Journal of Clinical Virology 115 (2019) 5–10

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Paper from the 21st ESCV meeting

Emerging arboviruses of medical importance in the Mediterranean region

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A R T I C L E I N F O

Keywords:
Arbovirus
Mediterranean countries
Emerging
Humans

A B S T R A C T

The epidemiology of viral infections transmitted by arthropods is changing due to a variety of parameters related to the virus, the host and the environment. The Mediterranean region is highly affected by changes in the intensity and extension of global-scale climate patterns, and, due to its location, it provides a vulnerable environment for emergence of arboviral diseases. The main arboviruses that pose currently a public health threat in the Mediterranean region are West Nile virus and Crimean-Congo hemorrhagic fever virus and, in less extend, tick-borne encephalitis virus. *Usutu virus* that affects mainly birds, can infect also humans, while Dengue and Chikungunya viruses showed that they are capable to cause sporadic autochthonous cases, and even outbreaks in the Mediterranean region. Sandly-transmitted viruses continue to have a public health impact, and novel ones have been identified. The presence of competent vectors (mainly mosquitoes), combined by arbovirus introduction through viremic travelers returning from endemic regions, prompt for increased surveillance to mitigate the risk for local transmission. In order to tackle efficiently and effectively the emerging arboviral diseases, an integrated "One Health initiative" is required to be maintained, involving public health, animal health and environmental authorities. Awareness of medical and veterinary staff and laboratory capacity are crucial for the early detection of pathogens, while reporting the unusual and enhance surveillance are important.

1. Background

Arthropod viruses (arboviruses) are viruses transmitted to hosts by arthropod vectors having a potential to cause diseases threatening the public and animal health. They are RNA viruses circulating in nature in an enzootic cycle between vectors and vertebrate hosts (birds or mammals) [1]. The emergence/re-emergence of arboviral diseases is associated with numerous interacting factors related to the virus (genome changes leading to more virulent and adaptive strains), to humans (urbanization, increased travel, trade and transport, and human activities creating favorable ecosystems for vector-host interaction), and to climate (temperature, humidity, precipitation, wind), which affect the spatial and temporal distribution and abundance of the arthropods, the characteristics of arthropod life cycles, the evolution rate of arboviruses and the efficiency with which they are transmitted from arthropods to hosts.

The Mediterranean Basin is the region around the Mediterranean Sea and includes many European, Asian and African countries. The unique Mediterranean climate pattern is characterized by dry summers with little or no rain, and mild, wet winters. Since Mediterranean region is located in a transitional zone, the climate is highly affected by changes in the intensity and extension of global-scale climate patterns, such as the North Atlantic Oscillation (NAO), El Niño Southern Oscillation (ENSO) and the monsoons [2]. Another characteristic of the region is the numerous wetlands where billions of migratory avian species stop twice a year for rest and food on their way to cross the narrow natural passages between continents. During these yearly migrations, birds have the potential of dispersing microorganisms that can be dangerous for public and animal health. Therefore, the area is vulnerable for emergence of arboviral diseases.

The main arboviruses that pose currently a public health threat in the Mediterranean region are seen in Table 1, and include West Nile virus (WNV) and Crimean-Congo hemorrhagic fever virus (CCHFV) and, in less extend, tick-borne encephalitis virus (TBEV) that is mainly endemic in central and northern Europe. Known phleboviruses, like Toscana virus (TOSV), sandfly Sicilian virus (SFSV) and sandfly Naples virus (SFNV), continue to cause numerous cases in all Mediterranean countries, while several novel sandfly-transmitted phleboviruses have been described recently, however, for most of them, their impact to public health remains to be elucidated. *Usutu virus* that affects mainly birds, can infect also humans, creating problems in diagnostics due to cross-reactivity with WNV. In addition, Dengue (DENV) and Chikungunya viruses (CHIKV) showed that they are capable to cause sporadic autochthonous cases, and even outbreaks in the Mediterranean region. Additional arboviruses, like Tahyna virus (TAHV), can cause disease in humans, but they cannot be considered as “emerging” viruses

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https://doi.org/10.1016/j.jcv.2019.03.007
Received 12 January 2019; Received in revised form 6 March 2019; Accepted 11 March 2019

Table 1
Emerging viruses which caused autochthonous cases in the Mediterranean region.

Virus	Family	Genus	Arthropod vector
West Nile virus	*Flaviviridae*	Flavivirus	Mosquito (mainly *Culex* spp.)
Dengue virus	*Flaviviridae*	Flavivirus	Mosquito (mainly *Aedes* spp.)
Usutu virus	*Flaviviridae*	Flavivirus	Mosquito (mainly *Culex* spp.)
Chikungunya virus	*Togaviridae*	Alphavirus	Mosquito (mainly *Aedes* spp.)
Crimean-Congo hemorrhagic fever virus	*Nairoviridae*	Orthonairovirus	Tick (mainly *Hyalomma* spp.)
Tick-borne encephalitis	*Flaviviridae*	Flavivirus	Tick (mainly *Ixodes* spp.)
Alkhurma hemorrhagic fever virus	*Flaviviridae*	Flavivirus	Tick
Phleboviruses	*Phenuiviridae*	Phlebovirus	Sandflies, mosquitoes

in the Mediterranean region as there are no reports available and probably they are unrecognized. It has to be mentioned that due to favorable ecosystem for several arthropod species in the region, there is an increasing concern about the potential spread of arboviral diseases from endemic countries by infected travelers.

The main arboviruses in the Mediterranean region are described in the following paragraphs divided according to their arthropod vectors.

2. Mosquito-transmitted viruses

2.1. West Nile virus (WNV)

WNV is the leading arbovirus in the Mediterranean region based on the number of human cases of the last decade. WNV surveillance is challenging, as the virus transmission cycle in nature between birds and mosquitoes is complex and most infected humans and other mammals are asymptomatic and dead-end hosts. Humans are infected through bite of infected mosquitoes (mainly *Culex* spp.), but also through blood (and blood products) transfusion, organ/tissue/cells transplantation, and breastfeeding. WNV-infected humans may present asymptomatic, mild, or severe neuroinvasive disease, with case fatality rate (CFR) approximately 10%. After an incubation period of 2–15 days, the patient presents a flu-like illness with fever, headache, and fatigue, often accompanied by rash; if the virus invades the nervous system, the patient presents symptoms of meningitis, encephalitis or acute flaccid paralysis (polio-like syndrome).

WNV infection in humans and equines are notifiable at the European Union (EU) level. Since 2010, numerous outbreaks of WNV infections occurred in Europe. Especially the year 2018 was characterized as WNV record year in Europe with an unusually early start and prolonged virus transmission season with increased number of human cases [3]. A 7.2-fold increase was observed, compared to the previous transmission season (2017). The first cases were notified by Greece in week 26, followed by notifications from Italy and Serbia. The incidence increased significantly in several countries, like France and Bulgaria. The 2018 season ended with a total number of 2,083 human cases reported to European Centre for Disease Prevention and Control (ECDC) by EU Member States (n = 1503) and EU neighbouring countries (n = 580), with 181 deaths (CFR 9%) [4]. Two third of cases were reported by Mediterranean countries: Italy (576), Greece (311), Israel (128), Croatia (53), France (27), Turkey (23) and Cyprus (1). Similarly, the number of equine outbreaks was 30% higher compared to 2017; more than half of the 285 reported equine outbreaks occurred in the Mediterranean countries [Italy (149), Greece (15), France (13), Spain (9), and Portugal (1)] [4]. The unusual high WNV activity was attributed to temperature and precipitation anomalies, such as milder than normal winter, high temperature in spring and extended rainfalls and flooding in summer, which favored the mosquito populations; additional factors may also have played a role.

In Greece, the number of neuroinvasive cases in 2018 (n = 241) exceeded that of 2010 (n = 197), when WNV emerged in the country attacking a naïve population [5,6]. The causative strain, Nea Santa-Greece-2010, belongs to the central European subclade of WNV lineage 2 [7,8]. Since 2010, similar strains were responsible for the subsequent large outbreaks in the Balkan countries and Italy. It is of note that one human case in 2018 in Greece was caused by WNV lineage 2 of the eastern European subclade (article submitted). This strain was initially identified in 2007 in the Volgograd region in Russia, and causes outbreaks in Russia and Romania [9,10]; it has been also detected in Italy, in 2014, but only in mosquitoes [11]. It seems that since 2004, when WNV lineage 2/central European subclade was first detected in Hungary, is replacing lineage 1, which caused all previous outbreaks in the Mediterranean region [12–14]. It is also replacing WNV lineage 2/eastern European subclade in Romania [15]. The WNV epidemiology is even more complex in Israel, where a variety of WNV lineages and subclades have been identified; however, the majority of the viruses in Israel belong to lineage 1 (Eastern European and Mediterranean clades of cluster 2 and cluster 4) and very few belong to lineage 2 [16]. WNV lineage 1 was the cause of the outbreak in 2018 in Israel [17]. Similarly, lineage 1 was detected during large WNV outbreaks in Tunisia [18–20].

2.2. Usutu virus (USUV)

The first detection of USUV in Europe was in 2001 during a severe bird die-off (mainly Eurasian blackbirds) in Austria [21]. However, a much earlier introduction in Europe was confirmed, when the virus was detected retrospectively in archived tissue samples from bird deaths in 1996 in the Tuscany region of Italy [22]. The current range of virus distribution in Europe suggests an initial introduction into western Europe (Spain) in the 1950s, followed by introduction of a separate strain into central Europe in the 1970s [23]. Currently USUV is detected mainly in central Europe and causes large outbreaks in birds. The virus was detected also in Tunisia, co-circulating with WNV [24].

In 2009 the first human cases of USUV infection were reported in two immunocompromised patients in Italy, and later in additional countries, with symptomatology similar to that of WNV infections (mild febrile illness or neuroinvasive disease) [25–27]. It was shown that USUV has a deleterious effect on human neuronal cells, even higher compared to ZIKV [28]. It cannot be excluded that USUV might be misdiagnosed as WNV when the diagnosis is based only on antibody detection, without testing by PCR or neutralization assays (due to cross-reactivity in serology) [29]. It has to be noted that misdiagnosis may be seen also when the molecular methods are not capable to distinguish between the two flaviviruses [30]. This is often seen during the screening of blood donations for WNV (some of the positives are USUV infections).

2.3. Dengue virus (DENV)

Being endemic in more than 100 countries, DENV is the most widespread viral mosquito-borne disease globally. Symptoms are usually mild, with fever, rash and arthralgia, but severe dengue may lead to life-threatening hemorrhagic fever or shock. *Aedes aegypti* mosquito is the primary vector of the virus, while *Aedes albopictus* can also serve as virus vector. This is of particular importance for Europe, as *A. albopictus* was introduced and is currently established in many

Mediterranean countries. Thus, locally acquired dengue (and other *Aedes*-transmitted diseases) is expected to occur when viremic travelers return in a Mediterranean country from endemic or epidemic regions during the season when the vectors are active. Examples are the autochthonous dengue cases reported in France in 2010, 2013– 2015 [31–33], while in 2010, following the dengue diagnosis in a German traveler returning from Croatia [34], it was shown that additional autochthonous cases occurred in Croatia [35]. Similarly, in early October 2018, six autochthonous dengue cases were reported in France, and other three were confirmed in Spain [36]. Furthermore, the mainland Portugal, and ten other European countries, detected imported dengue cases in 2012–2013, when a large outbreak was taking place in the Madeira islands; it has to be noted that the vector in the Madeira outbreak was *A. aegypti* [37]. Recent data from VectorNet (network supported by ECDC) show that *A. aegypti* (main vector also for CHIKV, Zika and yellow fever viruses) is now spreading from the Black Sea area to the west, along the coast of Turkey, suggesting that the risk for further spread in the Mediterranean countries is increasing. The presence of *A. aegypti* is confirmed also in Egypt, where dengue cases have been confirmed recently [38]. It has to be noted, that this is not a new introduction of *A. aegypti* in the Mediterranean Basin; once it was established and implicated in several dengue outbreaks, with the largest one occurred in 1927–1928 in Greece with more than one million cases [39–41]. During the 1940s, *A. aegypti* population was eradicated with the extensive use of dichlorodiphenyltrichloroethane (DDT).

2.4. Chikungunya virus (CHIKV)

First isolated in 1953 in the Makonde region in Tanzania, CHIKV invaded Asia and since 2013, the Americas, starting from the Caribbean island of St. Martin [42]. Symptoms of chikungunya fever are similar to those of dengue, but arthralgias are more prominent and extensive and may last for months or years. This symptom gave the name to the disease, since “chikungunya” in the Makonde language means "that which bends up". The risk of CHIKV emergence in Europe is similar with what was mentioned above for DENV. The first CHIKV outbreak in Europe occurred in 2007 in the Emilia Romagna region in Italy (205 confirmed cases) after the introduction of the virus by a viremic Indian patient who returned from Kerala [43]. Two additional CHIKV outbreaks occurred in 2017 in Italy and France. A total of 298 cases have been reported in Italy, and the virus belonged to the East/Central/South African lineage (strain identical with a strain from Pakistan), while 17 cases were reported in the south of France, with the strain belonging to the same lineage, but presenting genetic similarity with strains from Congo and Gabon [44,45]. Another mosquito-transmitted alphavirus, Mayaro virus, may also cause locally acquired cases in the Mediterranean counties, since imported cases have been reported in Europe, including France [travelers returning from Brazil (Amazon region) and French Guinea] [46,47].

2.5. Rift valley fever virus

Rift Valley fever virus (RVFV) is a mosquito-transmitted phlebovirus which affects mainly ruminants; however, humans may be infected by contact with blood of infected animals, and they present a flu-like illness which can progress to hepatitis and severe hemorrhagic disease. RVFV is endemic in Africa, with Egypt representing the northernmost extent of the virus; four epidemics occurred in Egypt (1977–2003) and resulted in human and animal mortality [48]. RVFV is a typical emerging zoonotic disease for the control of which a One Health approach is necessary [49].

2.6. Other mosquito-borne arboviruses

Sindbis virus (SINV) (genus Alphavirus, family *Togaviridae*) and several orthobunyaviruses (genus Orthobunyavirus, family *Peribunyaviridae*), like Tahyna virus (TAHV) and Batai virus (BATV), can cause disease in humans ranging from mild febrile illness and arthralgia to encephalitis. TAHV and other viruses of the California encephalitis serogroup are transmitted through *Aedes vexans* mosquitoes. Although this mosquito species is abundant in southern Europe, reports of TAHV infections are limited in the central Europe, especially the Czech Republic [50]. Similarly, there are no reports of BATV infections in the Mediterranean region, although the virus was isolated in 2009 from *Anopheles maculipennis* complex mosquitoes in Italy [51]. Cases may remain undiagnosed either due to mild symptomatology (patients do not seek clinical advice), or not inclusion of these viruses in the differential diagnosis, or because of diagnostic limitations (lack of commercial kits for serology for most of these viruses, or negative results in PCR due to short and low viremia).

3. Tick-transmitted viruses

3.1. Crimean-Congo hemorrhagic fever virus

First isolated during an outbreak in the Crimean peninsula, and later in Congo, Crimean-Congo hemorrhagic fever virus (CCHFV) is currently the most important of the tick-borne viruses in the Mediterranean region. The virus circulates unnoticed in nature between ticks and vertebrates. It is transmitted to humans by bite of infected Ixodid ticks (mainly *Hyalomma* spp.) or by contact with blood or tissues of viraemic patients or animals. Nosocomial infections are often reported. CCHFV-infected humans may present asymptomatic, mild, severe, or even fatal disease (CCHF) with CFR 10–40% in hospitalized patients. The clinical course usually follows four distinct phases: incubation (1–14 days), pre-haemorrhagic, haemorrhagic and convalescence. The disease (CCHF) is endemic in the Balkan countries and Turkey, while in 2016 it emerged in Spain; one additional case was reported in 2018 [52,53].

In Turkey the disease emerged in 2002 [54]; since then, more than 10,000 cases have been registered, with a peak being observed in 2008 and 2009 and a decrease thereafter [53]. Although CCHF endemic foci are present in the Balkan peninsula, only one human case has been reported in Greece; it occurred in 2008 and it was fatal [55]. However, the first detection of CCHFV in Greece was in 1975, when the AP92 strain was isolated from *Rhipicephalus bursa* ticks collected from goats in the northern part of the country [56]. AP92 is the prototype strain of lineage Europe 2, one of the 7 genetic lineages in which CCHFV strains are grouped on the basis of S RNA segment sequences. A high seroprevalence in humans in Greece (mean 4%, up to 14% in mountainous areas) has been attributed to the circulation of low pathogenic strain(s) [57,58]. AP92-like strains have been recently detected in ticks (mainly *Rhipicephalus* spp.) in Greece, Albania, Kosovo, Bulgaria, Turkey and Algeria, while very few mild CCHF cases associated with an AP92-like strain have been reported in Turkey [59–66]. Since all the severe CCHF cases in Balkans and Turkey belong to lineage Europe 1, the exact pathogenicity of lineage Europe 2 remains to be elucidated. It is noteworthy that the CCHFV strain detected in human cases and ticks in Spain belong to different lineages (lineage Africa 3), and it was suggested that they were introduced from Africa via migratory birds [52,67,68].

As all viral hemorrhagic fevers, CCHF is a notifiable disease in EU. Due to the high pathogenicity of the virus, the lack of specific drug or vaccine and the risk for person-to-person transmission and nosocomial infections, rapid diagnosis is essential for prompt implementation of appropriate infection control measures. Since competent tick vectors and suitable mammalian hosts are present in the favorable ecosystem of the Mediterranean countries, awareness and surveillance are needed for prompt detection of CCHF cases.

3.2. Tick-borne encephalitis virus (TBEV)

Initially isolated in 1937, TBEV is transmitted to humans mainly by bites of *Ixodes* spp. ticks. The subtype present in the Mediterranean countries is the European (or Western) one, transmitted by *I. ricinus* ticks (the other two are the Siberian and Far Eastern subtypes, both transmitted by *I. persulcatus* ticks) [69]. Humans can be also infected through consumption of unpasteurized milk from TBEV-infected animals, mainly goats. Following an incubation period of approximately one week, the disease (TBE) is usually biphasic; after an initial flu-like illness, and one asymptomatic week, a second phase of neuroinvasive disease may occur in one third of infections.

Since 2012, the disease is notifiable in EU countries. TBE endemic European countries are mainly located in central and northern part of the continent [69]. Among Mediterranean countries, cases are reported in France, Italy, and Croatia, while two cases have been confirmed in Greece [70,71]. The incidence of the disease is highly associated with tick abundance, which depends on several ecological and climatic factors. Better clinical awareness and surveillance are needed to avoid underdiagnosis of this vaccine-preventable disease.

3.3. Alkhumra hemorrhagic fever virus (AHFV)

AHFV is a tick-borne flavivirus initially detected in ticks in Saudi Arabia. It causes to humans a febrile disease, potentially accompanied by hemorrhagic manifestations; the CFR is up to 25%. The detection of AHFV in two travelers who returned in 2010 to Italy from Egypt (independent cases) suggest that the geographic distribution of this virus could be broader than previously thought [72].

4. Sandfly-transmitted viruses

Sandfly-transmitted viruses (genus phlebovirus, family *Phenuiviridae*) are widely distributed in all Mediterranean countries [73,74]. Besides the well-known Toscana virus (TOSV), Sandfly Sicilian virus (SFSV), and Sandfly Naples virus (SFNV), novel phleboviruses have been identified in recent years, some of them associated with disease in humans. The higher number of pathogenic phlebovirus species have been detected in Greece and Turkey [75–77]. Adria virus, initially detected in sandflies in Albania, was later detected in a febrile case in Greece, representing the first phlebovirus of the Salehabad serocomplex associated with disease in humans [77,78]. Two Sicilian-like phleboviruses were detected during human outbreaks in 2002 and 2008 in Cyprus and Turkey, respectively [79,80]. Phlebovirus infections were observed also in Algeria, Libanon, and Tunisia [74,81].

While SFNV and SFSV cause to humans a mild febrile illness (three-day fever), Toscana virus (TOSV) has neuroinvasive potential, and causes meningitis or meningoencephalitis. Especially in Italy, it is one of the most common causes of summer meningitis [82]. Three lineages of TOSV are currently identified, A, B and C, the last one detected so far only in Croatia and Greece [83–85].

Additional novel phleboviruses have been detected in sandflies [86,87]; therefore, it is not known whether they are pathogenic to humans. Since most phlebovirus infections cause mild symptoms, patients do not often ask medical advice, therefore, their incidence is underestimated. Furthermore, since the viremia is low-level and short-lasting, especially in mild cases, the diagnosis is based only on serology; thus, the exact phlebovirus species cannot be identified.

5. Concluding remarks

The geographic location of Mediterranean region and a combination of ecological, climatic and anthropogenic factors contribute to the spread of arboviruses in previous unaffected areas. The establishment of invasive mosquitoes is expected to play a critical role in the transmission of viruses with public health relevance. Therefore, entomological studies using molecular tools (i.e. barcoding characterization) should be conducted especially in the Mediterranean region. ECDC and EFSA maintain a common database on the presence and distribution of vectors and pathogens in vectors in Europe and the Mediterranean basin; exotic mosquito species, such as *A. aegypti, A. albopictus, A. japonicus, A. atropalpus* and *A. koreicus* are included in the database.

The presence of invasive mosquitoes, combined by virus introduction through viremic travelers returning from endemic regions, resulted in arbovirus emergence (like CHIKV) and re-emergence (like DENV), and has led to concerns that Zika virus (ZIKV) and other exotic viruses could also be introduced in a similar manner. Especially ZIKV became of global pandemic importance due to recent large outbreaks in America. Besides the imported cases, locally acquired ZIKV cases have been reported in several European countries, including France, Italy, and Spain, with transmission of the virus via a sexual route, while one case of mother-to-child transmission was reported from Spain [88–90]. Furthermore, two ZIKV-associated microcephaly cases were reported in Spain, with Colombia and Venezuela being the probable locations of infection [91]. A recent study showed that *A. albopictus* mosquitoes are competent vectors for ZIKV [92]. A similar concern is raised about yellow fever (YF), especially during the recent outbreak in Brazil [93]. As mentioned for dengue, YF outbreaks were common in Europe in the 19th century, including the Mediterranean Spain and France. Most of them, like the one in Barcelona in 1870, initiated after a ship arrival from endemic areas to European ports [94]. Thus, increased surveillance is required to mitigate the risk of local transmission following imported arboviral cases.

The recent data on the establishment of *A. albopictus* in southern Europe and on new settlements of *A. aegypti*, the emergence of CCHF in Spain and the large increase of WNV infections in Europe, together with exotic viruses knocking the door of Europe, call for enhanced surveillance activities and vector control measures. The rapid progress in molecular techniques facilitates the early detection of pathogens. It is expected that the wider use of next generation sequencing technology will enable the rapid detection of pathogens, including novel and emerging ones.

The efficiently and effectively tackling of emerging arboviral diseases is a difficult task and requires a maintained “One Health initiative” involving local, regional and national public health, animal health and environmental authorities. Awareness of medical and veterinary staff and laboratory capacity are crucial for the early detection of pathogens, while reporting the unusual and enhance surveillance are important for timely implementation of control and prevention measures.

Funding

The study is part of the EU Horizon 2020 research and innovation programme COMPARE (grant agreement No 643476).

Conflict of interest

None.

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