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IMPORTED CASES OF DENGUE IN POLAND AND THEIR DIAGNOSIS

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ABSTRACT

Infections with dengue virus are transmitted by mosquitoes. In tropical areas, it is mainly spread by *Aedes aegypti* while in countries with lower temperatures by *Aedes albopictus*. Since 2010, autochthonous cases of dengue are also reported in Europe. There are 4 serotypes of dengue virus (DENV). No correlation between clinical presentation of disease and virus type, however, were determined. Nevertheless, reinfection with different type of DENV may lead to a serious, life-threatening condition. An estimated 100 million persons are infected with dengue virus per year. Of them, approximately a half (mainly children) develop the symptoms of dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). Fatality is high in case of severe dengue. Dengue is a serious condition provided there is a presence of IgG antibodies directed against antigens of particular DENV serotypes, associated with primary infection caused by different serotype or transferred from infected mother to her child. For adequate dengue laboratory diagnosis, it is required to apply a set of various diagnostic methods. Within the family *Flaviviridae*, cross-reactivity is reported, which may lead to the occurrence of false-positive results. In Poland, differential diagnosis with different *Flavivirus* species is of special importance as it is an endemic area for tick-borne encephalitis (TBE). Thus, data regarding history of patient's immunization against TBE or yellow fever should be also taken into consideration as important in interpretation of results of serological examination.

Key words: *infection with dengue virus, introduction of dengue to Poland, infection diagnosis*

INTRODUCTION

A few billion persons live in dengue (DENV) endemic areas. *Aedes aegypti*, a mosquito living in tropical and subtropical regions, is the primary vector of dengue, transmitting the virus to humans. Infections caused by other mosquitoes belonging to the genus *Aedes* are reported less frequently. Infected persons serve as a reservoir of dengue virus for 4-5 days, maximum 12 days of symptoms, but also 2 days prior to their occurrence. In this period, virus may be transmitted from infected person to a feeding mosquito. Incubation of virus in mosquito organism lasts for 4-10 days, however, once infected mosquitoes are a source of the virus for the rest of their life [1,2].

Aedes albopictus may also transmit dengue virus. Primarily, it was living in Asia. Due to its tolerance to lower temperatures, however, now it is also present in North America and Europe. Expansion of *Ae.albopictus* to new areas may result in new geographical distribution of dengue viruses, consequently leading to a modified epidemiological situation of dengue in Europe. Currently, this mosquito lives on Madeira islands, where infections with dengue virus in humans were reported in 2012. Furthermore, *Ae.albopictus* has spread to the areas around the Mediterranean Sea and Black Seas [3,4], which are attractive for tourists from Poland.

This article aimed at presenting the issues concerning the diagnosis of imported dengue cases in Poland and assessing the risk of dengue introduction to Poland.

EPIDEMIOLOGICAL SITUATION OF DENGUE AROUND THE WORLD AND IN POLAND

Infections with dengue virus are reported in more than 100 countries worldwide, ranging from the South-East Asia Region, the countries of the Middle East, Africa, Central and South America to the countries of

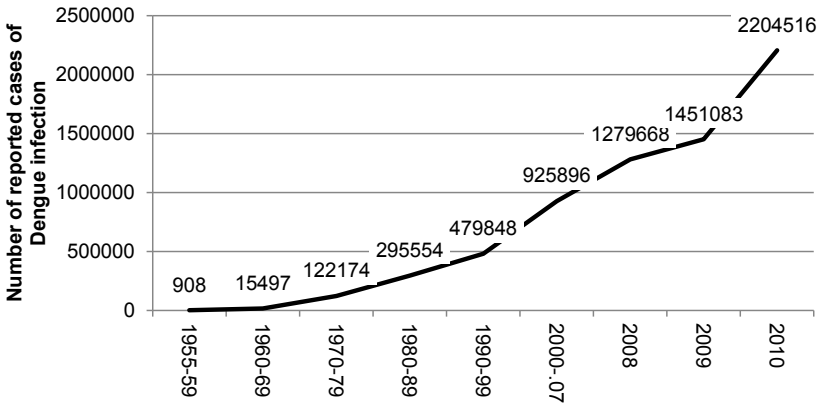


Figure 1. Number of reported to WHO cases of Dengue virus infections around the World [1,4] (DF = Dengue fever; DHF = Dengue Haemorrhagic fever).

Table 1. Dengue case classification for the purpose of epidemiological surveillance in Poland (according to the National Institute of Public Health – NIH)

Clinical criteria	Acute disease, accompanied by rash, high temperature lasting for 2-7 days, and at least two of the following symptoms: headache, retroorbital pain, myalgia, arthralgia, rash, haemorrhagic manifestation and leucopoenia.
Possible case	NA
Probable case	Any person meeting the clinical and laboratory criteria, i.e. the presence of dengue-virus-specific IgM antibodies in serum and/or high dengue-virus-specific IgG antibody titers in serum.
Confirmed case	Any person meeting the clinical criteria with at least one of the following laboratory results: • isolation of dengue virus from serum, plasma, leukocytes or autopsy tissue specimen • at least 4-fold increase of IgM or IgG titers, excluding cross-reactivity with other flaviviruses • detection of dengue virus antigen • detection of dengue virus genome

Western Pacific Region. According to the WHO, the geographical distribution of infections with DENV suggest that approximately a half of global population is at risk of contracting dengue. Since 50 years, a significant, sustained increase in the number of dengue cases notified to the WHO is observed. In 2010, a total of 2.3 million cases were reported. Compared to 2008, it was a 1.7-fold increase. Furthermore, an increasing tendency is still observed (Fig.1). In the 50s of the 20th century, dengue outbreaks were reported predominantly in the Philippines. Since the 70s, dengue fever cases of more severe course were notified in 9 countries while beginning from the 80s, large outbreaks were reported in the Caribbean, South America and Asia. In the USA, autochthonous dengue infections were first noted in the 21st century. Currently, the highest incidence occurs in the countries of the Americas (<50%) and Western Pacific (Fig.2) [1,4-8].

In Europe, dengue cases were reported in the Balkans and Mediterranean countries up to 1928. Since this

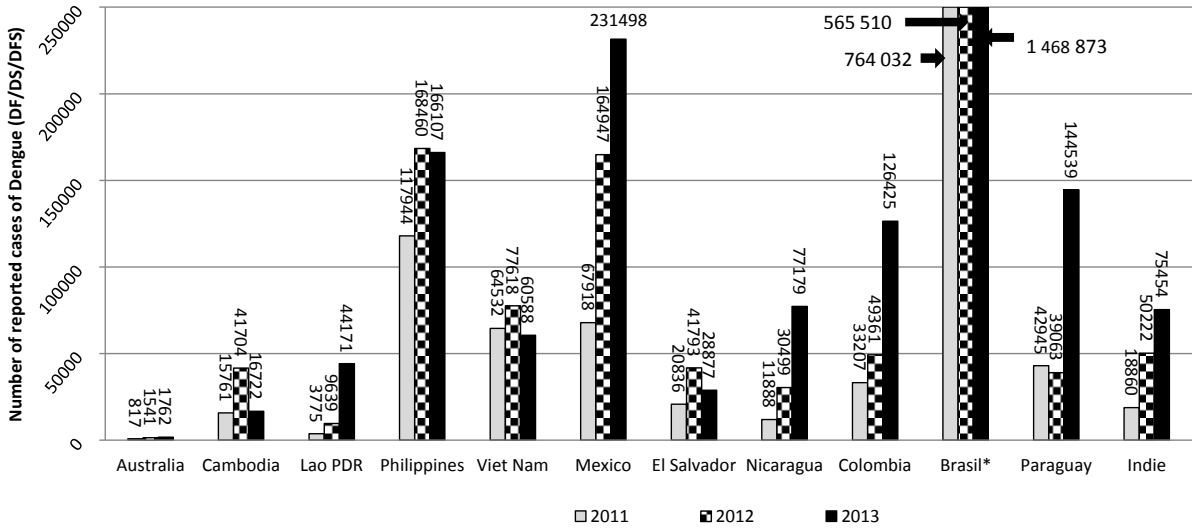


Figure 2. Number of reported denga fever (DF) and severe denga cases (DSS or DHF) in selected countries in 2011-2013 years [5-8].

year, only imported cases were noted until 2010, where local transmission of this infection occurred in Croatia and the south of France [4,9]. Infection was transmitted by *Aedes albopictus* which is tolerant to colder than tropical and subtropical climates and hibernation. In October 2012, dengue outbreak in Madeira islands was described, in which more than 2,000 human cases were identified from 26th September 2012 to 4th February 2013. Furthermore, a total of 78 cases imported to the European countries were notified, including: England – 23, Germany - 19, France – 3, Sweden – 5, Finland – 7, Denmark – 2, Austria – 2, Norway – 2, and one case in Croatia, Slovenia, Spain and Switzerland [3,4].

Increased human mobility across the countries of all continents poses a threat of dengue introduction to new areas. The number of dengue cases imported to the European countries, probably also to Poland, is still on the increase. Exposure to DENV during a stay in dengue endemic areas may progress into infection while being abroad or following return to the country. A proportion of these infections may remain undetected. Exclusively more severe cases are diagnosed and confirmed by laboratory methods. In 2009-2012, a total of 1,234 cases were laboratory confirmed in England, Wales and Northern Ireland, i.e. in 2009 - 177, 2010 - 449, 2011 - 235 and 2012 - 373 (including 20 infections acquired in Madeira islands). Between January and April 2013, a total of 141 cases were reported, including 3 persons infected during a stay in Madeira islands [10]. Unfortunately, in several European countries, i.a. Portugal, DENV infections are not subject to mandatory notification. Therefore, epidemiological data are incomplete.

In the light of aforesaid information, analysis of dengue cases reported each year in Poland probably suggest underestimation of data. In 2005-2013, a total of 29 cases were reported in Poland, including 6, 5, 5 and 0 in 2010, 2011, 2012 and 2013, respectively. Of 22 patients diagnosed with dengue and hospitalized in the Warsaw's Hospital for Infectious Diseases in 2002-2011, the majority were persons travelling to India and Indochina (Vietnam, Laos, Thailand) [11].

SYMPTOMS OF DENGUE (1,2,12)

There are three main clinical presentations of dengue:

1. Asymptomatic or influenza-like infection, clinically similar to cold
2. Dengue fever (DF)
3. Severe dengue (DS), including dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).

Dengue fever (DF) is an acute disease, accompanied by rash, high temperature (40°C) lasting for 2-7 days, and at least two of the following symptoms: severe headache, retroorbital pain, myalgia, arthralgia, rash, nausea, vomiting, enlarged lymph nodes and leucopoenia. Most frequently, dengue fever is diagnosed in infants, small children and adults. In case of children, it may be of fatal course.

Severe dengue (DS), formerly referred to as dengue haemorrhagic fever (DHF), is a potentially fatal presentation of disease due to fluid loss, renal and respiratory failure, accompanied by haemorrhagic manifestations and those indicative of multiple-organ failure. Early diagnosis and adequate medical treatment may decrease the risk of death from 20% to 1%.

For severe presentation of infection (including dengue haemorrhagic fever), a presence of IgG antibodies directed against antigens of particular DENV serotypes is required, which results from:

1. a past infection with virus of different serotype (irrespective of the presentation of primary infection/ past infection)
2. a past infection in pregnant woman or breastfeeding mother – in case of infants, the presence of maternal specific IgG antibodies modifies the primary infection in child to its severe presentation.

A list of other factors affecting the severity of dengue includes: immune status of infected person, associated with age (poorer response in case of very young and older persons), and co-existence of chronic diseases [2,13].

No correlation between a serotype of DENV (types 1-4) and severity of infection was found.

Predominantly, the number of notified cases refers to symptomatic infections. High fatality is reported mainly in dengue haemorrhagic fever and dengue shock syndrome. Furthermore, higher fatality rates are noted at the beginning of epidemics. An estimated 100 million dengue cases occurred in 2007, of whom approximately 500,000 were diagnosed and notified, and 12,500 died [1,4]. In the period between January and April 2014, more than 272,000 dengue fever cases and 2,708 infections of severe course, including 87 fatal cases, were reported in the countries of the Americas [7].

Due to the effect of specific IgG antibodies on the severity of infection, no active immunization is applied. A lack of specific treatment also raises difficulties. In severe cases, only supportive treatment is used.

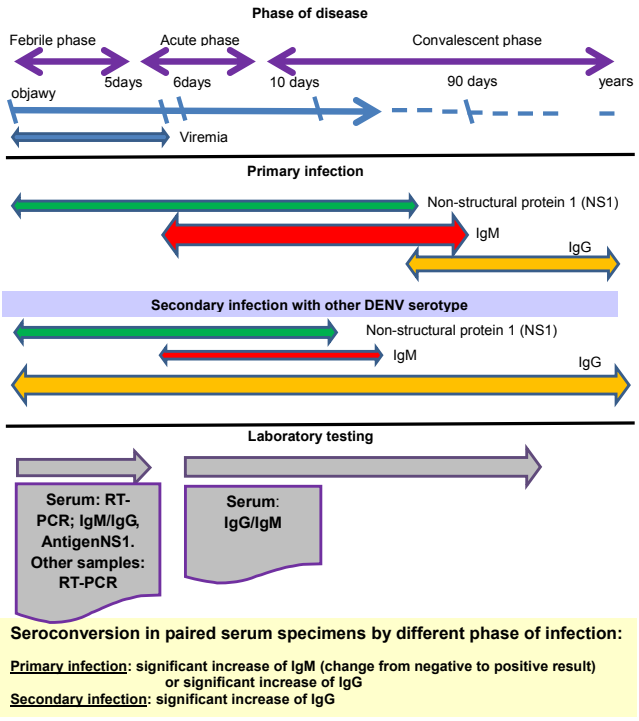
DENGUE LABORATORY TESTING

Infection with dengue virus should be suspected by a physician in Poland if a patient reports a stay in dengue endemic areas. Dengue laboratory testing is performed exclusively in case of symptomatic infections, i.e. dengue fever or severe dengue, including dengue haemorrhagic fever [1].

Differential diagnosis of these infections, based on medical history and physical examination, accompanied by microbiological and serological tests, allows for proper differentiating between infections of different viral aetiology. It results from the fact that haemorrhagic fever may be caused by other RNA viruses belonging to the families: *Arenaviridae*, *Bunyaviridae*, *Filoviridae*.

A separate problematic issue consists in differential diagnosis of infections caused by genetically or antigenically closely related *Flaviviruses*. Dengue virus, yellow fever virus or tick-borne encephalitis virus belong to the numerous *Flaviviridae* family, comprising of more than 50 species. Symptoms of infections with these viruses depend on pathogen-host interactions. They may range from mild fever, sometimes accompanied by rash, to organ damage and generalized immunopathologic changes, resulting in coagulation defects to central nervous system infection [13]. Laboratory confirmation of dengue is dependent on a number of factors, of which one of the most important is initial clinical diagnosis, based on epidemiological data, allowing for proper selection of laboratory methods [1,2,4,14]

Dengue laboratory testing includes the following methods (Fig.3):



1. virological tests (molecular tests, isolation of the virus *),
2. serological tests (detection of IgM, IgG),
3. identification of viral antigen.

Selection of methods as well as test results are affected by:

1. interval between the exposure and collection of specimen for testing,
2. type of specimen collected for testing,
3. specimen storage and transport conditions,
4. availability of diagnostic methods in particular laboratory.

Reverse transcription-polymerase chain reaction (RT–PCR) allows for identification of virus genome. Specimens for RT-PCR testing are serum, plasma, cerebrospinal fluid or tissue collected during acute phase of infection, accompanied by fever. DENV infection, using PCR, may be diagnosed during the first 5 days of symptoms.

As detection of viral RNA is possible exclusively in the first days of infection (when patient usually is abroad), the majority of imported cases is confirmed by serological testing. Immunological response to dengue infection results in the production of specific IgM and IgG antibodies which are mainly directed against the envelope proteins of virus. Immunological response may differ and is dependent on whether a patient is infected with DENV for the first or subsequent time as well as it is infection with the same or other serotype of virus. Due to antigenic affinity of *Flaviviruses*, the results of diagnostic tests may be significantly affected by a history of past infections or immunizations against *Flaviviruses*, including tick-borne encephalitis and yellow fever.

For primary infection with DENV, slow increase in IgM and IgG titers is characteristic. IgG antibodies are detectable at low titers at the end of week 1. Then, its titers slowly increase. During secondary infection, IgG titers increase very quickly and high cross-reactivity with antigens of other *Flaviviruses* is reported. IgM kinetics is more changeable. IgM titers are significantly lower in secondary DENV infection. Therefore, IgM negative results are sometimes reported in case of subsequent infections (Fig.3).

In case of primary and secondary infections, it is recommended to analyze paired serum specimens, collected at different stages of disease. Unfortunately comparison of paired serum specimens, i.e. acute and convalescent specimens, as well as monitoring of IgG and IgM titer dynamics is not always feasible in practice.

Cross-reactivity with other *Flaviviruses* in serum of infected patients should also be highlighted. Due to endemicity of tick-borne encephalitis in Poland and availability of immunization against TBE, cross-reactivity raises diagnostic difficulties. Furthermore, immunization against yellow fever, especially in travelers, is applied since many years. Differentiation between homologous (specific for dengue virus) and heterologous antibodies, cross-reacting with other

* - isolation of dengue virus in Poland and many European countries may be exclusively performed in biosafety level 3 or 4 (BSL3/4) laboratory.

flaviruses, requires methods based on virus neutralization (neutralization test, plaque reduction test), which are performed under conditions of increasedbiosafety level (BSL3/4) laboratory. [1,2,4,14].

Tests used to identify envelope antigen or non-structural protein 1 (NS1) of dengue virus are applied primarily in acute phase of primary infection (until day 9 of symptoms) as immunocomplex virus-IgG are present in the serum of patient during secondary infection. Furthermore, these tests do not allow for differentiation of virus serotypes. Currently introduced commercial kits, intended to identify NS1 (ELISA or tests based on histological techniques), are subject to assessment of their diagnostic accuracy and possibility of use during primary and secondary dengue infections [1,14].

Dengue case classification. Both interpretation of test results and classification of case by attending physician raise difficulties. Recently, the World Health Organization (WHO) introduced new schemes of case classification and diagnostic management which are verified in selected countries [4]. In Poland, a classic ECDC classification of 2008 is binding (Tab.1) [2].

SUMMARY

In Poland, dengue laboratory testing requires a special attention due to endemicity of tick-borne encephalitis (TBE). Infections with this virus and availability of active immunization may lead to the occurrence of false-positive results by serological tests (ELISA etc.), intended to detect anti-dengue antibodies, resulting from numerous cross-reactions within the viruses of family *Flaviviridae*.

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