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Case Report: First Case of Zika Virus Infection in a Returning Canadian Traveler

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Abstract A woman who recently traveled to Thailand came to a local emergency department with a fever and papular rash. She was tested for measles, malaria, and dengue. Positive finding for IgM antibody against dengue and a failure to seroconvert for IgG against dengue for multiple blood samples suggested an alternate flavivirus etiology. Amplification of a conserved region of the non-structural protein 5 gene of the genus *Flavivirus* yielded a polymerase chain reaction product with a matching sequence of 99% identity with Zika virus. A urine sample and a nasopharygeal swab specimen obtained for the measles investigation were also positive for this virus by reverse transcription polymerase chain reaction. Subsequently, the urine sample yielded a Zika virus isolate in cell culture. This case report describes a number of novel clinical and laboratory findings, the first documentation of this virus in Canada, and the second documentation from this region in Thailand.

The tourist industry has successfully promoted and popularized travel to countries, for the average North American traveler, that a few decades ago would not be considered vacation or travel destinations. Many of these locations are in tropical countries where mosquito-borne diseases, such as dengue and malaria, are endemic and highly prevalent. Early signs and symptoms of these travel-related infections can be similar and overlapping, therefore requiring differentiation through laboratory testing. In some regions, where multiple infectious agents co-circulate in mosquito vectors, it is no longer possible to infer with certainty which agent could be causing the disease on the basis of geographic location and clinical signs and symptoms.

We report the detection of Zika virus in a Canadian traveler who was classified as having a case of dengue fever based upon the clinical and epidemiologic history, and preliminary laboratory investigations. However, the atypical dengue serologic results prompted us to reconsider the possibility of another flavivirus infection.

The patient went to Thailand with a party of family and friends to attend a wedding and received travel counseling before her flight. She left Canada on January 20, 2013 and flew to Bangkok via Hong Kong where she stayed for eight days, and recalled being bitten by mosquitoes on a few occasions. She traveled to Kata Beach, Phuket Island, stayed for five days and noted a modest number of bites. She then returned to a hotel in Bangkok located near a river where she stayed for three days and recalled significant mosquito exposure day and night. She did not take medications for malaria, and she did not use a bed net at night. She flew back to Canada via Hong Kong, and noted a few mosquito bites during transit. During her flight she felt irritable, had a backache but no fever or chills, described her bitten areas as itchy, for which she took acetaminophen.

On her return to Canada, she returned to work the next day, and noted the onset of intermittent periods of fever and chills (day one of illness). Two days later, her mouth became sore and oral blisters developed. On day five of illness, a papular rash developed, which spread to her extremities and included her palms. The rash lasted four days, and in conjunction with a retro-orbital headache and fever and mild conjunctivitis, prompted a visit to an emergency department.

Blood, nasopharyngeal swab, and urine samples were collected for investigation of measles and other infectious causes as the differential work-up for travel associated pathogens. On day seven of illness, significant joint and muscle tenderness developed, which lasted for two complete days and then became episodic for an additional four days, followed by a gradual return to her normal well-being, which took an additional three days. The time from the prodromal period, which was marked by intermittent fever and chills, to the resolution of her symptoms, was approximately 16 days.

Initial laboratory investigations showed a hemoglobulin level of 131 g/L, a leukocyte count of 4.7×10^9 cells/L with a normal differential, but low platelet count of 81×10^9 cells/L (reference range = $150–400 \times 10^9$ cells/L). Levels of creatinine, electrolytes, alanine aminotransferase, and alkaline phosphatase were within reference ranges. Thick and thin blood smears were negative for malaria and other blood parasites, and blood cultures were negative for bacterial pathogens. Dengue IgM and IgG serologic analysis was performed by using kits from Focus Diagnostics (Cypress, CA) as specified by the manufacturer's protocol.

A number of blood samples were obtained in February and March to determine if this person had an acute dengue infection; these samples later indicated dengue seroconversion. The positive dengue IgM result from blood collected on day 10 of her illness was considered indicative of an acute infection, which was consistent with her other symptoms and collectively compatible with a clinical picture of acute dengue fever (Table 1). However, blood obtained on day 41, did not show IgG seroconversion, and IgM values for the previous serum sample (obtained on day 10) and this sample were fairly similar (Table 1). This inconsistency prompted us to investigate the possibility of another flavivirus infection.

The decision to use a reverse transcription polymerase chain reaction (RT-PCR) described by Ayers et al,[1] was based upon her onset of illness and availability of archived

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Table 1
Summary of samples collected and testing performed relative to onset of illness*

Sample type	No. days after onset†	Dengue EIA (IgM/IgG)	RT-PCR gel-based assay result	CDC results, Zika virus IgM EIA or PRNT‡
Blood	6	NT	Positive	IgM EIA: Equivocal
Urine and nasopharyngeal swab specimen	6	NA	Positive	
Blood	9	Negative/negative	Positive	IgM EIA: Equivocal, PRNT titer < 10
Blood	10	Positive (2.5)/negative	Negative	
Blood	41	Positive (1.5)/negative	Negative	
Blood	77	Positive (1.5)/negative	NT	IgM EIA: Strongly positive, PRNT titer = 1,280
Blood	114	Negative/negative	NT	–

*EIA = enzyme immunoassay; RT-PCR = reverse transcription polymerase chain reaction; CDC = Centers for Disease Control and Prevention; PRNT = plaque reduction neutralization test; NT = not tested; NA = not available.
†Number of days when samples were collected after onset of illness.
‡See text for description of testing.

serum samples from this period. This gel-based PCR targets the conserved nonstructural protein 5 gene region across numerous species of this genus, but enables subsequent discrimination between them because of characteristic sequence variations within the amplicon.

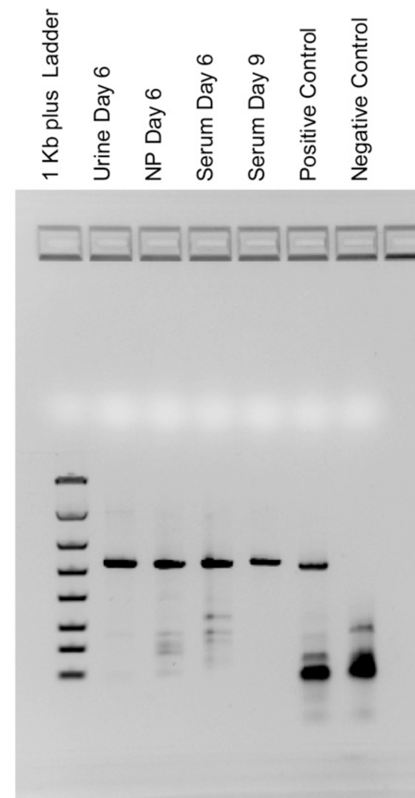
The results of the RT-PCR are shown in Figure 1 for blood, urine, and nasopharyngeal samples. The urine and nasopharyngeal swab specimen were included because nucleic acid extracts were available from the earlier measles RT-PCR testing, which showed negative results. Significant bands can be seen at the 800–900-base pair range expected for flaviviruses for the samples collected in the acute phase of her illness. The amplicon from each of these bands was sequenced and found to be identical between the various specimen types, and was highly homologous to the Asian lineage of Zika virus (GenBank accession no. JN60885) (Figure 2).

As a result, Zika virus serologic testing was referred to the Centers for Disease Control (CDC) in Fort Collins, Colorado, where IgM testing using an in-house enzyme immunoassay and a plaque-reduction neutralization test (PRNT) were performed; these results are shown in Table 1. Seroconversion to Zika virus was demonstrated by the CDC enzyme immunoassay (from equivocal to positive) and an increase in PRNT titers in acute-phase and convalescent-phase samples, complementing the molecular findings.

The National Microbiology Laboratory in Winnipeg, Manitoba, Canada, successfully isolated Zika virus from the urine sample by using a Vero E6 cell line. Sufficient viral RNA was present in the urine and nasopharyngeal samples to determine most of the genome, segments of which were complemented by sequencing templates from the cultured virus. The complete Zika virus sequence is deposited in GenBank under accession no. KF993678. A phylogenetic tree comparing this virus with other prototype flaviviruses is shown in Figure 2.

This case has a number of interesting features, notwithstanding that this is the first reported detection of Zika virus in Canada[2] and the second reported detection from this part of Thailand.[3] Furthermore, we isolated the virus in culture and determined the complete genome sequence.

The origins of Zika virus date back to its isolation from sentinel rhesus monkeys in the Zika Forest of Uganda.[4] In 2010, during syndromic surveillance of patients with fever in neighboring Cambodia, a case of Zika virus infection in a young child was detected by PCR which indicated its presence in this area of Southeast Asia.[5] An outbreak in Yap Island, Micronesia in 2007[6] and more recently in French Polynesia[7] in 2013 and 2014 illustrate the global distribution of this agent in Asia and the Pacific region, which in many respects are the same regions where dengue is also endemic. Various species of *Aedes* mosquitoes, including *Aedes aegypti* are the permissive vectors of Zika virus, and this mosquito species is also capable of transmitting dengue, making it



Legend
Lane 1: 1 KB plus Ladder
Lane 2: Urine collected Day 6 of illness
Lane 3: Nasopharyngeal swab collected Day 6 of illness
Lane 4: Serum collected Day 6 of illness
Lane 5: Serum collected Day 9 of illness
Lane 6: Positive Control: Calbertado virus in-vitro RNA
Lane 7: Negative Control: RNAase free water

Figure 1. Reverse transcription polymerase chain reaction assay showing location of genus *Flavivirus* bands. Lane 1, 1 kb ladder; lane 2, urine sample collected on day 6 of illness; lane 3, nasopharyngeal swab specimen collected on day 6 of illness; lane 4, serum sample collected on day 6 of illness; lane 5, serum sample collected on day 9 of illness; lane 6, positive control (Calbertado virus *in vitro* RNA; lane 7, negative control (RNase- free water).

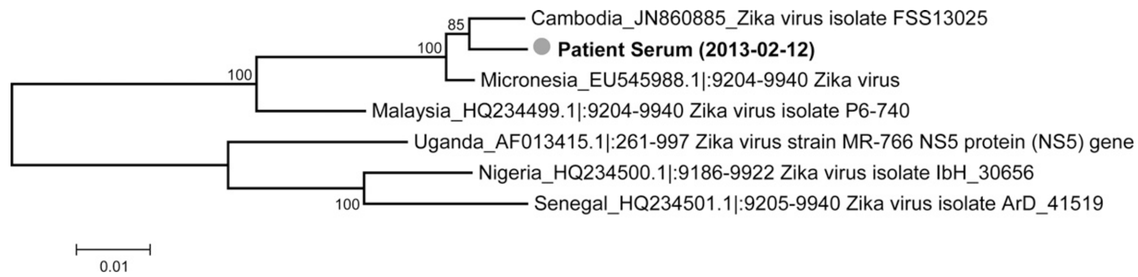


FIGURE 2. Phylogenetic analyses of Zika virus from the patient with representative sequences deposited in Genbank.[17] Scale bar indicates nucleotide substitutions per site.

plausible that Zika virus can also circulate in areas in which dengue is endemic.

The incubation period of flaviviruses, such as West Nile virus and dengue virus, is considered to be from 3 to 7 days (range = 3–14 days).[8] On the basis of the significant mosquito exposure of the patient in early February and onset of illness with fever and chills on February 6, the incubation period would be consistent with the reported range. The appearance of a rash on her fifth day of illness corresponds to the viremic phase, which is detectable by the gel-based assay, and her immune response to infection. Most notably, the presence of the virus in her nasopharynx and urine, together with culture of the virus from her urine, indicates significant levels of viral circulation and shedding and the possibility of person-to-person transmission in a close setting. There was a reported case of sexual transmission in a patient who was in the early stage of his infection.[9]

From a diagnostic perspective, the collection of urine and nasopharyngeal swab specimens at the onset of illness could also be used as alternative or complementary samples to blood for molecular detection of Zika virus, especially in persons returning from areas where both flaviviruses are endemic and for whom clinical signs and symptoms are not typical for dengue.

The likelihood of transfusion-related transmission has recently been investigated in mild or asymptomatic cases because of the brief viremic period (3–5 days) in the acute phase of the infection. A prevalence of 2.8% of blood donors were positive for Zika virus in the on-going outbreak in French Polynesia; furthermore, the sequences of the virus from these donors were similar to those from the circulating outbreak strain.[10] Similar events of transfusion-associated transmission have been reported with West Nile virus, another related flavivirus.[11]

The clinical signs and symptoms of infection with Zika virus can be easily confused with dengue, mainly because of the fever, headache, and generalized rash-like presentation. A non-purulent conjunctivitis is a unique feature of Zika virus infections, and was described in 55% of cases in the Yap Island outbreak,[6] and also a finding in our case, although not often reported for dengue unless it is a severe hemorrhagic presentation. Although speculative, infections with the Asian lineage of Zika virus are associated with a higher frequency of conjunctivitis because case reports from Africa make no reference to this feature.

The serologic tests performed at CDC showed an early IgM response, whereas the neutralizing antibody response detected by the PRNT can take somewhat longer. Presently, serologic assays for Zika virus are not commercially available, and our case suggests the possibility of using a combination of a positive IgM response to dengue virus and lack of an IgG seroconversion for convalescent-phase serum samples as a potential surrogate to investigate another flavivirus. However, when the dengue IgM response is positive, Zika virus is no longer detectable, and for our case the cross-reactive IgM response was detectable for at least one month. Thus, having blood samples obtained at the onset of illness and stored for molecular testing is necessary and increases the likelihood of detecting virus in clinical samples because the viremic period is brief. This finding contrasts with dengue virus infections when the viremic phase overlaps the period when the IgM is detectable.[12] Interestingly, the cross-reactive IgM response to West Nile virus, another flavivirus, was not seen for samples from this patient. A similar lack of cross-reactivity to West Nile virus was noted in the Yap Island outbreak,[13] and the case of the recent German traveler.[3] A positive dengue IgM result and the overlapping features of the infection can reinforce an incorrect classification of a Zika virus infection as dengue. A recent case report by Kwong and others[14] also commented on this possibility.

The availability of molecular testing using primers to conserved regions of the flavivirus genus has great merit in detecting a different etiologic agent, as in this particular case. Although dengue is considered to be the most prevalent agent causing typical fever and rash-like illness in the returning traveler, other viruses such as chikungunya[15] and possibly Zika virus, are also now co-circulating in some of these tourist destinations, where the management of the patient may be different. In chikungunya virus–infected patients, treatment with nonsteroidal anti-inflammatory drugs and corticosteroids may be considered when these patients have severe and disabling joint involvement, which can last for extended periods.[16] In contrast, Zika virus infections are, for the most part, self-limiting and without significant sequelae.[5,6]

The significant advantage of a positive molecular test result is that the result is definitive, and thus precludes follow-up samples to monitor for serologic conversions and multiple evaluations. Commercial molecular assays for dengue virus are now available, and it is possible to use these and the gel-based PCR in a stepwise algorithm to identify other probable cases of viral agents. One limitation of molecular-based assays is their dependence upon significant viremia for detection. Some travelers may be past this period if symptoms occur while they are in transit or there is a delay in seeking medical attention and collection of appropriate clinical samples.

In less-developed countries, financial and human resources to identify these agents through serologic or molecular epidemiology are either unavailable or restricted. Therefore, returning travelers from developed countries can serve as sentinels for

the presence of these emerging agents, especially if their onset of illness is within a few days of their return and the appropriate samples are collected and sent to a laboratory with advanced diagnostics.

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