

Dengue antibodies in Polish travellers returning from the tropics. Evaluation of serological tests

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ABSTRACT

Dengue is a viral disease caused by an RNA virus of the genus *Flavivirus*, family *Flaviviridae*, occurring as four serotypes (DEN-1, -2, -3, -4). It is transmitted to humans by the *Aedes* mosquitoes, mainly *A. aegypti*. The occurrence of dengue is strictly related with their preferred breeding areas. Dengue endemic regions are inhabited by some 2.5 billion people. 50–100 million cases of dengue fever and up to 1 million cases of dengue haemorrhagic fever are noted worldwide in more than 100 countries every year. The aim of the reported examinations was to diagnose dengue virus infections in returning travellers. In the years 2006–2009 serological tests were performed in 753 persons. In the diagnostics we used ELISA to find IgM and/or IgG class of antibodies against dengue virus, rapid immunochromatographic (cassette) test, NS1 viral antigen detection by ELISA, and virus RNA detection by RT-PCR method. IgM or IgG class antibodies, and both classes simultaneously, were detected in 19.8% of the examined cases. The greatest number of infections came from India and the Far East, next from South and Central America, and the smallest number from Africa. Sixteen patients with diagnosed dengue, including three cases of dengue haemorrhagic fever, were hospitalized.

INTRODUCTION

Dengue fever is one of the most widespread imported febrile diseases in European travellers returning from tropical regions [1]. It is caused by four RNA virus serotypes (DEN-1, -2, -3, and -4) of the genus *Flavivirus*, family *Flaviviridae*, “related” with viruses of West Nile fever, yellow fever, and Japanese encephalitis. The disease is transmitted by mosquitoes of the *Aedes* family, mainly *A. aegypti*, and less frequently by *A. albopictus* and *A. polynesiensis* [2]. The prevalence of dengue is strictly connected with the feeding areas of these mosquitoes. *Aedes spp.* mosquitoes can reproduce and survive only in the presence of water (natural and artificial fresh water containers) within specific temperature ranges. Frosts and freezing of water cause damage to breeding sites, thus restricting the spread of the disease to moderate climate zones.

More than 2.5 billion people, i.e. about 40% of the world’s population, inhabit dengue endemic areas in South-East and South Asia, the American continents, Central Africa, and the islands of West and East Pacific [2, 3].

Each year, from 50 to 100 million dengue incidents are documented as classic dengue fever (CDF) and up to 1 million cases of dengue haemorrhagic fever (DHF). The number of notified dengue incidents has increased several times over the last 50 years due to urbanization of tropical regions, global warming, and the possibility of rapid displacement of persons in the viraemic period and those infected with mosquito viruses, e.g. by air transportation facilities. The geographical range of dengue incidence is also expanding. While in 2001 dengue was registered in 69 countries, in 2008 it occurred in over 100 countries worldwide. Before 1970, DHF outbreaks were noted in 9 countries only. By

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1995 this number had increased more than fourfold [4, 5]. In view of the mild course of many dengue infections, the real number of dengue cases may be 5–10-fold higher. Every few years, an epidemic increase in dengue incidence is observed [6].

The sick people in the viraemic period, i.e. from the 1st to the 4th day from the onset of the disease, constitute the reservoir of the dengue. The incubation period amounts to 4–8 days. Past disease guarantees lifelong permanent immunity, specific for a given virus serotype [7, 8].

Each year there is an increasing number of Polish travellers to the tropics, i.e. to dengue fever prevalence. The number of imported dengue cases is increasing in Poland.

Until 2005, no diagnostic tests were performed in Poland regarding dengue virus infection. Therefore, in available literature there are no epidemiological data on imported dengue in Poland, the number of people with past infection or antibodies against the dengue virus after a stay in an endemic region, or on examination results allowing the assessment of infection risk during travel.

The aim of the investigation was to evaluate the frequency of occurrence of antibodies against dengue virus in persons returning from dengue-endemic areas, to assess the usefulness of other tests (detection of NS1 virus antigen by ELISA and of viral RNA by RT-PCR) in diagnosing dengue fever, and to evaluate the risk of dengue virus infection in Polish travellers to the tropics.

MATERIAL AND METHODS

The study group consisted of 753 patients (320 women aged 20–71 years and 433 men aged 19–77 years) of the Clinic of Tropical and Parasitic Diseases of the Medical University of Gdańsk and the Outpatient Clinic of Infectious, Tropical, and Parasitic Diseases of the University Centre for Maritime and Tropical Medicine in Gdynia, who were subjected to routine examinations after a stay in the tropics or were hospitalized in the Clinic for morbid symptoms in the years 2006–2009.

In the diagnostics of the infection, serological tests were performed by ELISA to detect IgM and IgG class antibodies against dengue virus (IBL International and Panbio). In the diagnostics of the infection, serological test were performed by ELISA to detect IgM and IgG class antibodies against dengue virus (IBL International and Panbio). When serological tests results were doubtful as to the specificity of antibodies detected (e.g. isolated presence of IgM class low-titre antibodies), the tests were repeated after four weeks [9]. This concerned 62 person. In febrile patients, rapid immunochromatographic (cassette) test for IgM/IgG (Panbio), detection of NS1 virus antigen presence by ELISA (Panbio), and viral RNA examination by RT-PCR were performed. Commercial tests were performed according to the manufactu-

rer's instructions. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using QIAGEN OneStep RT-PCR Kit (Qiagen) [10]. Sequences obtained from two positive patients showed 99.7% homology to the sequence from cosmopolitan Brunei DEN-2 virus strain [11] deposited in Gen Bank database (accession no EU179858).

RESULTS

The presence of IgG, IgG+IgM, and IgM class antibodies against dengue virus was found in 191 (23.37%) of the 753 persons examined. In 62 persons with isolated presence of low-titre IgM class antibodies, the tests were repeated after 4 weeks. No antibodies were detected in 42 cases, whereas in 20 the titre of IgM class antibodies against dengue virus was higher. IgG class antibodies also appeared. Thus, after verification and rejection of results regarded in 42 persons as unspecific in the first examination, dengue virus infection was diagnosed in 149 travellers to the tropics (19.8% of the study group). In 20 persons IgM class early antibodies were detected, and in 45 both IgM and IgG class antibodies were detected. In 84 cases the tests revealed only IgG class late antibodies. NS1 antigen presence was found in one patient for 8 febrile and 41 non-febrile patients examined. The results are summarized in Table 1.

The group of 149 persons in which antibodies were found was also analyzed with regard to the place and length of stay in the tropics (period of exposure to possible dengue virus infection), and the purpose of the journey. In 81 cases, epidemiological data in medical documentation indicated that the exposure to infection probably occurred in the following regions:

- Far East (Thailand, Vietnam, Laos, Cambodia, Korea, China) — 15 cases;
- South Asia (India, Afghanistan, Pakistan) — 16 cases;
- South and Central America (Brazil, Argentina, Bolivia, Peru, Cuba, Dominican Republic, Belize, Mexico) — 26 cases;

Table 1. Types of tests performed and their results

Type of test	Number and percentage of positive results
Antibodies (ELISA):	
IgM	20 (13.4%)
IgM+IgG	45 (30.2%)
IgG	84 (56.4%)
NS1 antigen in the blood	0/49 examinations
Virus RNA in the blood	2/14 examinations
Rapid immunochromatographic test	12 IgM+
IgM/IgG (cassette)	2 IgM+IgG+
	16 negative results

Table 2. The occupation of the patients

Occupation	Number of people examined	Occupation	Number of people examined
Missionaries	29	Travellers and journalists	5
Seafarers	3	Sportsmen	3
Regular soldiers	11	Bartenders and waiters	2
Surface miners	7	Participants of charitable missions	4
Workers of natural gas mining industry	3	Physicians and nurses	3
Marine drilling platform workers	3	Agricultural pilots	2
Engineers	4	Stewardesses	1
Geodesists	4	Artists	1
		Not established	25

— Central Africa (Angola, Cameroon, Nigeria, Ghana, Congo, Zaire, Tanzania, Kenya, Central African Republic, Zambia, Sudan) — 21 cases, Morocco — 3 cases.

The remaining 68 persons stayed in dengue-endemic areas, but the place of their stay was not established precisely.

The length of stay in dengue-endemic regions varied markedly: from one to several weeks for tourists, several months to a few years for persons working in the tropics, and from several months to as long as 33 years for missionaries.

The purposes of travel included contract and mission work, tourism (including visits to working relatives), and practising some kind of sport (rock and mountain climbing, participation in rallies, coral reef diving, cave exploration, etc.). Among 149 patients with detected antibodies against dengue virus, there were 39 tourists and 85 persons working in dengue-endemic regions. In 25 cases the type of occupation taken up was not established. The cross-sectional analysis of occupations is given in Table 2.

The study group included 16 febrile patients, for whom serological, and in two cases molecular, tests confirmed the diagnosis of dengue. These patients were hospitalized in the Clinic of Tropical and Parasitic Diseases. As regards the occupation, this group consisted of five tourists, five regular soldiers, four representatives of other professions, and two missionaries. Thus, people staying more than a few weeks in the tropics prevailed. The length of hospitalization of patients with dengue varied from 3 to 17 days. Six persons stayed in the Clinic more than ten days, three of them with diagnosed dengue haemorrhagic fever [12].

DISCUSSION

Results of serological tests performed in the University Centre for Maritime and Tropical Medicine indicate a serious risk of dengue virus infection during stays in endemic regions. This concerned about 1/5 of the patients examined. However, it should be emphasized that the group was not chosen at random. The people qualified for examina-

tions reported sick of their own accord due to ailments that might be related with their travel, or underwent compulsory medical examination as part of routine prophylaxis with regard to workers delegated to the tropics. It should be pointed out that although dengue requires mosquitoes as vectors and occurs primarily in regions of high population density where mosquito breeding sites are close to urban districts, antibodies were detected also in three seafarers and three marine drilling platform workers.

It is worth noting that in the group with antibodies against dengue virus, workers and missionaries prevailed over tourists: 57% vs. 26.2%. Taking into account that the risk factors for acquiring tropical diseases, including dengue, are: the length of stay in endemic regions, wet season favouring mass mosquito hatching, disregard of unspecific prophylaxis, and lack of awareness of the disease threat. Workers staying in the tropics year-long, for several years in particular, are most exposed to infection [13, 14]. It is understandable that tourists choose safer seasons, from November until the end of February in the northern hemisphere where the wet season lasts from August to October. In the southern hemisphere, the high tourist season lasts from May to August, and the wet season from February to April. Touristic stays usually last for 1–2 weeks and more rarely 3–4 weeks. Hence, dengue virus infection in only 26.2% of tourists is not surprising.

As known from WHO publications, the highest dengue virus threat exists in South-East Asia and on the Indian subcontinent, where the greatest dengue incidence is recorded, followed by South and Central America, and Africa, with the lowest number of cases in the Oceania region [4]. Our investigations have demonstrated that the patients examined were infected with dengue virus in Asia, South and Central America, and Africa in almost equal proportions [31, 26 and 24, respectively]. There were no travellers to the Oceania region in this group. Considerably different results of epidemiological examinations were reported by authors from Austria [15]. Among 93 patients hospitalized with a diagnosis of dengue in Vienna Municipal Hospital in the

years 1990–2005, 56% were infected with dengue virus in South-East Asia, 18% in India, 10% in Africa, and 3% in Oceania. In the years 2001–2004, 366 patients were examined in the USA with a suspicion of imported dengue, which was diagnosed in 77 persons. According to history data, for 30% of patients the destination and probable place of infection were Caribbean Islands, Pacific Islands for 21%, Asia for 17%, Central America for 15%, South America for 15%, and Africa for 2% [16, 17]. The above data indicate that depending on the country, there are different preferences as to the destination of the journey. In our investigations, a markedly higher number of infections contracted most likely in Africa was noted compared to that usually reported. This is probably due to the considerable proportion of missionaries, working mainly in Africa, in our study group.

In the diagnostics of dengue virus infection, serological methods (determination of NS1 antigen, IgG, and IgM class antibodies) and in some cases virus RNA identification by RT-PCR were employed. According to the test producer's information, it should be taken into account that the isolated small increase in IgM class antibody level may result from an unspecific reaction, for example, because of the cross-reactions with other flaviviridae. In such cases the test should be repeated for verification after a week or more. It was found that as many as 42 tests from 62 verified were regarded as false-positive. This may be the reason for dengue "overdiagnosing" by inexperienced physicians [18]. There is a possibility of cross-reactivity of antibodies to a yellow fever virus among Polish travellers going to South and Latin America, and Africa, who were vaccinated. There is no vaccine for the West Nile Virus fever; in addition, the morbidity caused by this fever is not reported in Poland, with just one exception described in 2007. Vaccination for Japanese encephalitis is not commonly available in Poland. Only small groups of workers going to the endemic regions of this disease are occasionally vaccinated. Therefore, cross-reactions between the two viruses mentioned above can be considered only among travellers frequently visiting the tropics. Unfortunately, these data were not available in medical records.

Rapid IgM/IgG cassette proved very useful in diagnosing dengue in the early infection stage in febrile patients because the result of the test is obtained after just 15 minutes, allowing differentiation between the primary and secondary infection. It is also quite specific since it is compatible in over 90% of cases, with results obtained by ELISA in classic determination of IgG and IgM antibodies, and it is inexpensive.

Thirty tests were performed, in 14 cases yielding a positive result for IgM antibodies, corresponding to the early stage of dengue virus infection. In two cases IgM+IgG class antibodies were also found, which suggests infection with a second or successive dengue virus serotype. Such a result

is of prognostic significance since dengue haemorrhagic fever does not occur in persons infected for the first time. It may develop upon subsequent infection, probably with virus serotype 2. RT-PCR method allows identification of the virus type responsible for the disease.

Over 49 serological tests were performed to find NS1 antigen of dengue virus in the blood of febrile patients returning from the tropics. No positive result was obtained, even in patients with diagnosed dengue. Since the virus antigen remains in blood until the 4th–8th day from the onset of the disease, and the return from the dengue-endemic region usually lasts 1–2 days, the antigen frequently becomes indeterminable by diagnostic tests. The usefulness of this method for Polish tourists diagnosed in Poland seems doubtful. Virus RNA determination in the blood by RT-PCR, allowing RNA detection after 4–6 days from the infection, is more useful and sensitive. Nonetheless, it is more expensive, less readily available, and is not performed as a matter of urgency.

Dengue is rarely diagnosed in Poland, although 50–100 million cases are reported worldwide, and the real number is much higher. In the majority of cases the course of dengue is mild and almost symptomless, resulting in disregard of the disease and abandonment of diagnostics. However, haemorrhagic fever symptoms are sometimes observed. In view of the lack of a vaccine against dengue, knowledge about unspecific prophylaxis is indispensable, particularly among people sent to work in dengue-endemic areas.

Despite the continual increase in the number of diagnosed cases of imported dengue in Poland, the experience of Polish physicians in prevention, diagnosis, and treatment of this disease is still insufficient. In the current epidemiological situation it seems that the diagnostic methods discussed in the present paper and offered in our centre are effective for proper dengue diagnosis.

CONCLUSIONS

1. Serological tests have demonstrated that 149 travellers to the tropics (19.8% of 753 cases examined) might have been infected by dengue virus.
2. Almost half (43.6%) of the persons with detected antibodies showed the presence of early antibodies in blood.
3. In 16 patients (2.1% of the study group) dengue fever was diagnosed, with dengue haemorrhagic fever in 3 cases.
4. It seems that the risk of dengue virus infection when visiting the tropics is quite high, particularly during longer (several months or years) stays in regions endemic for this disease, frequently connected with work performed.
5. Travellers to the tropics should be acquainted with unspecific prophylaxis against dengue.

REFERENCES

1. Eisenhut M, Schwarz TF, Hegenscheid B. Seroprevalence of dengue, chikungunya and Synbis virus infections in German aid workers. *Infection* 1999; 27: 82–85.
2. Centers for Disease Control and Prevention, Epidemiology, clinical guidance and diagnostic testing of dengue, November 10, 2009, <http://wwwnc.cdc.gov/travel/content/outbreak-notice/dengue-tropical.html>, January 17, 2010.
3. WHO, DengueNet – WHO's Internet-based System for the global Surveillance of Dengue Fever and Dengue Haemorrhagic Fever (Dengue/DHF), *Weekly Epidemiological Record* 2002; 77: 300–304.
4. World Health Organisation, Dengue and dengue haemorrhagic fever. March 2009, Fact Sheet No 117. <http://www.who.int/media-centre/factsheets/fs117/html> 2010.01.17.
5. Outbreak Notice, Update: Dengue, Tropical and Subtropical Regions, July 08, 2008, <http://wwwn.cdc.gov/travel>, March 13, 2009.
6. Porter KR, Beckett CG, Kosasih H et al. Epidemiology of dengue and dengue haemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. *Am J Trop Med Hyg* 2005; 72: 60–66.
7. Leitmayer KC, Vaughn DW, Watts DM et al. Dengue virus structural differences that correlate with pathogenesis. *J Virol* 1999; 73: 4738–4747.
8. Vaughn DW, Green S, Kalayanaraj S et al. Dengue in the early febrile phase: viremia and antibody responses. *J Infect Dis* 1997; 176: 322–330.
9. Koraka P, Suharti C, Setiati TE et al. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with outcome of infection. *J Clin Microb* 2001; 39: 4332–4338.
10. Bakonyi T, Ivanics E, Erdelui K et al. Lineage 1 and 2 Strains of Encephalitic West Nile Virus, Central Europe. *Emerg Infectious Dis* 2006; 12: 618–623.
11. Osman O., Fong M.Y., Devi S. Complete genome sequence analysis of dengue virus type 2 isolated in Brunei. *Virus Research* 2008; 135: 48–52.
12. Teichmann D, Gobels K, Niedrig M, Sim-Brandenburg JW, Lage-Stehr J, Grobusch MP. Virus isolation for diagnosing dengue virus infections in returning travellers. *Eur J Clin Microbiol Infect Dis* 2003; 22: 697–700.
13. Jelinek T. Dengue fever in international travelers. *Clin Infect Dis* 2000; 31: 144–147.
14. Rigau-Perez JG, Gubler DJ, Vorndam AV, Clark GG. Dengue: a literature review and cause study of travelers from the United States. *J Travel Med* 1997; 4: 65–71.
15. Laferl H, Szell M, Bischof E, Wenisch C. Imported dengue fever in Austria 1990–2005, *Travel Medicine and Infectious Disease* 2009; 4: 319–323.
16. Ayala A, Rivera A, Johansson M, Muñoz-Jordan JL. Travel-Associated Dengue-United States. *JAMA* 2005; 55: 700–702.
17. Beatty ME, Vorndam V, Hunsperger EA, Muñoz JL, Clark GG. Travel-Associated Dengue Infections – United States, 2001–2004. *JAMA* 2005; 294: 421–422.
18. Tran TN, de Vries PJ, Hoang LP. Enzyme-linked immunoassay for dengue virus IgM and IgG antibodies in serum and filter paper blood. *BMC Infect Dis* 2006; 6: 13–21.