CASE REPORT

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First documented case of Zika virus infection in a Polish patient – diagnostic traps. Case report

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Abstract

Zika virus (ZIKV), a mosquito-borne virus of the Flaviviridae family, has been reported in 89 countries. While ZIKV is not endemic in Poland, travel to regions with Aedes mosquitoes prevalence, presents a risk. This case report describes Poland's first ZIKV case in a patient with past Dengue virus (DENV) infection, following travel with his wife in 2016 to the Dominican Republic. Symptoms included fever, maculopapular rash, muscle pain, enlarged cervical lymph nodes, and lumbar pain. Hospital tests confirmed ZIKV, as well as possible simultaneous DENV infection. Further RT-PCR and serology tests were performed to exclude cross-reactivity. After 12 weeks, ZIKV RNA was detected in seminal fluid. Elevated IgG levels indicated prior DENV infection, while Dengue virus' antigen of non-structural protein 1 (NS1) antigen tests ruled out acute DENV. These findings impacted the couple's reproductive plans due to potential ZIKV transmission and patient's risk of secondary infection.

Key words

Zika virus, Dengue virus, Aedes, Zika virus infection, mosquito-borne diseases, Flaviviridae Infections

INTRODUCTION

Zika virus (ZIKV) is an enveloped positive-strand ribonucleic acid (RNA) virus belonging to the genus *Flavivirus* and the family *Flaviviridae*, which includes other clinically important viruses such as Yellow fever virus (YFV), Dengue virus (DENV), West Nile virus (WNV) and Tick-borne encephalitis virus (TBEV) [1]. The vectors of ZIKV, like many other Flaviviruses, are mosquitoes. ZIKV is primarily transmitted through the bites of several mosquito species, especially Aedes spp. Among these, Aedes aegypti noted for its diurnal activity and the less human prone Aedes albopictus are considered the principal vectors contributing to ZIKV transmission [2, 3]. In addition to vector-borne transmission, non-vector routes have also been documented, including vertical (foetal/neonatal), sexual, transfusion-related, and urinary transmission [4].

ZIKV was first discovered in Uganda in 1947 where it was isolated from the serum of a rhesus monkey. For about 60 years, the virus remained within the equatorial zone throughout Africa and Asia. However, it first occurred in 2007 on Yap Island in the Caroline Islands of the Western Pacific Ocean, and expanded east at the turn of 2013–2014 to French Polynesia and other Pacific Islands. The virus emerged in Latin America, causing an outbreak in 2015, primarily in Brazil, and subsequently spread in 2016 to Central and North America [5]. Accord8ing to the World Health Organization (WHO), 89 countries and territories have reported evidence of mosquito-transmitted Zika virus infection [6].

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Although the majority of ZIKV patients are asymptomatic, nevertheless, approximately 20 – 25% of patients develop symptoms [4], typically resembling a flu-like illness. The estimated period of incubation ranges from 4 – 10 days [7]. Low-grade fever, itchy maculopapular rash on soles and trunk, fatigue, arthritis or arthralgia, and non-purulent conjunctivitis are listed as the most common symptoms [5, 8]. The virus poses a particular threat during the preconception and prenatal periods, as maternal infection may result in severe foetal complications. Infection occurring during pregnancy can lead to congenital microcephaly and other congenital central nervous system malformations, meningitis, and even miscarriages and stillbirths [4].

Additionally, autoimmune disorders such as Guillain-Barré syndrome may occur in adults following ZIKV infection. In laboratory findings complete blood count is often normal, though nonspecific changes can be observed, such as mild lymphopenia, mild neutropenia, and mild-to-moderate thrombocytopenia. Moderate elevations of inflammatory markers or liver enzymes have been described. These symptoms also manifest in many other viral infections, such DENV and Chikungunya virus (CHIKV) infections. Consequently, none of these clinical presentations definitively differentiate the infections [8]; thus, further diagnostic measures should be undertaken.

CASE REPORT

This report presents the case of a 31-year-old male who was admitted in 2016 to the Department of Infectious Diseases in Bytom, Poland, with the suspicion of tropical disease infection after a 2-week trip with his wife to the Dominican Republic

in the Caribbean. In the last days of his vacation, he began to experience sudden fever and muscle pain. Later, during his first day of hospitalization, four days after symptom onset, a maculopapular rash without itchiness appeared on his torso and arms. Additionally, there was bilateral enlargement of cervical lymph nodes, which were sensitive to touch and were movable. He also complained about severe pain in the lumbar area, his fever was irregular with the highest temperature of 39 °C, but decreased to normal after taking antipyretic drugs.

There were no comorbidities in the patient's medical history and had completed vaccination against hepatitis B, hepatitis A, rabies, tetanus, diphtheria, yellow fever, and tick-borne encephalitis, which all were up-to-date during examination. He had frequently travelled to countries in Africa and Asia for work purposes, and claimed to have experienced multiple episodes of high fever infections after travelling. However, no diagnostic tests for tropical diseases had been conducted previously. He admitted to being quite disciplined in protecting himself against mosquito bites during his trips, even though he observed several of them on his skin. His wife did not exhibit any of symptoms, either during or after the vacation which, however, had taken place during the ZIKV epidemic in Latin America.

It is worth noting that the couple had also been for over a year trying to conceive a child.

The patient's blood was taken for laboratory testing, the results of which which showed a slightly lowered leukocyte count (3.46 x10^9/L) in the blood count, elevated levels of basophils (11.6%) in the blood smear test. C-reactive protein (CRP) and procalcitonin levels were within the normal range. The patient was also tested for Human Immunodeficiency Virus (HIV) and syphilis. The results were negative.

According to recommendations of the WHO, Centres for Disease Control and Prevention (CDC), European Centre for Disease Prevention and Control (ECDC), and Pan American Health Organization (PAHO) regarding diagnostics of patients who visited countries of Central America, South America and Caribbean Region, blood samples were also to be tested for malaria, DENV, ZIKV, and CHIKV. It is necessary to take into account the possibility of a patient getting infected with TBEV immediately before a vacation because both ZIKV and TBEV can exhibit similar symptoms, and may occur simultaneously.

Due to a pending diagnostic process, the patient received symptomatic treatment. After four days, the maculopapular rash faded away and the patient decided to discharge himself from the hospital. He had been thoroughly informed about the potential health consequences related to the possible sexual transmission of ZIKV infection. It has been emphasized that there is a need for sexual abstinence or the consistent and correct use of barrier methods of contraception until test results are obtained. In the event of positive results, preventive measures against infection through this route should be extended in accordance with the recommendations. The patient had been advised about the possible need to undergo serological and molecular control tests on urine and semen t the Department of Virology of the National Institute of Public Health – National Institute of Hygiene in Warsaw. The possible validity of testing for Zika virus infection and gynaecological and obstetrics consultation in terms of further pregnancy planning for his wife was recommended. Diagnostic tests for DENV, ZIKV, CHIKV and malaria conducted during hospitalization yielded the following results:

- Malaria – negative;
- DENV-Ab-IgM – 10.85 NTU (doubtful), DENV-Ab-IgG – 68.98 NTU (positive);
- ZIKV-IgM – 1.6 ratio (positive), ZIKV-IgG – 12.7 RU/ml (negative);
- CHIKV-IgM – 0.4 ratio (negative), CHIKV-IgG – 0.82 RU/ml (doubtful).

Because of the need for clarification of possible cross-reactions in serological tests of the patient and assessment of the actual situation, further ambulatory polymerase chain reaction (RT-PCR) tests were performed using VIASURE *Tropical Panel I* Real Time PCR Detection Kit (Certest Biotec S.L., Spain), with serological monitoring. The results are shown in the Table below.

Subsequent serum samples collected at 12-, 22-, and 46-weeks following symptom onset revealed following results:

- steady and significant decrease in IgM antibody level against ZIKV (in the second sample – a drop of more than 30%), together with a too high level of IgG against ZIKV in all samples (in the second sample, an increase of more than 30% in comparison to the first sample);
- high level of IgG against DENV increase in the second sample (10%), and stable low level of IgM against DENV (insignificant). No NS1 DENV antigen was detected;
- low level of antibodies IgM and IgG against CHIKV(insignificant).

The serological test results indicated that the patient was infected with the ZIKV. This diagnosis was confirmed by RT-PCR results – the genome of the ZIKV was detected in the patient's semen samples after 12 weeks after symptom

Table 1. Results of virological examination in samples collected from the patient (WS) and his wife.

Patient	Period from onset	Serological tests for Zika infection		Serological tests for Dengue infection		Antigen NS1 of Dengue virus	Serological tests for Chikungunya infection		Real-time RT-PCR for ZIKV	
		IgM to NS1 of ZIKV	IgG to NS1 of ZIKV	IgM to DENV	IgG to DENV		IgM to CHIKV	IgG to CHIKV	Urine	Semen
WS	5.5 weeks	1.03 (equ)	2.42 (pos)	0.38 (neg)	3.7 (pos)	0.22 (neg)	0.4 (neg)	0.82 (equ)	np	np
WS	12 weeks	0.7 (neg)	3.22 (pos)	0.43 (neg)	4.1 (pos)	np	np	0.7 (neg)	neg	pos
WS	22 weeks	0.12 (neg)	4.1 (pos)	np	3.25 (pos)	np	np	nt	np	neg
WS	46 weeks	0.17 (neg)	2.15 (pos)	np	1.7 (pos)	0.16 (neg)	0.42 (neg)	0.52 (neg)	np	neg
Wife		0.3 (neg)	0.07 (neg)	np	0.08 (neg)	np	np	0.03 (neg)	neg	np

Interpretation of results: negative result < 0.8 R; equivocal result 0.8–1.09 R; positive result ≥ 1.1R; np – not performed

onset. Positive results of IgG antibodies to DENV may have been the result of previous infection due to DENV, or cross-reactions observed among Flaviviruses [9].

The case was not previously reported because the patient was observed for an extended period due to his wife becoming pregnant a few months after receiving the ZIKV test results. As the ZIKV was present in the patient's semen, there was concern about the possibility of transmitting the virus to the foetus. During pregnancy, tests also revealed a positive result for toxoplasmosis. For that reason, the newborn infant was monitored for three years and showed no abnormalities in development during that time.

DISCUSSION

ZIKV remains a public health threat which, in 2016, was given the status of Public Health Emergency of International Concern, considering Zika-related microcephaly. Although the number of people infected has been decreasing year by year since the outbreak in 2016, ZIKV is still an active concern in some countries. Due to worldwide trade and growing numbers of travellers since the COVID-19 outbreak, the number of cases might start to increase. It is worth mentioning that rising global annual temperature can lead to the invasion of Aedes spp. mosquitos in new countries and territories. In 2023, PAHO reported that 36,738 cases of ZIKV disease were reported in both Americas, with the majority of incidents occurring in Brazil (95%) [10]. In Europe, in their Annual Epidemiological Report for 2021, the ECDC stated that seven cases of ZIKV had been reported. All the infected people had travelled outside the EU/EEA region [11]. Moreover, Aedes albopictus, which is a vector of many viruses, including ZIKV, has been noted in some regions of Europe, mostly on the Mediterranean coast in France and Spain, and the Adriatic coast of Croatia [12]. As the ZIKV is mosquito-borne, this route of transmission is considered the most common.

The first reported case of sexually-transmitted ZIKV infection dates back to 2011 when the wife of an American scientist, who had worked in Senegal, exhibited Zika-like symptoms of infection nine days after returning home. Grischott et al. [13] found that in four out of 10 reported cases of possible sexual transmission, ZIKV RNA had been detected in semen samples. In one of them, ZIKV RNA was detected in semen 62 days after the onset of the disease. In another case report, RNA of ZIKV was detectable for up to six months after the onset [14]. As in the presented case report, all the men with positive ZIKV testing in seminal fluid, showed Zika symptoms. This described route of infection could be a threat for couples trying to conceive a child as ZIKV in pregnant women leads to multiple congenital malformations in the foetus, with microcephaly listed as the most frequent. In Brazil in 2014, the annual number of microcephaly cases ranged from 150 – 200, but at the beginning of 2016, this number increased to almost 4,000 due to an outbreak of the ZIKV [7]. Miscarriages occurred in 14% among foetuses exposed to ZIKV [15]. Due to the possible risk, travellers returning from countries and territories with evidence of ZIKV should remain cautious during sexual intercourse. Male travelers should use condoms or abstain from sex for at least three months, and females should avoid sex or use condoms for at least two months from the onset of symptoms, the date of return from the territories with ZIKV prevalence, or the date of diagnosis, even if they do not present any symptoms. Use of a constant barrier method as a form of contraception can significantly reduce the risk of ZIKV sexual transmission [16]. Although ZIKV RNA was been detected in the seminal fluid of the patient in the current case report, tests performed on his wife did not reveal any trace of the ZIKV, despite the couple being sexually active during their stay in the Dominican Republic and planning to conceive.

Interpretation of test results for ZIKV infections and DENV should be based on comprehensive laboratory diagnostics, taking into account the complexity of the problem. PCR tests for ZIKV infection on acute-phase serum samples detect viral RNA [17]. RT-PCR in serum is the main test in the diagnostic approach of viral RNA of ZIKV, CHIKV, and DENV during the initial viremic phase [18]. However, this method has limited diagnostic time because RT-PCR in serum confirms infection only in the first days from infection (max. 7 – 10 days) [19]. Nevertheless, long-term persistence of ZIKV RNA has been observed in urine and sperm samples through RT-PCR testing [20]. Serological testing is recommended in the not-acute phase of infection (2 – 12 weeks from onset), since ZIKV IgM may not be detectable earlier [8].

ELISA test for non-structural protein 1 (NS1), which is a protein secreted by cells infected with flaviviruses, presents practically no cross-reactivity with NS1 of DENV, providing high specificity reactivity [21]. Another serological assay that offers high specificity among flaviviruses is a neutralizing test, which detects virus-specific antibodies. The plaque reduction neutralization test (PRNT) is considered the gold standard in quantifying and detecting the levels of neutralizing antibodies against flaviviruses, and assists in differentiating between viral infections, although some studies have reported cross-reactivity with previous DENV infection [22].

The diagnostic of *Flavivirus* infections, however, is more complex. One of the well-known phenomenon is viral haemorrhagic fever due to secondary infection by another serotype of DENV, and the problem of diagnosis of the secondary case. It is possible that similar mechanisms could be observed during ZIKV infection, as seen following other *Flavivirus* infections (e.g. Dengue fever), and even asymptomatic cases [23]. In such a situation, the possibility of false negative results or false positive results in serological tests to antibodies to structural antigens of *Flaviviruses* might be observed; therefore, the detection of species specific NS1 or antibodies to NS1 seems to be a crucial test.

It is worth noting that in the patient in the presented case repot, the different laboratory techniques used for diagnosis – RT-PCR and serology – allowed differentiation of *Flavivirus* infections and recognized the Zika infection. The diagnosis, however, presented were some serious implications for the patient's future. Analysis of serological tests results may indicate not only current ZIKV infection but also probable previous DENV infection. As the patient used to travel to countries in Africa and Asia, it is most likely he became infected with DENV with asymptomatic or subclinical and unspecific symptoms, during one of the trips. Primary infection with one of four serotype of DENV provides a lifelong immune response and protection against this serotype. After becoming infected with a different DENV serotype during secondary infection (in next 5 – 15 years)

there is a possibility of developing Dengue haemorrhagic fever/dengue shock syndrome. Clinical manifestation shows four major symptoms – severe fever, haemorrhage, often with hepatomegaly, and even circulatory failure. There is also significant thrombocytopenia with <100,000 platelets/μL, abdominal pain, and plasma leakage caused by increased vascular permeability. Dengue haemorrhagic fever/dengue shock syndrome is associated with high mortality rate [21, 24].

It is worth mentioning that the 'Dengvaxia' vaccine is available for patients aged 6 – 45 as protection against the virus. It can be administered only to those previously infected with any serotype of DENV, in order to minimalize the risk of secondary infection. The vaccine contains an attenuated YFV backbone combined with genes of four DENV serotypes [25, 26]. Other vaccines are still in the process of development. Even so, patients should remain immensely cautious when travelling abroad to regions of DENV surveillance, and use adequate protection against mosquitos in order to avoid becoming infected with the virus which can lead to severe complications.

Although immune plays a major role in fighting infection, in the case of ZIKV it can also lead to inducing an autoimmune response. Guillain-Barré syndrome associated with Zika fever is estimated to be 2 – 3 per 10,000 infections, which is compatible with the risk of Campylobacter infection. Other autoimmune disorders, such as thrombocytopenia purpura or autoimmune retinopathy, have also been related to ZIKV infection [15]. Furthermore, in studies completed on macaques, primary infection with ZIKV induced protective immunity, and the critical part played by CD8 T cells [27]. Therefore, the patient in the presented case report should be aware of possible complications considering the immune system. Because of the presence of ZIKV RNA in the patient's semen, he and his wife should postpone attempting to conceive for about six months, or until the molecular test no longer presents any residue of ZIKV. It is also highly important for him to use a barrier method of contraception in order not to infect his wife with the virus.

As the vaccines against the ZIKV are still in the process of development, travellers to countries and regions of virus surveillance should remain cautious and obey the guidelines of healthcare agendas. Poles travelling to areas where the virus is present should follow recommendations from the Chief Sanitary Inspector, which also apply to other tropical diseases transmitted by mosquitoes. It is recommended to use repellents containing such substances as DEET, icaridin/picaridin, and IR3535, during the day and night, both outdoors and indoors. Additionally, wearing appropriate clothing to protect against mosquito bites and using mosquito nets at night are advised. Lastly, staying indoors during times of the day when mosquito activity is highest, preferably in air-conditioned or fan-equipped rooms, is recommended [28].

CONCLUSION

The presented case highlights the complexity of the diagnostic process and treatment of patients with ZIKV infection, and highlights the challenges posed by overlapping symptoms and intricacies of using both molecular and serological methods in diagnosing travellers returning from endemic regions. Due to absence of an approved vaccine for ZIKV, the increased popularity of travel to endemic counties, climate change, and expansion of vectors, ZIKV might become an emerging concern in countries previously free from the virus.

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