu-like prodrome, mild fever and rash in the face and trunk, as well as ocular pain and arthralgia; the symptoms alleviated after a few days. He contacted occupational health and due to suspicion of dengue, a serum sample was taken 24 June, and it was positive for dengue virus IgG (titer 1:1,280, in-house immunofluorescence assay (IFA) test) and IgM (1.9/ cut-off 1.0, Dengue Virus IgM Capture DxSelect ELISA, Focus Diagnostics, USA), but negative for dengue virus (DENV) non-structural (NS) 1 antigen (Dengue NS1 Ag Strip Bio-Rad, France). Along with the serum sample, a urine sample taken on 25 June, the following day, was received for flavivirus RNA detection using a real-time pan-flavivirus NS5 nested RT-PCR [1,2]. RNA from serum and urine samples was extracted using QIAamp Viral RNA Mini Kit (Qiagen).

From the urine (but not the serum) an amplification product was detected and subsequently sequenced (160bp excluding primers, available from the authors upon request). A BLAST search identified the sequence as Zika virus (ZIKV) identical to Asian lineage strains originating from Easter Island 2014 [3], French Polynesia 2013 (GenBank KJ776791), Brazil 2015 (GenBank KU321639) and Thailand 2013 [4] and in phylogenetic

analyses the sequence clustered with these strains (Figure). A PCR contamination in the laboratory is further ruled out as no work with ZIKV has ever been conducted, or any positive samples analysed previously in the laboratory - or in the country as a whole.

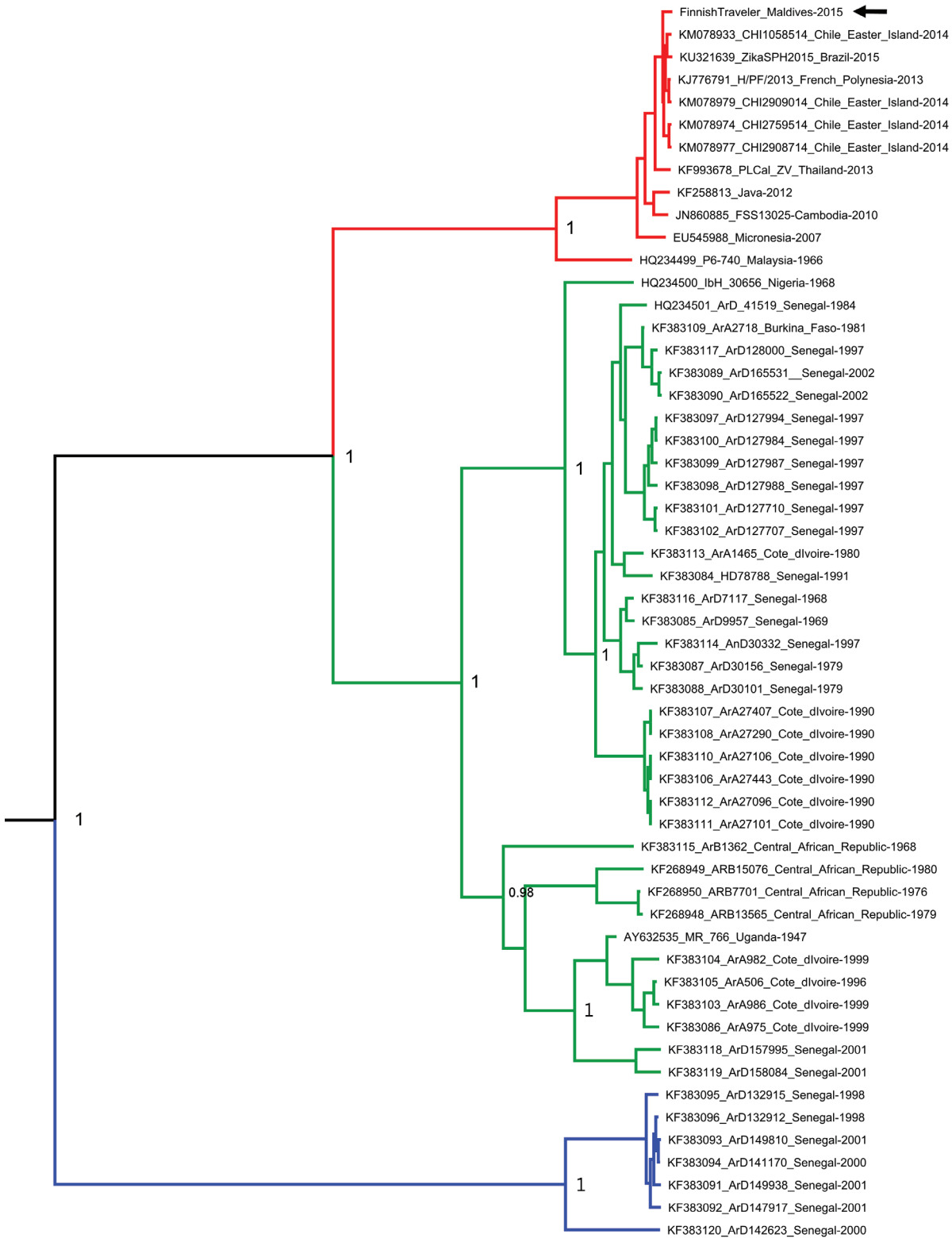
The Asian cluster is shown in red and African clusters as green and blue. Posterior probabilities are shown only for basal nodes. All ZIKV sequences were downloaded from GenBank (5.1.2016) and sequences overlapping the partial NS5 gene sequence were included in the analysis. The sequences were aligned using ClustalW algorithm implemented in MEGA version 6. For the sake of clarity, the identical sequences from Easter Island were removed from the data set. The best-fit substitution model was sought using MEGA version 6. The phylogenetic tree was constructed using Bayesian Monte Carlo Markov Chain (MCMC) method implemented in BEAST version 1.8.0 using Tamura-Nei (TN93+G) model of substitution, strict molecular clock and constant population size demographic model. The Bayesian analysis was run for 50 million states and sampled every 1000 states. Posterior probabilities were calculated with a burn-in of 5 million states and checked for convergence using Tracer version 1.6.

## Investigation of family members

Similar disease and mosquitoes were frequently reported in the area at the beginning of the rainy season and generally interpreted as dengue. The patient's wife had experienced a mild febrile illness a couple of weeks before departure. Serum samples obtained on 8 July, two weeks after confirmation of Zika virus in our patient, from the patient's wife and three children all of less than 10 years of age, were negative for flavivirus in pan-flavivirus NS5 nested RT-PCR. The children were also DENV seronegative, but the wife had low positive DENV IgG titer (1:20) and low positivity (1.3/ cut-off 1.0, Dengue Virus IgM Capture DxSelect ELISA, Focus Diagnostics, USA) in DENV IgM test.

**FIGURE**

Maximum clade credibility tree of partial Zika virus NS5 sequences



The Asian cluster is shown in red and African clusters as green and blue. Posterior probabilities are shown only for basal nodes. All ZIKV sequences were downloaded from GenBank (5.1.2016) and sequences overlapping the partial NS5 gene sequence were included in the analysis. The sequences were aligned using ClustalW algorithm implemented in MEGA version 6. For the sake of clarity, the identical sequences from Easter Island were removed from the data set. The best-fit substitution model was sought using MEGA version 6. The phylogenetic tree was constructed using Bayesian Monte Carlo Markov Chain (MCMC) method implemented in BEAST version 1.8.0 using Tamura-Nei (TN93+G) model of substitution, strict molecular clock and constant population size demographic model. The Bayesian analysis was run for 50 million states and sampled every 1000 states. Posterior probabilities were calculated with a burn-in of 5 million states and checked for convergence using Tracer version 1.6.

## TABLE

Verified acute Zika virus infections from South/Southeast Asia, 1977–2015

Country of origin	Year	Number of patients	Reference
Indonesia	1977–1978	7	[27]
	2013–2015	2	[31,32]
Cambodia	2010	1	[28]
Philippines	2012	1	[29]
Thailand	2012–2014	10	[4,23,30,33]
Malaysia	2014	1	[22]

## Background

ZIKV is a mosquito-borne flavivirus originally isolated in Uganda, 1947 [5]. ZIKV was associated with mild febrile disease and maculo-papular rash in tropical Africa and some areas of South East Asia. Since 2007 ZIKV has caused several outbreaks outside its former distribution area in islands of the Pacific Ocean: in 2007 on Yap island (Federated States of Micronesia) [6] and since 2013–14 in French Polynesia [7,8]. Since 2015, outbreaks have been reported for the first time in South America (Brazil, Columbia) [9,10]. Two lineages of ZIKV, an African (subdivided to West African and East African) and Asian lineage, which emerged in the Pacific and the Americas, respectively, have been identified on the basis of NS5 gene sequences [11].

The main transmission occurs in an urban cycle similar as for dengue and chikungunya, with *Aedes (Stegomyia)* mosquitoes as vectors [12]. Probable sexual transmission has been associated with ZIKV infection [13] and ZIKV has been isolated, and ZIKV RNA detected from semen samples [14]. Also transplacental transmission of ZIKV during childbirth has been reported in the French Polynesian outbreak [15].

Association with Guillain-Barré syndrome and more recently to an emerging epidemic of congenital microcephaly have increased the public health impact of ZIKV infections [16,17].

## Discussion and conclusions

As in our case, the DENV serology may be positive in ZIKV patients due to cross-reactions between other flaviviruses, and the ZIKV-specific RNA detection methods or sequencing are best for confirmation. The RT-PCR positivity from urine but not serum may suggest that urine is better as a sample material for RNA detection of ZIKV, and indeed the viral load in urine has been shown to be higher and detectable for a longer period, as compared to serum [18] in parallel to dengue [19]. Our patient was found negative in DENV NS1 Ag test, which in most DENV patients is positive during the acute phase [20] yet a negative NS1 result does not exclude DENV infection.

The short sequence obtained from the patient confirmed the etiology of the infection as Zika virus and suggested that the virus strain present in Maldives is of the Asian lineage of ZIKV, and indistinguishable within the amplified short fragment from the epidemic strains reported from e.g. Easter Island and Brazil. Yet, as we have so far been able to sequence only a short part of the NS5 gene, more sequence information is evidently needed.

ZIKV is an emerging arbovirus and it seems to fit well to the transmission cycles of DENV and CHIKV [21], which both have been earlier detected from the Maldives. ZIKV infections have earlier been imported from Asia, South America, French Polynesia and the Caribbean to Europe [22–26]. In Asia, there have been no previous verified ZIKV cases anywhere near Maldives (Table).

With this demonstration of ZIKV transmission in the Maldives, it remains to be elucidated if the circulation of ZIKV is already widespread in the area or geographical vicinity, as clinical manifestations of DENV, CHIKV and ZIKV as well as serological test results for DENV and ZIKV may be similar, or a risk of a larger Zika epidemic remains a possible future threat. The most prevalent symptoms associated with ZIKV, based on the reports from cases transmitted in Asia, are fever, rash, gastrointestinal symptoms, conjunctivitis, arthralgia, sore throat, headache and myalgia [4,22,23,27–33]. The recent potential associations of ZIKV with microcephaly and Guillain-Barré syndrome [16,17] highlight the need for ZIKV recognition and detection. The differentiation of DENV and ZIKV infections is a challenge for both clinicians and diagnostic laboratories.

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## Conflicts of interest

None declared.

## Authors' contributions

Wrote the manuscript: EMK, EH, TS, HKK, MR and OV; performed laboratory investigations: EMK, EH; performed phylogenetic analyses: EH, TS; managed the patient: MR; collected diagnostic and clinical data HKK, MR, OV.

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