Importation of dengue, Zika and chikungunya infections in Europe: the current situation in Greece

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Abstract

Although several arboviruses—such as dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) viruses—are not endemic in Europe, they have the potential to emerge following importation of the virus, taking advantage of the favourable climate and ecosystem. DENV, ZIKV and CHIKV are transmitted by Aedes species mosquitoes and are amongst the most common travel-associated arboviruses. Furthermore, they are linked to sporadic, local outbreaks, especially in the southern parts of Europe. In this review we present in brief the DENV, ZIKV and CHIKV cases imported to Greece during the last 6 years (2013–2018), and we describe the recent laboratory data obtained from the Hellenic Pasteur Institute and the National Reference Centre for Arboviruses. We report 21 imported cases of DENV, ZIKV and CHIKV infections in travellers arriving in Greece. The probable origins were south-eastern Asian (71%) and north-central American (29%) countries. Furthermore, we stress the importance of early and accurate diagnosis in spite of a plethora of diagnostic challenges that clinicians and virologists have to face. Altogether, with the authorities’ awareness and the preventive measures to be applied, local transmission events can be successfully avoided, especially in summer when the temperature is favourable for mosquito-borne infections.

Keywords: Aedes, arbovirus, chikungunya, dengue, diagnosis, Greece, imported, outbreak, prevention, Zika

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Introduction

Arthropod-borne viruses (arboviruses) of the families Flaviviridae (genus Flavivirus) and Togaviridae (genus Alphavirus) are transmitted to humans by arthropod vectors, mainly mosquitoes. Some arboviruses need a vertebrate animal reservoir as the main amplifying host, while for others—including the flaviviruses dengue (DENV) and Zika (ZIKV) and the alphavirus chikungunya (CHIKV)—humans are the main reservoir, leading to the risk of local outbreaks. Diseases caused by arboviruses account for more than 100 000 000 cases, 100,000 deaths annually, and numerous chronic complications worldwide [1]. Most of these viruses are originally found in tropical regions such as Africa, South America and Asia; however, their geographic distribution has expanded and dispersed widely. During the last decade several arboviruses have emerged in southern European countries, taking advantage of the favourable climate and ecosystem [2].

Mosquito-transmitted arboviruses represent a real threat in Europe. Besides malaria and acute diarrhoea, DENV, ZIKV and CHIKV are the most common pathogens of travel-associated infections in Europe [3,4], and are linked with local outbreaks [5]. Greece is a non-endemic country for these viruses, whereas West Nile virus (WNV) is an example of a mosquito-borne flavivirus that has established an endemic circulation in the country [6].

In this mini-review, we report the imported cases of DENV, ZIKV and CHIKV infections in Greece after summarizing the current situation of autochthonous cases in Europe. Special attention is drawn to the significance of early and accurate diagnosis and measures to be applied by the authorities (especially during the time of the year when mosquitoes are active), which appear to be the main pillars for a successful prevention and control strategy in non-endemic countries like Greece.
Vectors of DENV, ZIKV and CHIKV in Europe and in Greece

DENV, ZIKV and CHIKV outbreaks highlight the role of the vectors Aedes aegypti and Aedes albopictus in their dispersal. While competence studies have proved their ability to transmit all three viruses, A. albopictus is considered to be a secondary vector only for DENV transmission [7]. The presence of A. aegypti in the area close to the Black Sea, and its colonization of the island of Madeira since 2004, pose a risk for its potent importation to Europe [8]. Although A. aegypti was introduced in The Netherlands in 2010, control measures applied immediately led to its efficient elimination from the country [9].

A. albopictus has already been established in several European countries, mainly in the Mediterranean basin, including Italy, southern France, south-eastern Spain, Slovenia, Croatia, Bosnia-Herzegovina, Albania and Greece [10]. A. albopictus was first detected in Europe in 1979 in Albania [11]. The quite recent autochthonous dengue cases reported in Spain [12] and France [13] have drawn attention to how A. albopictus circulates and spreads in those countries. Recent data have shown that A. albopictus has expanded into the region of Extremadura in western Spain [14] after its well-known establishment in the Spanish south-eastern coast since 2004. In France, A. albopictus was first detected in the region of Vallauris in 2004, and since then it has been spreading towards the western and northern parts of the country, reaching the Rhone region in 2013 [15].

In Greece, A. albopictus was first recorded in 2003 in the north-western part of the country [16], and subsequently it has gradually spread all over the mainland. Greece is characterized by high summer temperatures and humidity that facilitate mosquito abundance and mosquito–human contact, enabling arbovirus transmission. In addition, the probability of imported arbovirus infections has increased during recent years due to increased numbers of persons coming from endemic areas (as travellers or refugees).

Since 2010, when WNV emerged in Greece and resulted in a large outbreak in humans [17], WNV infections occur almost every year; therefore, the Greek public health authorities have implemented a mosquito surveillance programme on an annual basis to monitor mosquito populations, their geographical distribution and possible expansion, in order to lead the mosquito control programmes [18]. Surveillance studies show that A. albopictus is now established in the capital city of Athens, in Central Macedonia, Thrace, Thessaly and Peloponnese [19]. In 2014 and 2015 A. albopictus was detected for the first time in Crete and Lesvos Islands, respectively [18], suggesting that its expansion in Greece is an ongoing process. This spread has been particularly favoured in urban areas and it is continuously increasing due to the changing climatic conditions. For this reason, imported DENV, ZIKV and CHIKV infections from visiting travellers constitute a risk for virus spread in competent vectors that have been adapted to an urban human–mosquito–human cycle [20]. To this extent, the possibility for autochthonous DENV, ZIKV and CHIKV transmission following introductions from endemic areas is increasing along with the increasing numbers of imported cases reported annually in European countries.

Autochthonous cases of DENV, ZIKV and CHIKV infections in Europe

DENV is the most prevalent member of the genus Flavivirus (family Flaviviridae) and occurs in four antigenically different serotypes (DENV 1–4). After an incubation period of 4–10 days, although many individuals remain asymptomatic, DENV infection is characterized by a wide variety of clinical manifestations ranging from a mild febrile illness to a severe and often fatal haemorrhagic illness reflecting the three phases of the disease: the febrile, the critical and the recovery [21]. After the onset of the disease, DENV can be detected in whole blood or plasma for 4–5 days and, consequently, can be ingested by female mosquitoes during feeding on a viraemic patient. IgM antibodies start to increase 5 days post onset of symptoms, reaching a peak at 2 weeks and declining to undetectable levels after 3 months [22]. In 1927–1928, the largest European dengue epidemic took place in Greece, with more than 1 000 000 documented cases [23]. The large extent of the epidemic was due to the abundance of the main virus vector, A. aegypti, and the totally naïve human population. Since then, A. aegypti has disappeared from Europe, while A. albopictus is responsible for the emergence of sporadic events of local transmission during the last decade. Specifically, several autochthonous dengue cases were reported in southern France in 2010 [24], 2015 [25], 2018 [26] and 2019 [13], in Croatia in 2010 [27,28], and in Spain in 2018 [13]. It is of importance that the Spanish authorities reported for the first time in 2019 the likelihood of sexual transmission of DENV in an area without the presence of vector mosquitoes, especially in men having sex with men [12]. Probable sexual transmission of DENV has been reported only once, in 2013, in a heterosexual couple from South Korea [29]. The latest epidemic was in 2012 in Madeira, located at a Portuguese archipelago in the North Atlantic Ocean where A. aegypti is prevalent [30].

ZIKV (genus Flavivirus, family Flaviviridae) is a recent example of an arbovirus that resulted in a large human outbreak in the Americas in 2015 following its introduction into naïve areas.
where competent vectors were present [31]. After an incubation period of 3–14 days, ZIKV can be detected in blood for up to 1 week. Subsequently, IgM antibodies start to increase and are detectable for at least 12 weeks post infection [32]. Initially, ZIKV infection was considered to be associated only with modest symptoms; however, severe neurological complications (Guillain–Barré syndrome) were reported during the large outbreak in 2013–2014 in French Polynesia [33], and a dramatic increase in severe congenital malformations (mainly microcephaly) was seen during the outbreak in Brazil in 2015–2016 [34]. In October 2019, France reported three autochthonous ZIKV cases for the first time in Europe. They were identified in the Var region, without any report of imported cases in the close vicinity or any evidence of sexual transmission [35].

CHIKV (genus Alphavirus, family Togaviridae) has three main genotypes: West African, Asian and East/Central/South African (ECSA); the latter is divided into at least two sublineages: the Indian Ocean and Indian [36]. Although A. aegypti is the main vector of CHIKV, a single mutation in the E1 glycoprotein (E1-A226V) is associated with efficient transmission of the virus by A. albopictus as well [37]. Following a 3–12-day incubation period, CHIKV infection is characterized by a rapid-onset febrile disease, often accompanied by arthralgia, which in some cases remains for a long period [2]. Molecular diagnosis in whole blood is performed during the first 5 days of illness; after that, IgM antibodies are detectable in serum. Several local transmission events occurred in Europe, initiated by imported cases. One outbreak took place in 2007 in Italy [38], while two more events occurred in France in 2010 [39] and 2014 [40] linked to two imported cases from India and Cameroon, respectively. Recently, in the summer of 2017, autochthonous CHIKV cases were identified in central Italy and south-eastern France. CHIKV was detected in A. albopictus mosquitoes trapped near the location where the two cases occurred [41,42].

### Original imported DENV, ZIKV and CHIKV cases in Greece

In collaboration with the National Public Health Organization (NPHO) in Greece, the Public Health Laboratories of the Hellenic Pasteur Institute in Athens and the National Reference Centre for Arboviruses in Thessaloniki receive samples for identification of possible imported cases of DENV, ZIKV and CHIKV infections. During 2004–2012 only one imported DENV case was reported in Greece (in 2004), while 21 imported cases of DENV, ZIKV and CHIKV infections were reported during 2013–2018. In that period (2013–2018), 27,289 cases of overall mandatory reporting of infections (both endemic and imported) have been recorded in Greece, and 1,047 of them were fatal (data obtained from NPHO Mandatory Notification System). Specifically, the two laboratories received samples from 155 patients to be tested for DENV, ZIKV and CHIKV. Among the 21 cases, DENV, ZIKV and CHIKV were responsible for 12, five and four cases, respectively (Table 1). Four cases (two with CHIKV and two with ZIKV infections) were published [3,43,44]. The annual total number of tested cases with suspected DENV, ZIKV or CHIKV infection and the corresponding number of cases diagnosed are shown in Fig. 1.

### Table 1. Characteristics of the 21 imported cases of dengue virus (DENV), Zika virus (ZIKV) and chikungunya virus (CHIKV) infections in chronological order, Greece, 2013–2018

| Case  | Symptom onset | Probable country of origin | PCR Blood | PCR Urine | Serology Genotype | Serology IgM | Serology IgG 1st | Serology IgG 2nd | Serology NS1 | Reference |
|-------|---------------|-----------------------------|-----------|-----------|-------------------|-------------|------------------|------------------|-------------|-----------|
| 1. DENV | Sep 2013      | India                        | +         | –         | –                 | –           | –                | –                | –           |           |
| 2. DENV | May 2014      | Indonesia                    | +         | +         | –                 | +           | –                | –                | –           |           |
| 3. DENV | Oct 2014      | India                        | +         | –         | –                 | –           | +                | –                | –           |           |
| 4. CHIKV | Jun 2014      | Dominican Republic           | +         | +         | Asian             | +           | +                | –                | –           |           |
| 5. DENV | Jul 2014      | India                        | +         | –         | –                 | –           | +                | –                | –           |           |
| 6. DENV | Jul 2014      | India                        | +         | +         | –                 | –           | –                | –                | –           |           |
| 7. DENV | Aug 2015      | Philippines                  | +         | +         | +                 | –           | –                | –                | –           |           |
| 8. DENV | Nov 2015      | India, Vietnam               | +         | +         | –                 | –           | +                | –                | –           |           |
| 9. DENV | Jun 2016      | Thailand                     | +         | +         | +                 | –           | +                | –                | –           |           |
| 10. DENV | Aug 2016      | Thailand, Malaysia           | +         | +         | –                 | –           | –                | –                | –           |           |
| 11. ZIKV | Nov 2016      | Cuba                         | +         | +         | +                 | –           | –                | –                | –           |           |
| 12. ZIKV | Nov 2016      | Cuba                         | +         | +         | +                 | –           | +                | –                | –           |           |
| 13. CHIKV | Sep 2016      | India                        | +         | +         | –                 | –           | +                | –                | –           |           |
| 14. CHIKV | Feb 2016      | Brazil                       | +         | +         | ECRA              | –           | –                | –                | –           |           |
| 15. DENV | Jun 2017      | India                        | +         | +         | +                 | –           | –                | –                | –           |           |
| 16. ZIKV | Aug 2017      | Cuba                         | +         | +         | Asian             | +           | +                | –                | –           |           |
| 17. ZIKV | Jan 2018      | Cuba                         | –         | +         | +                 | –           | –                | –                | –           |           |
| 18. DENV | Jun 2018      | Myanmar                      | +         | +         | –                 | –           | +                | –                | –           |           |
| 19. DENV | Jun 2018      | Thailand                     | +         | +         | Asian             | +           | +                | –                | –           |           |
| 20. ZIKV | Jun 2018      | India                        | +         | +         | +                 | +           | –                | –                | –           |           |
| 21. CHIKV | Sep 2018      | India, Nepal                 | –         | +         | +                 | –           | +                | –                | –           |           |

ECSA, East/Central/South African genotype.  

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Laboratory testing was performed based on a combination of molecular and serological methods, as described previously [3,43–45]. A patient with positive viral antigen or RNA in blood, plasma or urine was considered as a confirmed case, while a patient with IgM antibody seroconversion (negative to positive) was considered as a probable case. In total, 20 were confirmed cases and one was probable (sample 7-DENV in Table 1). For DENV and CHIKV nucleic acid detection, whole blood was the most commonly used sample type, while in the case of ZIKV urine was also used (Table 1), since viruria is usually more prolonged than viraemia [46]. In most cases (81%), IgM antibodies were also detected, while convalescent samples were often not available (for the detection of IgG antibodies) (Table 1).

Most imported cases were observed between May and September. Patients’ age ranged from 10 to 73 years (median 35 years). The reported probable countries of infection were 24. In all genotyped cases, the obtained sequences were identical to sequences available in GenBank database from viral strains circulating in the country of origin. The number of the travellers returning to Greece with DENV, ZIKV or CHIKV infection and the country from where they came are graphically depicted in Fig. 2. Most countries of origin were south-eastern Asian countries (71%): India (33.3%), Thailand (12.5%), Indonesia (4.2%), Philippines (4.2%), Vietnam (4.2%), Malaysia (4.2%), Myanmar (4.2%) and Nepal (4.2%), while 29% of travellers had returned from the Americas: Cuba (20.6%), Dominican Republic (4.2%) and Brazil (4.2%).

**Diagnostic challenges**

The emergence of DENV, ZIKV and CHIKV infections in Europe, and the increasing number of imported cases in Europe (including Greece) during the past few years, have drawn the attention to the need for advanced laboratory preparedness and response measures. Diagnosis of arboviral infections is challenging for both the clinician and the laboratory virologist. Symptoms are usually non-specific, and clinical manifestations are overlapping, especially in the early phase of the disease [47]. The challenging clinical differential diagnosis underscores the importance of early and accurate laboratory diagnosis using techniques with high sensitivity and specificity. These include detection of viral antigens and nucleic acids, detection of virus-specific antibodies and/or isolation of the virus [48]. Each of these techniques presents advantages and disadvantages and is characterized by a range of sensitivity and specificity levels. Molecular tests have significantly advanced the diagnosis of arboviral infections, conferring high sensitivity and specificity, and are considered the reference diagnostic standard [22,49]. Detection of viral RNA is feasible in blood or plasma during the first few days after symptom onset, while viruria may last longer. A later and larger window for diagnosis is provided by the use of serological tools, but these have the drawback of cross-reactivity among viruses belonging to the same family, which might lead to diagnostic difficulties and misinterpretation of the laboratory results [50]. Thus, the knowledge of the number of days after symptom onset is of great importance in selecting the most appropriate sample type and laboratory method and in interpreting the results [3,51]. To increase diagnostic confidence, a combination of methods is recommended.

The situation gets even more complex in non-endemic regions where the emergence of arboviral infections is unexpected. The patient’s travel history of the previous month, combined with knowledge of global epidemiology, facilitates the inclusion of the diseases in the differential diagnosis. Vaccination history is also
important to differentiate between vaccine memory response and primary and/or secondary infection. Nevertheless, the high proportion of mild and asymptomatic arboviral infections can impede rapid identification of potential outbreaks.

**Control and prevention of local transmission events**

The number of imported arboviral infections in Europe is increasing, and this applies also to Greece. The risk of further spread of related diseases and the initiation of local outbreaks is higher during the summer months when mosquitoes are active. Among the 21 imported cases of DENV, ZIKV and CHIKV infections reported over the last 6 years in Greece, 16 were observed in summer and early autumn months (May–September). The fact that *A. albopictus* is continuously active in Greece for over 8 months of the year from mid-spring to the end of December, with the greatest abundance during summer and autumn months [52], poses a high risk for initiation of autochthonous transmission. Moreover, vulnerability and receptivity to arbovirus infections are increased in Greece due to increased numbers of persons coming to the country either as tourist travellers or, more recently, as refugees. Besides the dengue outbreak in Greece during 1927–1928, no autochthonous cases caused by DENV, ZIKV or CHIKV were observed in the country.

Prior to their departure, travellers visiting an endemic area have to be informed and be made aware of the clinical signs and symptoms of DENV, ZIKV and CHIKV infections, in order to request medical advice in case they present any symptoms. Since no global vaccines or drugs are available for these diseases, personal protective measures (e.g. use of mosquito repellents or staying within air-conditioned areas) are the main preventive strategy. Since sexual transmission has been reported for ZIKV [53], travellers returning from a ZIKV-endemic area have to take precautions and follow guidelines concerning sexual activities or pregnancy [54]. All suspected cases have to be reported to the NPHO, and in case they are confirmed, guidelines are given to the patients, and mosquito surveillance and control programmes are put in place in order to diminish the risk of further virus spread. Therefore, the early diagnosis of imported arboviral cases is critical, and when accompanied by physicians’ increased awareness and active surveillance measures by the authorities, autochthonous transmission events can be prevented.

**FIG. 2.** Origin and number of travellers with dengue virus (DENV), Zika virus (ZIKV) or chikungunya virus (CHIKV) infections returning to Greece during the years 2013–2018. (Map source: Free Vector World Map from 365PSD.com.)
Conclusions

The number of imported cases of DENV, ZIKV and CHIKV infection in Europe has been rising during recent years due to increased global travel and the large CHIKV and ZIKV outbreaks in the Americas (2014–2017) [55,56]. A surveillance study of ZIKV infection in the European Union/European Economic Area conducted in 2016 showed that 2133 confirmed cases of ZIKV infection were reported between June 2015 and January 2017 by 21 countries, and that 71% of them were infected in the Caribbean [55]. The number of imported DENV, ZIKV and CHIKV cases is relatively low in Greece. However, this number increased during the last 6 years, and although almost all imported cases up to 2015 were DENV infections, DENV, ZIKV and CHIKV were equally responsible for the cases imported during 2016–2018, mostly related to the outbreaks in the Americas. Actually, the case of a CHIKV infection in a patient who returned from the Dominican Republic in 2014 [3] occurred at the early stage of the CHIKV outbreak in the Caribbean [57], while all travellers with ZIKV infection returned from Cuba (Table 1).

Although the risk for ZIKV transmission in Europe is currently low, since A. albopictus seems to be not an efficient vector for the virus, importation of DENV, ZIKV and CHIKV poses a risk for local spread and outbreaks, especially in southern European countries where A. albopictus is established. Therefore, clinician awareness, prompt and accurate laboratory diagnosis of suspected cases, and effective surveillance systems are crucial to tackle the emergence and spread of travel-associated diseases.

Author contributions

MEm contributed to the conception of the review, MEm and MEv contributed to the analysis and drafting the article, AP and AM contributed to interpretation of data and critical revision. All authors finally approved the version to be submitted.

Transparency declaration

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