Imported Chikungunya fever case in Greece in June 2014 and public health response

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We report about the first imported case of Chikungunya fever in Greece in a Greek traveler returning from the Dominican Republic and the associated public health response. We investigated the case and performed focused epidemiological and entomological investigation in all areas the patient visited during the infectious period, to identify the targeted interventions needed. Entomological investigation revealed the occurrence of the competent vector *Aedes albopictus* (Diptera: Culicidae) in the environment surrounding the hospital where the patient was admitted and in her workplace. All captured mosquitoes tested negative for Chikungunya virus. We further conducted clinical and laboratory examination of the patient's co-travelers, gave advice on appropriate personal preventive measures against mosquito bites to the patient and co-travelers and on vector control, and raised awareness among health professionals throughout Greece. The risk of introduction and local transmission of Chikungunya and other arboviruses in Greece and other European countries is present, as the competent vector exists in many parts of Europe. Public health professionals, travel medicine specialists and clinicians should maintain awareness regarding this possibility of importation of arbovirus cases in order to provide the appropriate advice, seek the prompt diagnosis, and implement appropriate interventions. Mobilization of various stakeholders will lead to enhanced epidemiological and entomological surveillance that will allow for improved risk assessment in each area.

Keywords: Public Health, Vector-borne diseases, Arboviruses, Medical entomology, Viral infection, Alphaviruses

Introduction

Chikungunya fever is a febrile viral disease caused by the Chikungunya virus (CHIKV) (genus Alphavirus, family *Togaviridae*) that may either be acute, lasting from 2 to 5 days or persist and become chronic.¹ In addition to fever, patients frequently complain of headache, myalgia, rash, and arthralgias. CHIKV is transmitted by several species of mosquitoes, including *Aedes albopictus* Skuse (Diptera: Culicidae) and *Aedes aegypti* (Linnaeus) (Diptera: Culicidae)²; both of these mosquito species can serve as vectors for Dengue virus³ and the re-emerging (in the Americas), Zika virus^{4–6}). The first described local transmission of CHIKV in the Caribbean is associated with

an ongoing outbreak that has accounted for local transmission in 45 countries or territories throughout the Americas with more than 1.7 million suspected cases.^{7–9}

At the same time the CHIKV vector, *Ae. albopictus* has been first detected in Greece in 2003–2004, and since then it has been invading an ever expanding geographical area, including the Athens metropolitan area.^{10–12}

This report describes the first imported case of CHIKV infection in Greece from a traveler returning from the Dominican Republic, the associated clinical, laboratory, and epidemiological investigation and the public health response to it.

Methods

Case investigation

The Office for Vector-borne Diseases of the Hellenic Center for Disease Control and Prevention (HCDCP)

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Table 1 Case definition used for the investigation of the imported case with Chikungunya (CHIKV) virus infection

CHIKV infection	Definition
Suspect case	- Any person with fever and - no alternative diagnosis that could explain the symptoms and - history of travel to, or residence in, an area with documented ongoing transmission of CHIKV within the 3 weeks preceding the symptoms
Probable case	- Any person with fever and - no alternative diagnosis that could fully explain the illness and - with CHIKV-specific IgM antibodies detected in serum
Confirmed case	- Any person with one of the following laboratory criteria, irrespective of clinical manifestations: - isolation of CHIKV from a clinical specimen; - detection of viral RNA from a clinical specimen; - detection of CHIKV-specific IgM antibodies in a unique serum sample and confirmation by neutralization - seroconversion or fourfold increase in CHIKV-specific antibodies titers in paired serum samples

conducted a case investigation and obtained through a structured case report form details regarding demographics, travel history, contacts during the transmission period in order to identify the risk of further transmission of the virus and communicate the appropriate preventive measures. The case definition used is shown in Table 1.

Laboratory investigation

The presence of CHIKV IgM antibodies was tested using indirect immunofluorescence test (IIFT) (Anti-Chikungunya virus IIFT – IgM), while for the presence of CHIKV IgG antibodies a commercial ELISA test was applied (Anti-Chikungunya virus ELISA – IgG) following the instructions of the manufacturers (both kits were purchased by Euroimmun Medizinische Labordiagnostika AG, Lübeck, DE). Molecular testing was performed using a commercial Real Time RT-PCR kit (Real Star Chikungunya RT-PCR kit 1.1 from Altona Diagnostics GmbH, Hamburg, DE). An in-house RT-nested PCR using generic alphavirus primers was also applied.¹³ Sequencing of the PCR product was followed by phylogenetic analysis. Virus isolation was attempted in Vero E6 cells from serum sample of the patient obtained on the third day of illness. Flasks were tested daily for cytopathic effects.

Entomological investigation and testing

HDCP collaborated with Benaki Phytopathological Institute (BPI) and the Department of Parasitology, Entomology and Tropical Diseases of the National School of Public Health (NSPH) for a focused entomological investigation in all areas the patient visited during the time it was estimated that she was infectious, as *Ae. albopictus* mosquitoes have been already detected in several areas of Athens.^{10,12} For this investigation, four mosquito BG-Sentinel traps baited with lactic acid (two in each area) and ten ovitraps (five in each area) were set up by BPI in the areas around the patient's residence, and in the yard of the Attikon University Hospital (in places favorable for mosquito breeding as suggested by the entomologists) and five ovitraps at the patient's workplace, following relevant ECDC guidelines.¹⁴ Four BG-Sentinel traps were operated for 1 day, from 8.00 to 8.00 pm, at a distance

at least 200–250 m apart. On the days of removing the BG-Sentinel traps an ovitrap network was set up in the patient's residence and the Attikon University Hospital. For the patient's workplace, only oviposition traps were set up. Each ovitrap network consisted of five (5) small black plastic buckets (about 1 l) filled with water to two-thirds. Ovitrap networks were placed at least 150–200 m apart and each trap had an oviposition substrate (a non-sterilized wooden tongue depressor) in its internal surface. All ovitrap networks operated for 5 days.

The collected eggs from ovitraps (strips) were transferred in the laboratory in order to be hatched and reared to the adult stage. This technique is essential since the eggs of *Ae. albopictus* and *Ae. cretinus* cannot be separated using morphological characteristics (rearing room at 25–26 °C and 85% RH, using the adult stage for identification, for more information see also Giatropoulos *et al.*¹²). The adult mosquitoes captured with BG-Sentinel traps as well as those emerged after rearing in the laboratory at BPI were identified to the species level using dichotomical keys.¹⁵ Molecular testing for the presence of CHIKV was performed at the NSPH, with Real Time RT-PCR at *Ae. albopictus* pools.¹⁶

Results

Brief clinical and epidemiological data

The patient was a 26-year-old female that had a short trip (with 2 other co-travelers) from Athens to Santo Domingo de Guzmán, the capital of the Dominican Republic, via Paris, France, between 12 and 15 June 2014. She reported several mosquito bites overnight on 13 June. The other two travelers reported no bites. The patient resumed her regular daily activities (including work) up until 18 June 2014, when she developed a petechial rash over her torso accompanied by arthralgias, back pain and fever with chills. She was admitted at a tertiary academic hospital. The only significant laboratory results included a WBC count of 4000 μl^{-1} with lymphocytic pleiocytosis (43.5%). Infectious disease specialists raised the suspicion of a vector-borne disease either Chikungunya or Dengue fever and recommended isolation since multiple mosquitoes were present in the regular ward the day after admission. The

patient was discharged on 25 June 2014 with persistent arthralgia that was evident on the 15- and 20-day follow-up visit but resolved at the 6 month follow-up.

Laboratory investigation

CHIKV RNA was detected in both serum and plasma samples obtained on the third day of illness by the use of real-time RT-PCR (Ct 24.89 and 24.96, respectively) and nested RT-PCR performed at the National Reference Centre for Arboviruses and Hemorrhagic Fever viruses in the Aristotle University of Thessaloniki, Greece. Sequencing of the PCR product confirmed the PCR results, and the obtained sequence presented 100% identity to the respective ones previously detected in the Dominican Republic (such as the sequence with Accession Number KR559498, CHIKV strain WHCHK29, collection date March 2014). The CHIKV strain that has arrived to the Americas and is causing the current outbreak belongs to the Asian genotype.¹⁷ CHIKV-specific IgM and IgG antibodies, not detected in serum available from the 3rd day of illness, were positive on samples obtained on the 7th and 15th day of illness. A CHIKV strain was isolated in cell culture, with cytopathic effects becoming evident on the 5th day after inoculation. No antibodies were detected in samples obtained from the two co-travelers.

Entomological investigation

The entomological survey revealed the presence of the known vector *Ae. albopictus*. From the four BG-sentinels only those at the Attikon University Hospital captured mosquitoes. Specifically, two female adults and twelve male adults *Ae. albopictus* were captured at the Attikon University Hospital, while no *Aedes* mosquitoes (adults) were captured at the patient's residence. Regarding oviposition traps, *Aedes* spp. eggs were collected from the patient's workplace and the Hospital area (25 and 55 eggs respectively) and no *Aedes* spp. eggs were recorded at the patient's residence. All eggs were hatched and larvae reared to the adult stage were identified as *Ae. albopictus*.

Public health actions

The patient was given advice for personal protective measures against mosquito bites during the maximum transmission period (for the immediate 10 days after admission) since it was considered possible that she could be at the final stages of viremia and still be infectious).

Her two colleagues that traveled together to the affected area were interviewed for the presence of any symptoms compatible with the disease and were advised to be on alert for such symptoms for the maximum incubation period (up to 12 days after their arrival in Greece). They did not develop any compatible symptoms. Moreover, they were advised to exercise strict personal protective measures against mosquito bites for a more extended period (up to 4 weeks after their arrival) in the event they had been infected and remained asymptomatic. Finally, they were all

subjected to serological testing for CHIKV-specific antibodies at the National Reference Centre for Arboviruses and Hemorrhagic Fever viruses, 3 weeks after their arrival and were negative. Close contacts of the patient (relatives staying at the same house) were advised to exercise personal protective measures against mosquito bites and be on alert for symptoms for two months.

The Office for Vector-borne Diseases of HCDCP communicated with the patient's working place (Hellenic Police, Athens, Greece) and gave advice on appropriate personal preventive measures against mosquito bites for all travelers to endemic areas and for all persons residing or working at the Department of police; in addition advice was provided on the need for a systematic vector control program for the area surrounding the Police Department in collaboration with the local prefecture authorities.

Information about the disease and the vector was disseminated by the HCDCP to health care professionals at a nationwide level through specific written guidance, emails, and website postings.

Furthermore, the Travel Medicine Office of HCDCP systematically issues – through weekly outbreak report uploaded in the website – current through a weekly ongoing disease outbreaks, travel-related information and advice to travelers, such as mosquito bite prevention, self-monitoring of health after return and medical advice in case of symptoms. Travel health advice is provided by travel health consultants to travelers going to areas with ongoing outbreaks of Chikungunya fever, in particular those travelers at increased risk for more severe disease, such as people with underlying medical conditions and pregnant women.

A formal risk assessment considered the case of national interest since a large wave of tourists was anticipated from the affected areas to Greece. Given the presence of the competent vector in many areas in Athens, the risk of local transmission was considered possible. Autochthonous cases of CHIKV would be considered a public health emergency. Any further imported case in areas where there is known presence of the *Ae. albopictus* vector should trigger targeted surveillance activities, such as case and entomological investigation. In areas with no available entomological data, entomological investigation would first target the identification of the presence of the competent vector, such as mosquitoes belonging to genus *Aedes*.

Discussion

Herein we describe the first imported case of CHIKV infection in Greece in a traveler to the Dominican Republic. No further Chikungunya fever cases were identified among the co-travelers or other individuals of close contact. Entomological investigation revealed the presence of the competent vector *Ae. albopictus* in the environment surrounding the hospital where the patient was admitted and her workplace. The HCDCP emphasized instructions to the public on personal protective measures against mosquito bites all over the country, taking advantage of the

ongoing annual campaign for the prevention of West Nile virus infection.

CHIKV has re-emerged and caused large outbreaks in humans in Africa, southern and southeastern Asia, and the Indian Ocean Islands where it caused a large epidemic after 32 years.^{18–22} The introduction of the CHIKV in other countries through viremic travelers returning from endemic countries increases the risk of the virus transmission and establishment in new areas, where the competent vectors exist.^{23,24} Such introduction has been described frequently over the last few years.^{25–33} It is believed to have led to an outbreak of the disease in the province of Ravenna, Italy in 2007, with more than 300 suspect and over 200 laboratory confirmed cases reported. The index case was believed to be a foreigner coming from an affected area in the Indian subcontinent.^{34,35}

More recently and relevant to our case, the first recorded local transmission of CHIKV in the Western Hemisphere has been described in December 2013 in the Caribbean and since then the outbreak has spread to 45 countries or territories throughout the Americas with more than 1.7 million suspected cases.^{7–9} The overflow to European countries from returning travelers was anticipated by Public Health Authorities in Europe and has been described recently in European countries like Spain, France, the Netherlands, and England.^{36–39} As an example, on October 2014, France reported a family cluster of 4 cases, in the area of Montpellier, France, and in total 11 autochthonous confirmed cases and 1 probable case.⁴⁰ The cases had no history of travel or residence in an endemic area but lived in the vicinity of a CHIKV case imported in France from Cameroon.⁴⁰

Clinicians should be aware of the CHIKV geographical distribution around the world, and especially the ongoing outbreaks, such as those in the Caribbean region and Central/South America, given the potential for the virus introduction through viremic patients in naïve countries where the competent vector is established. The same is true for potential Dengue^{41,42} and Zika^{5,43–45} virus introductions. Concurrent infection with more than one such pathogen should not be overlooked and has been recently described.⁴⁶ Although we cannot overlook the possibility of missing a bitten asymptomatic case we have not seen any further cases in Greece during 16 months of follow-up. Thus, spread that occurred through such a case is considered unlikely. To optimize the response to similar type of events inter-sectoral collaboration is needed to ensure better entomological data (knowledge of mosquito populations, distribution of species, feeding habits and dynamics of replication), enhanced surveillance, laboratory capacity for diagnosis, and appropriate risk and crisis communication in order to mobilize the community in vector control efforts. The role of government and local Public Health authorities in intensifying such efforts should be emphasized. However, it may be difficult to maintain vector-related activities in times of financial constraints;

continuous risk communication is necessary to maintain national activities regarding vectors instead of just targeted focused reactive actions that follow the initial event and may miss the spread of an epidemic infectious disease.

Ae. albopictus is an invasive mosquito species (IMS), monitored in several countries and believed to be expanded by the climate change and the international travel and trade. It is capable of transmitting not only CHIKV, but also Dengue virus³ and the re-emerging Zika virus^{4–6}. Although all collected mosquitoes (adults and eggs) were identified as *Ae. albopictus*, it is known that a close related indigenous mosquito species, *Aedes (Stegomyia) cretinus* Edwards (Diptera *Culicidae*), is also present in Greece. This species has been placed along with *Ae. albopictus* into the same subgroup (*albopictus*) of the group *scutellaris* which belongs to the subgenus *Stegomyia* of the genus *Aedes* Becker. *Ae. cretinus* has a limited distribution across the world (Greece, Cyprus, Georgia, and Turkey) and little appears to be known about its biology and vector capacity.¹⁵ Prior to the entrance and establishment of *Ae. albopictus*, *Ae. cretinus* has been reported in Crete, Macedonia and five suburbs of north-eastern Attica, in a distance of 10–20 km far from the city of Athens.⁴⁷ Entomological surveillance in Athens, employing ovitraps for the period 2009–2010, showed that *Ae. albopictus* had developed higher population densities than *Ae. cretinus*.^{12,48} More recent entomological reports demonstrate that *Ae. albopictus* has been established in many areas in Greece, including the Peloponnese peninsula, the Ionian Islands, the Central Macedonia, Attica, and Crete and it has become a primary nuisance mosquito.⁴⁹

The management of invasive mosquito species requires the involvement of the residents in each area in order to avoid the development of the larvae in their houses (private area) with immediate targeted vector control activities (e.g. insecticidal spraying for adult mosquitoes) and is currently suggested by entomologists in Greece following the notification of imported CHIKV and for the same matter Dengue or Zika virus cases. A factor taken into account in all instances is the presence or not of the competent vector and the risk assessment of local transmission of the virus.

Significant public health implications as well as the risk of economic loss for a touristic country like Greece may arise in the event of a CHIKV outbreak associated with such an introduction. In our case, of major public health importance was the early recognition of the disease by the astute physicians involved in this case, as well as the prompt response of the Public Health Authorities, with careful investigation and management of the case and the two co-travelers along with the entomological investigation. Furthermore, laboratory preparedness and testing interpretation is appreciated and should be maintained. Finally, the awareness raised among the health professionals all over the country could be a valuable tool for enhancing the passive surveillance of the disease.

Taking into consideration the rapidly evolving climate change and the huge global travel numbers as well as migration movements, it is imperative to maintain awareness for the emerging vector-borne diseases. Public health professionals, travel medicine specialists and clinicians should maintain awareness regarding this possibility of importation of arbovirus cases in order to provide the appropriate advice, seek the prompt diagnosis, and implement appropriate interventions. Mobilization of various stakeholders will lead to enhanced epidemiological and entomological surveillance that will allow for improved risk assessment in each area.

Conflicts of interest

None declared.

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