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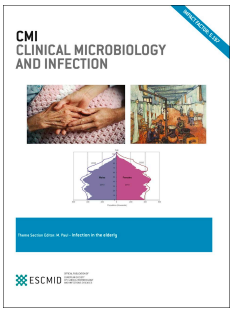

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Emerging souvenirs - clinical presentation of the returning traveller with imported arbovirus infections in Europe

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Abstract

Background

Arboviruses are an emerging group of viruses that are causing increasing health concerns globally, including in Europe. Clinical presentation usually consists of a non-specific febrile illness that may be accompanied by rash, arthralgia and arthritis and/or with neurological or haemorrhagic syndromes. The range of differential diagnoses of other infectious and non-infectious aetiologies is broad, presenting a challenge for physicians. While knowledge of the geographic distribution of pathogens and the current epidemiological situation, incubation periods, exposure risk factors and vaccination history can help guide the diagnostic approach, the non-specific and variable clinical presentation can delay final diagnosis.

Aims and Sources

This narrative review aims to summarize the main clinical and laboratory-based findings of the three most common imported arboviruses in Europe. Evidence is extracted from published literature and clinical expertise of European arbovirus experts.

Content

We present three cases that highlight similarities and differences between some of the most common travel-related arboviruses imported to Europe. These include a patient with chikungunya virus infection presenting in Greece, a case of dengue fever in Turkey, and a travel-related case of Zika virus infection in Romania.

Implications

Early diagnosis of travel-imported cases is important to reduce the risk of localized outbreaks of tropical arboviruses such as dengue and chikungunya and the risk of local transmission from body fluids or vertical transmission.

Given the global relevance of arboviruses and the continuous risk of (re-)emerging arbovirus events, clinicians should be aware of the clinical syndromes of arbovirus fevers and the potential pitfalls in diagnosis.

Keywords (5-10): Arbovirus, imported febrile illness, dengue fever, chikungunya virus, Zika virus, travel-imported illness

Introduction

With an increase in global travel rising to around 950 million persons per year, physicians are frequently confronted with patients potentially infected with exotic pathogens (1). Besides malaria, infection with an arbovirus is a common cause of fever in travellers returning to Europe (2). Arboviruses are a large group of emerging RNA viruses spanning different viral families and genera that are responsible for human disease worldwide in the range of hundreds of million cases annually (3-5).

In Europe, several endemic arboviruses are of clinical importance such as tick-borne encephalitis, West Nile fever, Crimean-Congo haemorrhagic fever and sandfly fever (6). However, there is heterogeneity in the surveillance of endemic, well-known arboviruses and uncertainty about the true burden of related illness in Europe (6). The situation is aggravated by (re-)introduction of vectors as well as introduction of viraemic patients following travel (5-8). Limited autochthonous outbreaks have been described for dengue (DENV) (9-13) and chikungunya (CHIKV) virus in Europe (14-16). The endemic areas for DENV and CHIKV are found in tropical and subtropical regions of the world. DENV is the most successful arbovirus in terms of emergence in recent decades, with an estimated 390 million infections per year globally (17). The main endemic areas are in Asia and South America with less data in some areas, particularly in Africa. Returning travellers presenting with DENV can serve as "sentinels" and a recent study estimated the proportion of cases in Africa to be in the same range as in Latin America (17-19). CHIKV is mainly found in Asia and Africa, with recent large outbreaks on the Indian Ocean islands and spread to the New World, particularly the Caribbean and the Americas (20-22).

Zika virus was considered to be a flavivirus of low interest, until its dramatic emergence in French Polynesia in 2013 and subsequently in South America in 2015. While Zika usually causes mild disease in adults, it can lead to congenital malformations when infecting pregnant women and is also associated with Guillain-Barré syndrome (23). This has led to increased awareness of the potential risk of other neglected arboviruses (5). Fortunately, no vector-borne transmission of Zika virus has been recorded in Europe to date (6). However,

sexual transmission of Zika virus has been reported and is an additional source of introduction besides vectors and diseased travellers (24, 25).

While dengue, Zika and chikungunya account for the vast majority of travel-imported arbovirus cases in Europe, there is a plethora of other, less well-known arboviruses capable of causing human disease. These include viruses such as Jamestown Canyon, Mayaro, Oropouche, Tahyna, and Usutu viruses and many more, which are not known to most clinicians (26-28). The increasing importance of arboviruses in Australia, such as Kunjin virus, Murray Valley fever and Ross River fever poses an under-recognized hazard for travellers from Europe (29, 30).

For clinicians, the diagnosis of travellers presenting with syndromes of fever, rash, myalgia, arthralgia and headache is challenging, due to their non-specific nature and the wide range of potential differential diagnoses. Diagnostic test strategies for arboviruses can be complex due to the short viraemic period, pitfalls in serology such as high levels of antibody cross-reactivity, and patchy access to specialized arbovirus diagnostics. Despite similarities in the disease presentations, there are differences which, together with travel and vaccination history, can help guide the identification, sampling and differential diagnostics.

We present three cases to highlight the difficulties which European clinicians face in recognizing travel-related imported arbovirus illnesses and we discuss similarities and differences in clinical and laboratory findings.

Imported arbovirus infections to Europe

Case 1: An imported chikungunya case to Greece

In the spring of 2016, a woman in her twenties returned to Greece from Recife, Brazil, where she had stayed since November 2015. During her return travel she developed myalgia and arthralgia for 9 days followed by development of high fever (40°C) and mild headache upon arriving in Greece. There was no significant past medical or surgical history. On physical examination there was no rash, hepatosplenomegaly or conjunctivitis, but she had swelling of both knees and the left wrist. Her white blood cell count was 3.2 x 10^9/L with 50% neutrophils, 34% lymphocytes and 13% monocytes, haematocrit 39.5%, platelet count 247 x 10^9/L, and C-reactive protein 10 mg/dL (normal <5). All other tests were unremarkable. Her fever subsided over the following 72 hours, with normalization of her laboratory tests and she was discharged after 3 days of hospitalization. She was advised to adopt safe sex practices until results for ZIKV were received.

Molecular testing for DENV, ZIKV and CHIKV was performed at the National Reference Centre for Arboviruses in Greece on the samples taken on the 2nd day of illness using commercial Real Time RT-PCR kits (Altona Diagnostics GmbH, Hamburg, DE). CHIKV RNA was detected in serum and blood. An in-house RT-nested PCR using generic alphavirus primers (31) obtained a sequence clustering in the ESCA genotype. The sequence showed 100% identity to sequences from Brazil (32). The presence of CHIKV IgM and IgG antibodies was tested using indirect immunofluorescence test and ELISA, respectively (Euroimmune, Lübeck, DE). A weak positive result was obtained only for CHIKV IgM antibodies in the initial sera, while both IgM and IgG antibodies were detected in a convalescent sample taken on March 1. Serology for DENV and ZIKV remained negative. CHIKV was isolated from her blood in Vero E6 cells, with cytopathic effects seen on the 2nd day after inoculation. The patient had an unremarkable recovery, however arthralgia persisted for 3 more months.

Case 2: An imported dengue fever case in Turkey

A 24-year-old French national presented in November 2017 at the Koç University Hospital, Istanbul with a three-day history of fever, fatigue and malaise, starting four days after returning from a nine-month residence in Cambodia.

On admission, his temperature was 38.9 °C, his blood pressure was 130/70 mmHg and he had a right subconjunctival haemorrhage. No rash or hepatosplenomegaly was detected and other physical examination findings were normal. Laboratory tests revealed a total white cell count of 3.65 x 10^9/L (normal 4.4-11.5), platelet count 176 x 10^9/L (normal range 100-400), mildly elevated AST with peak level 100 U/L (normal range 0-31), ALT peak 67 U/L (normal range 0-31), LDH peak 200 U/L (normal range 135-225) and GGT peak 244 U/L (normal range 8-61), CRP peak 27.6 mg/L (normal range 0-5). A further tropical diagnostic workup was requested and blood samples taken on 2 of hospitalization (day 5 of illness) were sent to the Public Health Institute of Turkey, Ankara. On the 3rd day of hospitalization he became afebrile but his temperature increased again 2 days later. Dengue IgG and IgM antibodies (Immunofluorescence test, Euroimmune) and PCR (Reverse transcriptase PCR, Multiplex) were positive, confirming acute dengue infection. He was discharged on the 7th day of hospitalization (day 12 of illness) and had made a full recovery with normalization of blood tests at outpatient review on day 14 of illness. Paired serology remained negative for leptospirosis.

Case 3: An imported Zika virus case in Romania

A man in his thirties presented in the summer of 2016 at the University Hospital of Infectious Diseases, Cluj-Napoca, Romania with a 4-day history of fever, headache, fatigue, myalgia and rash spreading from the neck and thorax before becoming generalized. Symptoms started 5 days after he left the Dominican Republic, where he had stayed for seven days.

On physical examination, his temperature was 36.8℃ and he had conjunctivitis of the right eye, a generalized non-pruritic macular rash and diffuse erythematous pharyngitis. There were no other significant findings. Laboratory tests revealed mild thrombocytopenia of 135 x 10^9/L (normal range 150-450), with normal haemoglobin, inflammatory markers and renal

and liver biochemical parameters. Malaria was ruled out by rapid diagnostic test (Malaria MBPan Mascia Brunelli) and thin and thick blood film examinations. Urinalysis, blood cultures and viral and bacterial pharyngeal swab tests did not reveal any pathological findings. A further diagnostic work-up for tropical diseases was requested and serum and urine samples (taken 5, 8 and 18 days after start of symptoms) were sent to the Reference Laboratory, Cantacuzino Institute in Bucharest. NS1 dengue antigen was negative, ELISA testing (Euroimmune, Lübeck, DE) for Zika IgM antibodies showed borderline values on the first two samples (index value 0.901 [(negative< 0.8, positive >1.1]) with seroconversion by day 18 of illness (index value = 2.02). ELISA for Zika IgG antibodies was negative in the first two (index = 0.1) and borderline positive in the last serum samples (index = 1.064). Real time PCR (in-house test) was positive for ZIKV RNA in the urine samples taken 5 and 8 days after symptom onset but negative in the serum samples taken at the same time. Symptomatic treatment was recommended and counselling was provided regarding sexual transmission and the need to avoid pregnancy for 6 months for his partner. The rash disappeared after 3 days, his platelet count normalized in one week and no other signs and symptoms appeared during the 6-month follow-up. His wife was not tested for Zika virus infection and did not develop clinical illness, but avoided pregnancy for 6 months.

Discussion

Arboviruses are found worldwide and more than 150 are documented to cause disease in humans (1, 5). Overall, vector-borne diseases imported to Europe through travel are increasing, among them arbovirus infections such as dengue and chikungunya (2). The clinical presentation of an acute arbovirus infection can range from asymptomatic or mild disease, up to severe life-threatening courses and death, with a high disease burden in endemic countries. Due to the broad spectrum of differential diagnoses, a rapid and targeted diagnostic approach is necessary.

The clinical syndromes of arbovirus disease can generally be divided in four main syndromes consisting of (1) fever alone or fever with (2) rash and arthralgia, (3) neurological symptoms and/or (4) haemorrhagic symptoms (1). Most arboviruses are associated with one or more than one of these syndrome complexes, with fever as a common feature for all of them, with the exception of Zika, where fever is not always present (33, 34). However, there is significant overlap between the syndromic groups [Fig 2]. Most arboviruses cause a biphasic illness with initial non-specific symptoms for a few days followed by improvement then either resolution or more severe features starting about a week after symptom onset. Common laboratory features of all three arboviruses are decreased white cell counts and platelet counts, less pronounced in Zika virus infection. For a comparison of clinical and laboratory findings in chikungunya, dengue and Zika virus infections, see Tables 1 and 2.

Chikungunya infection (Case 1) is usually associated with abrupt onset of fever and malaise after an incubation period of 3-7 days, although this can extend to 12 days. Most (more than 75%) infected patients develop symptoms (21). Distinction from dengue may be difficult, especially in travellers returning from areas where both infections are circulating (35). Typical symptoms include fever that can exceed 39℃ and polyarthralgia. Symmetrical bilateral arthralgia is found in most patients and is usually located in the peripheral joints, appearing shortly after the onset of fever (2-5 days). There may also be visible or palpable swelling. A macular or maculopapular rash is commonly seen, in up to 75% of patients. Other symptoms

include pruritus, conjunctivitis, headache, myalgia and gastrointestinal symptoms (36, 37). Laboratory abnormalities that are commonly seen in chikungunya infection include lymphopenia, thrombocytopenia and elevated aminotransferase levels (36, 37).

The viraemic period can range from 2-10 days with a total duration of acute illness around 7 to 10 days. Some patients experience persistence of relapse of arthralgia for months with the risk of developing chronic joint symptoms or even chronic inflammatory polyarthralgia (38, 39). Alopecia and depression are also reported as long-term sequelae (40).

Dengue virus infection (Case 2) is classified by the WHO in the following categories: dengue without warning signs, dengue with warning signs and severe dengue (41). During the course of disease, three phases can be seen that consist of a febrile phase, a critical phase and a recovery phase (41). Travellers are usually seen in the febrile phase, which lasts about 2-7 days. Patients present with high fever accompanied by headache, vomiting, myalgia, arthralgia and a transient blanching discrete or coalescent macular rash. This rash often has "islands of white in a sea of red" (5). Pruritus may be present initially. Severe headache, pain behind the eyes, back pain, and myalgia and arthralgia are reported in up to 70% of cases (42), with rash in around 50%. Other symptoms may include gastrointestinal manifestations such as diarrhoea, vomiting, pain and nausea and symptoms resembling a respiratory tract infection (cough, running nose, sore throat, injected pharynx). Mild haemorrhagic manifestations like petechiae and mucosal membrane bleeding from gum or nose can occur (41). In this phase, clinical features are indistinguishable between severe and non-severe dengue and are also difficult to distinguish from other non-dengue febrile illnesses. The tourniquet (Hess) test is advocated to identify severe disease early: a blood pressure cuff is inflated to between systolic and diastolic pressure for 5 minutes and the resulting petechiae are counted. However, the specificity and sensitivity of this test is moderate (43). Laboratory abnormalities include a decreased white blood count, particularly neutropenia. The febrile phase may followed by defervescence around day 3-7 of illness and is characterized by increase capillary permeability and in severe illness bleeding and significant plasma leakage.

Laboratory findings in this phase include increased haematocrit, thrombocytopenia and leukopenia, particularly neutropenia. The risk of having complications and progressing to severe dengue is highest in this phase. The recovery phase that follows is characterized by fluid resorption and gradual recovery.

In Zika infection (Case 3), symptoms are the most non-specific of all three viruses and differential diagnosis remains a challenge. A large proportion of infections remain asymptomatic and only 20-25% of infected individuals present with symptoms (44).

Acute Zika virus infection is usually characterized by low-grade fever (up to 38.5℃), rash, arthralgia and conjunctivitis. Other symptoms include myalgia, headache, eye pain and asthenia (45, 46). A maculopapular rash is seen in around 90% of patients, but fever is less pronounced and less common than in chikungunya and dengue infections, affecting 40-75% of patients and thrombocytopenia is also much less common. As highlighted in case 3, asymptomatic infections have to be considered in case pregnancy is planned after travel of the patient or their partner to an endemic area. Current recommendations do not recommend testing of asymptomatic returning travellers; therefore emphasis should be put on pre-travel advice for couples that are planning a pregnancy (47, 48).

In the diagnostic approach for the three arboviruses presented here, both serology and direct virus demonstration (such as PCR testing) are useful but several characteristics of the viruses influence time and cost-effective diagnostic work-up. Serological testing includes detection of IgG and IgM antibodies that are usually present by a week after onset of symptoms of all three viruses. Dengue virus and Zika virus are both flaviviruses, so cross-reactivity of antibodies may be problematic. Positive antibody findings may be due to acute infection, but could also result from previous infection with the same or another flavivirus, or following previous immunization against yellow fever or tick-borne encephalitis (49). This cross-reactivity can complicate the interpretation of results, and previous exposure history and immunization history should be checked and the information should be delivered to the laboratory. Frequent travellers who have received immunizations against yellow fever,

Japanese encephalitis or tick-borne encephalitis can show a considerable antibody background for flaviviruses that can mimic a wild-type flavivirus infection.

Direct virus detection by PCR is another option for diagnosing flaviviruses, which is highly specific when positive. However, the duration of viraemia in flavivirus infections is short and therefore PCR test positivity is confined to the first few days of illness. This window is often missed in returning travellers, especially if their symptoms start while still abroad. For dengue, direct detection of the virus antigen NS-1 in blood that can prolong the window up to 7 days (41). Of wider interest, it has recently been shown that detection of virus RNA in urine can prolong the window for PCR diagnosis for up to several weeks after onset of symptoms of dengue and other arboviruses (50-52). Diagnostic testing for other, more rare arboviruses beyond the three common ones presented here is mostly limited to specialized laboratories.

Conclusion

The identification and diagnosis of acute arbovirus infections can be challenging and laborious for both physicians and clinical virologists, although the combination of epidemiology, clinical syndromes and findings in basic blood tests such as the blood count may provide useful clues. As arboviruses are an emerging group of viruses in Europe and beyond and access to highly specified diagnostics is often limited, recognition of suspected arbovirus infections should be addressed both in clinical training as well as in research on diagnostics and therapeutics.

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Transparency declaration

The authors declare no conflicts of interest.

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455 **Figure legends**

456 **Figure 1.**

457 Blanching rash of dengue fever in a returning traveller.

458

459 **Figure 2.**

460 Summary of arbovirus syndromes together with fever: Central nervous system; Fever
461 arthralgia rash; Viral haemorrhagic fever. (a) alphavirus; (c) coltivirus; (f) flavivirus;(b)
462 bunyavirus; (n) nairovirus; (p) phlebovirus. CCHF Crimean Congo haemorrhagic fever; CHIK
463 chikungunya; CTFV Colorado tick fever; DEN dengue; EEEV Eastern equine encephalitis; JE
464 Japanese encephalitis; LACV La Crosse virus; MVEV Murray Valley encephalitis; ONNV
465 O'nyong nyong; RRV Ross River fever; RVFV Rift Valley fever; SLEV St Louis encephalitis;
466 TBEV tick borne encephalitis; VEEV Venezuelan encephalitis; WEEV Western equine
467 encephalitis; WNV West Nile fever; YFV yellow fever; ZIKV Zika virus. Adapted (with
468 permission) from Solomon T, Chapter 40 in eds Beeching N, Gill G, Lecture Notes Tropical
469 Medicine, Wiley 2014; p 274.

470

471 **Table 1.** Comparison of selected clinical findings in chikyungunya, dengue and Zika

472 infections

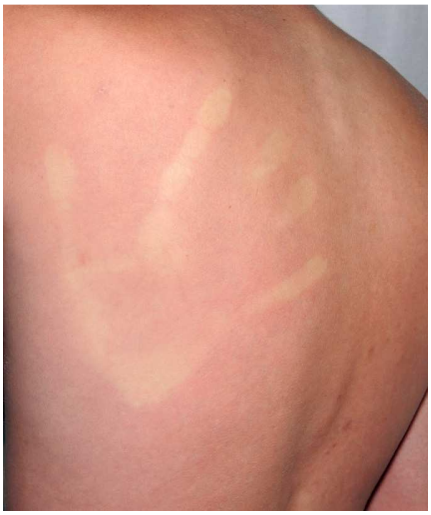
Clinical presentation	Chikungunya	Dengue	Zika
Fever	+++	+++	+
Rash	++	++	+++
Myalgia	+	+++	+
Arthralgia	+++	+	++
Oedema	-	-	++
Retro-orbital pain	+	++	+
Conjunctivitis	+++	-	+++
Lymphadenopathy	++	++	+
Hepatomegaly	+++	-	-
Haemorrhage	-	+	-

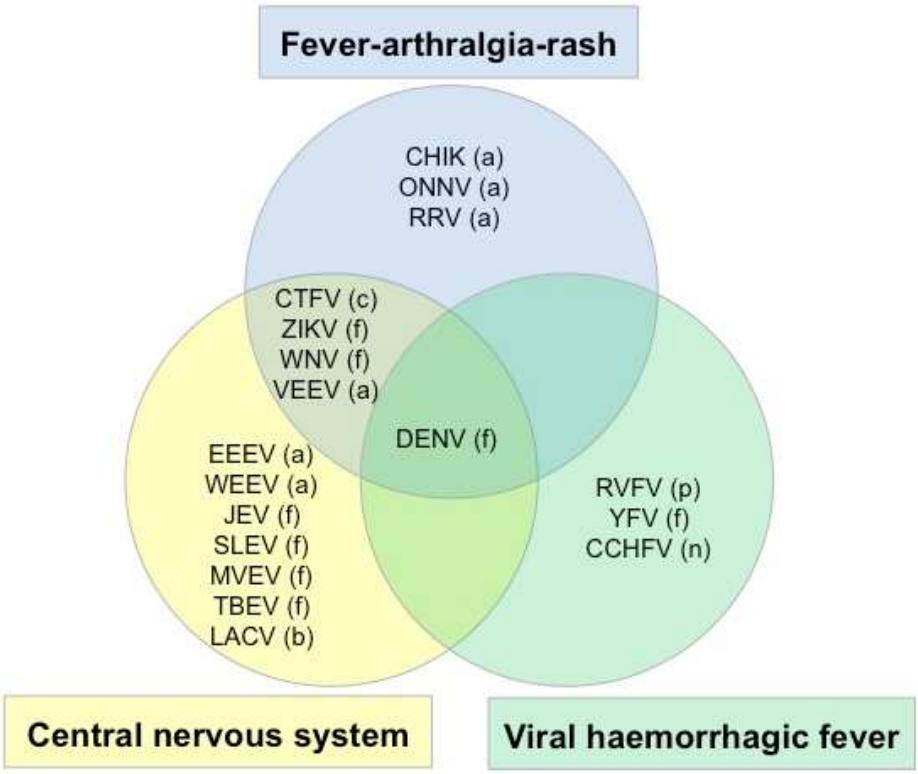
473 +++ (very common), ++ (frequently observed), + (sometimes observed), - (no typical
474 symptom)
475 Table adapted and modified from (33, 53).
476

Table 2. Comparison of baseline laboratory findings in chikyungunya, dengue and Zika infections

Laboratory findings	Chikungunya	Dengue	Zika
Anaemia	+	-	-
Leucopenia	++	+++	-/+
Neutropenia	+	+++	-
Lymphocytopenia	+++	++	-/+
Thrombocytopenia	+	+++	-/+*
Increased CRP	++	+++	-
Increased ALT	++	+++	-

+++ (very common) ++ (frequently observed), + (sometimes observed), - (no typical symptom); *if observed, thrombocytopenia is only mild.
Table adapted and modified from (33), additional data from (54, 55).




Fever-arthralgia-rash
CHIK (a)
ONNV (a)
RRV (a)
CTFV (c)
ZIKV (f)
WNV (f)
VEEV (a)
DENV (f)
EEEV (a)
WEEV (a)
JEV (f)
SLEV (f)
MVEV (f)
TBEV (f)
LACV (b)
RVFV (p)
YFV (f)
CCHFV (n)
Central nervous system
Viral haemorrhagic fever