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

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24 Abstract**25 Background**

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

34 Aims and Sources

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

38 Content

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

43 Implications

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

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

50

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

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53 **Introduction**

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

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

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

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

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

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

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

100 **Imported arbovirus infections to Europe**

101 **Case 1: An imported chikungunya case to Greece**

102 In the spring of 2016, a woman in her twenties returned to Greece from Recife, Brazil, where
103 she had stayed since November 2015. During her return travel she developed myalgia and
104 arthralgia for 9 days followed by development of high fever (40°C) and mild headache upon
105 arriving in Greece. There was no significant past medical or surgical history. On physical
106 examination there was no rash, hepatosplenomegaly or conjunctivitis, but she had swelling
107 of both knees and the left wrist. Her white blood cell count was $3.2 \times 10^9/L$ with 50%
108 neutrophils, 34% lymphocytes and 13% monocytes, haematocrit 39.5%, platelet count $247 \times$
109 $10^9/L$, and C-reactive protein 10 mg/dL (normal <5). All other tests were unremarkable. Her
110 fever subsided over the following 72 hours, with normalization of her laboratory tests and she
111 was discharged after 3 days of hospitalization. She was advised to adopt safe sex practices
112 until results for ZIKV were received.

113 Molecular testing for DENV, ZIKV and CHIKV was performed at the National Reference
114 Centre for Arboviruses in Greece on the samples taken on the 2nd day of illness using
115 commercial Real Time RT-PCR kits (Altona Diagnostics GmbH, Hamburg, DE). CHIKV RNA
116 was detected in serum and blood. An in-house RT-nested PCR using generic alphavirus
117 primers (31) obtained a sequence clustering in the ESCA genotype. The sequence showed
118 100% identity to sequences from Brazil (32). The presence of CHIKV IgM and IgG antibodies
119 was tested using indirect immunofluorescence test and ELISA, respectively (Euroimmune,
120 Lübeck, DE). A weak positive result was obtained only for CHIKV IgM antibodies in the initial
121 sera, while both IgM and IgG antibodies were detected in a convalescent sample taken on
122 March 1. Serology for DENV and ZIKV remained negative. CHIKV was isolated from her
123 blood in Vero E6 cells, with cytopathic effects seen on the 2nd day after inoculation. The
124 patient had an unremarkable recovery, however arthralgia persisted for 3 more months.

125

126 **Case 2: An imported dengue fever case in Turkey**

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

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

145

146 **Case 3: An imported Zika virus case in Romania**

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

151 On physical examination, his temperature was 36.8°C and he had conjunctivitis of the right
152 eye, a generalized non-pruritic macular rash and diffuse erythematous pharyngitis. There
153 were no other significant findings. Laboratory tests revealed mild thrombocytopenia of $135 \times$
154 $10^9/L$ (normal range 150-450), with normal haemoglobin, inflammatory markers and renal

155 and liver biochemical parameters. Malaria was ruled out by rapid diagnostic test (Malaria
156 MBPan Mascia Brunelli) and thin and thick blood film examinations. Urinalysis, blood cultures
157 and viral and bacterial pharyngeal swab tests did not reveal any pathological findings. A
158 further diagnostic work-up for tropical diseases was requested and serum and urine samples
159 (taken 5, 8 and 18 days after start of symptoms) were sent to the Reference Laboratory,
160 Cantacuzino Institute in Bucharest. NS1 dengue antigen was negative, ELISA testing
161 (Euroimmune, Lübeck, DE) for Zika IgM antibodies showed borderline values on the first two
162 samples (index value 0.901 [(negative < 0.8, positive > 1.1]) with seroconversion by day 18 of
163 illness (index value = 2.02). ELISA for Zika IgG antibodies was negative in the first two (index
164 = 0.1) and borderline positive in the last serum samples (index = 1.064). Real time PCR (in-
165 house test) was positive for ZIKV RNA in the urine samples taken 5 and 8 days after
166 symptom onset but negative in the serum samples taken at the same time. Symptomatic
167 treatment was recommended and counselling was provided regarding sexual transmission
168 and the need to avoid pregnancy for 6 months for his partner. The rash disappeared after 3
169 days, his platelet count normalized in one week and no other signs and symptoms appeared
170 during the 6-month follow-up. His wife was not tested for Zika virus infection and did not
171 develop clinical illness, but avoided pregnancy for 6 months.

172 Discussion

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

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

191
192 Chikungunya infection (Case 1) is usually associated with abrupt onset of fever and malaise
193 after an incubation period of 3-7 days, although this can extend to 12 days. Most (more than
194 75%) infected patients develop symptoms (21). Distinction from dengue may be difficult,
195 especially in travellers returning from areas where both infections are circulating (35). Typical
196 symptoms include fever that can exceed 39°C and polyarthralgia. Symmetrical bilateral
197 arthralgia is found in most patients and is usually located in the peripheral joints, appearing
198 shortly after the onset of fever (2-5 days). There may also be visible or palpable swelling. A
199 macular or maculopapular rash is commonly seen, in up to 75% of patients. Other symptoms

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

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

207
208 Dengue virus infection (Case 2) is classified by the WHO in the following categories: dengue
209 without warning signs, dengue with warning signs and severe dengue (41). During the
210 course of disease, three phases can be seen that consist of a febrile phase, a critical phase
211 and a recovery phase (41). Travellers are usually seen in the febrile phase, which lasts about
212 2-7 days. Patients present with high fever accompanied by headache, vomiting, myalgia,
213 arthralgia and a transient blanching discrete or coalescent macular rash. This rash often has
214 "islands of white in a sea of red" (5). Pruritus may be present initially. Severe headache, pain
215 behind the eyes, back pain, and myalgia and arthralgia are reported in up to 70% of cases
216 (42), with rash in around 50%. Other symptoms may include gastrointestinal manifestations
217 such as diarrhoea, vomiting, pain and nausea and symptoms resembling a respiratory tract
218 infection (cough, running nose, sore throat, injected pharynx). Mild haemorrhagic
219 manifestations like petechiae and mucosal membrane bleeding from gum or nose can occur
220 (41). In this phase, clinical features are indistinguishable between severe and non-severe
221 dengue and are also difficult to distinguish from other non-dengue febrile illnesses. The
222 tourniquet (Hess) test is advocated to identify severe disease early: a blood pressure cuff is
223 inflated to between systolic and diastolic pressure for 5 minutes and the resulting petechiae
224 are counted. However, the specificity and sensitivity of this test is moderate (43). Laboratory
225 abnormalities include a decreased white blood count, particularly neutropenia. The febrile
226 phase may followed by defervescence around day 3-7 of illness and is characterized by
227 increase capillary permeability and in severe illness bleeding and significant plasma leakage.

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

232

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

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

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

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

258 Direct virus detection by PCR is another option for diagnosing flaviviruses, which is highly
259 specific when positive. However, the duration of viraemia in flavivirus infections is short and
260 therefore PCR test positivity is confined to the first few days of illness. This window is often
261 missed in returning travellers, especially if their symptoms start while still abroad. For
262 dengue, direct detection of the virus antigen NS-1 in blood that can prolong the window up to
263 7 days (41). Of wider interest, it has recently been shown that detection of virus RNA in urine
264 can prolong the window for PCR diagnosis for up to several weeks after onset of symptoms
265 of dengue and other arboviruses (50-52). Diagnostic testing for other, more rare arboviruses
266 beyond the three common ones presented here is mostly limited to specialized laboratories.

267

268 **Conclusion**

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

276

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289

290 Transparency declaration

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292

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454

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 chikungunya, 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

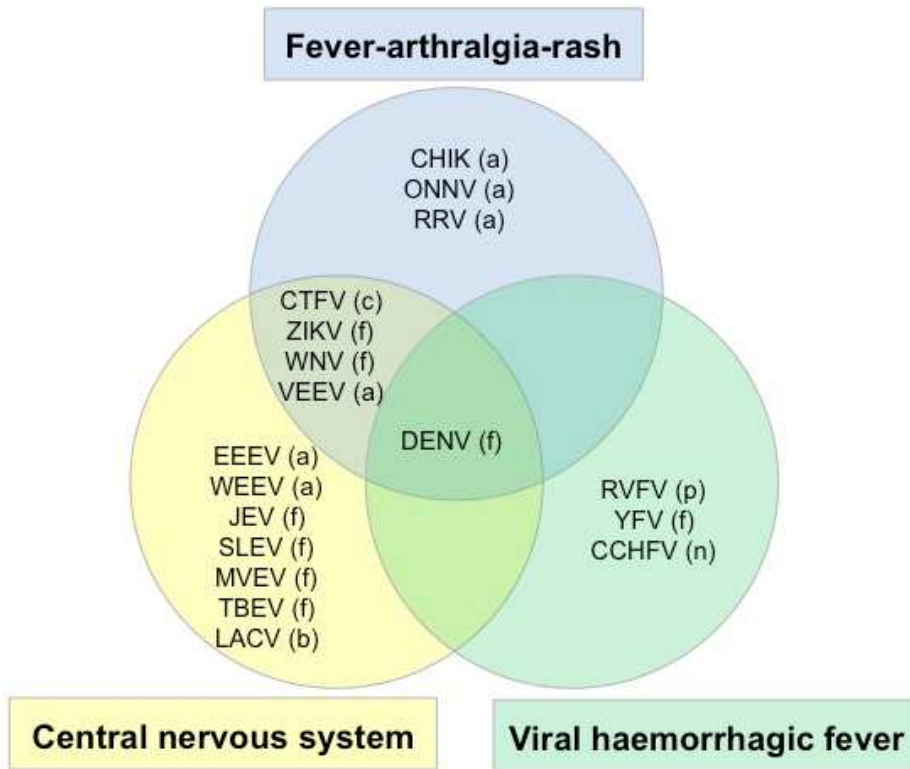
477 **Table 2.** Comparison of baseline laboratory findings in chikungunya, dengue and Zika
 478 infections

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

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



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