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Imported cases of dengue fever in Russia during 2010–2013

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ABSTRACT

Objective: To confirm dengue infection among Russian tourists returned from Southeast and Mexico in 2010–2013 with clinical signs of infection. **Methods:** Blood and serum samples from patients were collected. NS1 antigen and human IgM/IgG antibodies to dengue virus were identified using commercial tests manufactured by "Standard Diagnostics, INC.", Korea. ELISA test was used for the quantitative analyses of human IgM/IgG antibodies to dengue virus ("Orgenics Ltd.", Israel). Viral RNA was detected using commercial real-time PCR tests manufactured by "Genome Diagnostics Pvt. Ltd.", India and "Vector", Russia. Genotypes of revealed dengue viruses were determined employing nucleotide sequencing and phylogenetic analysis of 5'-UTR of the viral genome. **Results:** A total of 98 collected blood samples were analyzed. Fifty samples were positive for at least one of four markers of dengue infection. IgM to dengue virus were revealed in 38 samples, in 25 samples IgM were combined with IgG. NS1 antigen was detected in 43 samples. 22 serum samples were positive for dengue virus RNA. The majority of samples (12 patients) from tourists returned from Thailand were positive for genotype 1 of dengue virus, 2nd and 4th genotype were identified each in 1 patient. **Conclusions:** Due to laboratory confirmed cases of imported dengue fever in Russia, the differential diagnosis of dengue is strictly recommended for tourists returning from endemic areas.

1. Introduction

Dengue fever is endemic in more than 100 countries in Africa, the Americas, Eastern Mediterranean, Southeast Asia and the Western Pacific. According to the WHO data, up to 100 million people are infected every year worldwide with dengue fever^[1,2]. However, recent studies show that the total number of cases might range from 217 to 392 million. Asia countries account about 70% of this cases (India–33%), 16% happen in Africa, about 14% in the Americas (where more than half of infections occur in Brazil and Mexico)^[3]. In Southeast Asia and Western Pacific Ocean about 1.8 billion people (more than 70% of total population) are at risk for dengue virus infection. Dengue fever epidemics are a major public health problem for India, China, Indonesia,

Myanmar, Sri Lanka, Thailand and Timor–Leste while in urban and rural areas of these countries mosquito *Aedes aegypti* (*Ae. aegypti*) is breeding^[4].

Russian Federation is not endemic for dengue fever and the cases of infection were not recorded until 2010^[5], despite the fact that 40 species of mosquitoes of the genus *Aedes* (*Ae.*) reside in the country. This genus inhabit area that covers 5 regions of Federation and hatching of *Ae. aegypti* L. and *Aedes albopictus* Skuse is possible around the settlements of southern Russian (the coast of Black Sea, Caucasus). Mosquito of the genus *Aedes* become infectious in 8–12 days after blood-sucking. Their ability of being infectious continuous lifelong, *ie.* 1–3 months. However, the replication of the virus in mosquitoes doesn't happen if the temperature is below 22 °C, therefore the area of high virus spread is less than inhabitable area of dengue mosquitoes and limited to 42° north and 40° south latitude^[6]. In view of permanently high level of dengue fever morbidity worldwide and habitat expansion of mosquitoes of the genus *Aedes*

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due to global warming, the imported cases of dengue fever in non-endemic areas registered more often [7–9]. In recent years, the number of Russian citizens visiting the dengue-endemic countries rose exponentially, consequently the risk of the pathogen importation to the Russian Federation from abroad is dramatically increased.

The research seeks to prove the significance of dengue fever threat for such non-endemic territories like Russia. The purpose of the work was to confirm dengue infection among Russian tourists returned from Southeast and Mexico in 2010–2013 with clinical signs of infection.

2. Materials and methods

Blood and serum samples from patients who got ill within 21 days after returning from Thailand, Indonesia, Vietnam and Mexico were collected in hospital communicable diseases units in 15 regions of Russia. Considering involvement of humans in this research, all activities were undertaken under ethical clearance issued by the ethical committee of hospitals where patients were monitored. All patients gave an informed consent on investigations.

In order to identify dengue virus NS1 antigen and human IgM/IgG antibodies to dengue virus “SD BIOLINE Dengue NS1 Ag + Ab Combo Test” and “Dengue NS1Ag + Ab Combo” (“Standard Diagnostics, INC.”, Korea) tests were used according to the manufacturer’s instructions. For the quantitative analyses of human IgM/IgG antibodies to dengue virus “ImmunoComb® II Dengue IgM/IgG Bispot” (“Orgenics Ltd.”, Israel) ELISA test was used. To determine primary dengue infection and reinfection in serum samples collected in febrile phase IgM/IgG ratio was calculated. Acute patients with primary infection have a higher IgM/IgG ratio than found in secondary infections, when the index is over 1.78 the infection considered primary, less-secondary [10,11].

Viral RNA was extracted using “QIAamp Viral RNA Mini Extraction Kit” (“Qiagen”, Netherlands). Real-Time PCR was performed using “Geno-Sen’s DENGUE 1–4 Real Time PCR Kit” (“Genome Diagnostics Pvt. Ltd.”, India) and “Vector-PCR_{RT}-Dengue (1–4)-RG” (“Vector”, Russia) according to the manufacturer’s instructions.

In accordance with protocol for differential diagnosis of dengue fever approved by WHO all the samples were tested for markers of infections caused by Lassa, Machupo, Junin, Marburg, Ebola, Japanese encephalitis, Chikungunya viruses using PCR kits (“Vector”, Russia). Also all the samples were tested for markers of West Nile fever, tick-born encephalitis using “AmpliSense” PCR kits (Central Research

Institute of Epidemiology, Russia) and “Vector-PCR-RT-RG” (“Vector”, Russia) according to the manufacturer’s instructions.

DNA fragments obtained from PCR amplification were analyzed by gel electrophoresis using 2 % agarose and purified for subsequent determination of the nucleotide sequence using “Gel Extraction Kit” (“Qiagen”, Netherlands). All the samples were analyzed twice in independent experiments.

Determination of the nucleotide sequence was carried out on ABI 3130xl DNA Analysis System (“Hitachi”, Japan) using “BigDye Terminator v3.1 Cycle sequencing Kit” (Applied Biosystems, USA). Phylogenetic analysis carried out using the software MEGA 5 (USA) and Lasergene 9 (“DNASTAR”, USA).

3. Results

In total 98 samples were analyzed for markers of dengue fever from May 2010 to May 2013 year. The samples were mostly collected from November to April (some samples were collected in May and June). This could be explained by active visiting of Southeast Asian countries by tourists, where the peak holiday season is from November to February.

These cases with dengue fever were from 15 regions of Russian Federation, including 3 cases in 2010, 4 cases in 2011, 13 cases in 2012 and 30 cases in 2013. Female cases accounted for 45%, with average age as 35 yrs; while male cases accounted for 55%, with average age as 42 yrs. At least 1 of 4 markers of dengue fever infection (viral RNA, NS1 antigen, IgM/IgG antibodies to dengue virus) was found in 50 samples. The positive rate of NS1 antigen in the blood was 78%. In most samples titer of IgM antibodies (70% , $M_{cp}=188\pm 71$) was higher than IgG (44% , $M_{cp}=45\pm 67$) (the IgM/IgG ratio ranged from 2 to 64). In two patients in febrile phase the IgM/IgG ratio was found lower than 1.78 (4%). And the positive rate of PCR was 28%.

None of studied samples was positive for Lassa, Machupo, Junin, Marburg, Ebola, Japanese and tick-born encephalitis, Chikungunya, West Nile viruses.

More than 40 of the observed patients were treated in hospital communicable diseases unit, while the physical condition of 28 patients was evaluated as moderate complexity, and 14 patients had severe physical condition. All cases had favorable prognosis. From outpatient cards is known that all the infections were accompanied by typical signs of dengue fever: fever, arthralgia, myalgia, weakness,

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