



Chikungunya and Dengue Risk Assessment in Greece

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Abstract

Objective: The aims of the study were:

- (1) To assess the mutated CHIKV E1-A226V and DENV II infection and dissemination rates of an *Ae. albopictus* population established in Athens (Greece)
- (2) To assess the risk of outbreaks in four Greek localities based on *Ae. albopictus* population density whose estimate was based on the number of eggs laid in ovitraps.

Methods: Under laboratory conditions females were offered blood meal infected with the CHIKV titer of 1×10^6 TCID₅₀/mL and DENV II titer of 1.76×10^6 TCID₅₀/mL; at day 11 after oral infection, females were sacrificed, legs were removed and processed for PCR analysis to assess the presence of viral replicates. In order to evaluate the risk of outbreak of CHIKV and DENV II, a pilot monitoring program was started in three Greek localities and in Chania (Crete), to estimate the adult female population density on the base of the number of eggs in the ovitraps.

Results: We proved the vector competence of the Greek *Ae. albopictus* strain for E1-A226V mutated CHIKV and DENV II. Combining the data on the vector competence with those on the female population density, based on the egg density data, the estimated risk of outbreak was relatively low but not negligible.

Conclusion: As the vector competence estimated under laboratory conditions was obtained by offering females moderately low initial virus titers, it can be expected a higher vector competence in the field. This consideration, together with a possible increase of the mosquito population due to the global warming effects, make the quantitative ovitrap-based monitoring a necessary and useful tool to estimate the risk of outbreaks.

Keywords

Aedes albopictus; Chikungunya; Dengue; Infection rate; Dissemination rate; Field risk assessment

Introduction

Globalization of trade and travel has facilitated the spread of non-native species across the earth. A proportion of these species become established and cause serious environmental, economic and human health impacts. These species are referred to as invasive, and several

invasive mosquito species (IMS) were inadvertently introduced in Europe, where they found favorable environmental conditions enhanced by the climate change [1]. The predicted increases in temperature (1.4 to 5.8°C by 2100) and rainfall are likely to extend the distribution of mosquitoes and associated pathogens, in addition to shortening the development time of mosquito larvae and the extrinsic incubation period of pathogens [1,2]. Warmer and wetter weather is likely to result in longer seasonal activity of mosquitoes, while sea level rise will produce new wetland habitats suitable as breeding sites by gradually inundating coastal regions [1,3]. The related sanitary risks include the reappearance of mosquito-borne diseases such as chikungunya, dengue, and West Nile fevers which are currently emerging in different European countries, and the possible emergence of new diseases, like those caused by USUTU virus, circulating among *Ae. albopictus* populations in Northern Italy [4] and by the possible diffusion of the zika virus (ZIKV) [5], for which *Ae. albopictus* vector competence is under investigation (author's unpublished data).

Aedes albopictus was first detected in Albania in 1979 [6] and its establishment has been reported in 13 further European countries to date: Croatia, France, Greece, Italy, Malta, Monaco, Montenegro, San Marino, Serbia, Slovenia, Spain, Switzerland and the Vatican City [7-9]. The species has also been detected in Belgium, Bosnia and Herzegovina, Germany and the Netherlands [8,9]. Distribution models predict that *Ae. albopictus* will continue to expand depending on transport, environmental and climatic changes [7,10,11]. After its first record in Northwest Greece in 2003-2004, *Ae. albopictus* occurs in many other Greek regions [12], with high populations detected in Athen's urban areas [13]. This mosquito shows an aggressive nuisance behavior during the day and is considered competent to transmit at least 22 arboviruses [14]. The first chikungunya outbreak in a temperate region was reported from Northeastern Italy, with *Ae. albopictus* incriminated as the only vector [15]. Indigenous cases of chikungunya and dengue have been reported in France and Croatia in 2010 [16-18]. Up today, no chikungunya human case occurred in Greece, while in September 2012 the Hellenic Centre of Disease Control notified a case of dengue in an 84-year-old patient, who died. The most serious documented dengue virus epidemic in Europe occurred during the summers of 1927 and 1928, when about 90% of the population of Athens was infected and more than 1,000 persons died [19]. *Aedes aegypti* was responsible for those outbreaks, but in the last decades of the past century its presence had not been recorded in Europe. Now it is considered established in Madeira [20], Southern Russia, Abkhazia and Georgia [21]. Current models estimate some risk for dengue and chikungunya transmission in the Mediterranean basin related to climate change impact [22], as *Ae. albopictus*, whose vector competence has been demonstrated, could replace *Ae. aegypti*, being already present with abundant and increasing populations.

Primary aim of this study is the assessment of the infection and dissemination rates [23,24] of the *Ae. albopictus* Greek strain for DEN II virus and for the CHIK E1-A226V mutated virus and the risk of outbreak depending on the vectorial capacity and on the density of *Ae. albopictus* population in Athens (Greece) and in Chania (Crete).

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Materials and Methods

Infection and dissemination rates

Trials were performed in a BSL-3 laboratory (28 ± 1°C, 80% RH, 14:10 L:D) by using *Ae. albopictus* females obtained from field collected eggs in Athens, Greece. The CHIKV isolate (GenBank, access code EU244823.2) was provided by IZSLER of Brescia (5° P Vero cell, initial titer 1X10⁶ TCID₅₀/mL), while the DENV II isolate was provided by CRREM of Bologna (7° P, initial titer 1.76 X 10⁶ TCID₅₀/mL). Three suspensions were prepared for each virus, as described in Table 1. At day 11 after oral infection, females were individually anesthetized on ice; legs were separated from body and separately stored into criovials containing 180 ul of PBS in liquid nitrogen. Details concerning rearing conditions, methods of females' oral infection and quantitative virus analysis (QRT-real time PCR) are described in Bellini et al. [25]. The infection rate (proportion of females with virus positive bodies) and the dissemination rate (proportion of females with virus positive legs) were calculated. Paired comparisons (Fisher tests) were used to compare the number of positive/negative body samples (infection rate) and leg samples (dissemination rate) among the three virus titers of CHIKV and DENV II.

Field risk assessment

In order to estimate the risk of an outbreak to occur it is necessary to know the average number of bites per day per person and the vector competence for the specific pathogen. Statistically significant correlations were found between the mean number of eggs/ovitraps (which is the cheapest monitoring method for *Ae. albopictus*) [26] and the average number of bites per day per person estimated by Human Landing Collection (HLC). Therefore, the data obtained by an ovitraps-based monitoring system allow to estimate the number of biting females, a key factor for the calculation of the number of new cases which can arise from a singular case (R₀), as follows [26].

$$R_0 = \frac{m a^2 V P^n}{-\log_e P}$$

m is the average number of bites per day per person based on the number of eggs per ovitraps; a² indicates the propensity of the vector to bite humans; V is the vector competence; P is the daily survival rate of the vector; n is the length of the extrinsic cycle (the time from female infection to pathogen transmission). Epidemics occur when the calculated R₀ value is higher than 1. In order to evaluate the risk of outbreak of CHIKV and DENV II, a pilot monitoring program was started in three Greek localities and in Chania (Crete), to estimate the adult female population density on the base of the number of eggs in the ovitraps.

Results

Infection and dissemination rates

Figure 1 presents the rates of infection and dissemination obtained for the three CHIKV and DENV II titers. The infection rate for CHIKV decreased dramatically between C1 and either C2 or C3 (C1 vs C2, P = 0.0001; C1 vs C3, P = 0.0017), while no statistically significant difference was found between C2 and C3 (P = 0.4369). The decrease of the dissemination rate was less noticeable (C1 vs C2, P = 0.0174; C1 vs C3, P = 0.0640; C2 vs C3, P = 0.4470). The infection rates for DENV II were much lower than those measured for CHIKV and decreased significantly between D1 and D3 (P < 0.0001), D2 and D3 (Fisher test P < 0.0001), but

not between D1 and D2 (P = 0.1444). The dissemination rate was not different between D1 and D2 (P = 0.6487), while it slowed down to zero for D3 (D1 vs D3, P = 0.1476; D2 vs D3, P = 0.1729).

Field risk assessment

Based on egg density data, no risk of outbreak for the mutated CHIKV was found both in the Piraeus harbor (Port) and in the Athens International Airport "Eleftherios Venizelos" (Airport), while in Rizoupoli area (Athens) a low risk was found in August, 2014 and in the period July-October, 2015. In Chania (Crete) a low risk level was estimated in August-September, 2015 (Figure 2). In 2014 and 2015, no risk of outbreak was found for the DEN II virus in the four localities (Figure 3).

Conclusions

The laboratory trials confirmed the vector competence of Greek *Ae. albopictus* females for E1-A226V mutated CHIKV and DENV II. At 11 days post-infection, the CHIKV infection rate was consistent with the results obtained with an *Ae. albopictus* strain from the Alpes-Maritimes (Southern France) [23]. The mutated CHIKV dissemination rate was very similar to the value estimated for the strain collected in Romagna (Northern Italy) during the 2007 CHIKV outbreak [24] and for the strain collected in La Reunion [26]. The results obtained for DEN II were consistent with results obtained by Vega-Rua et al. [23] at 9 days post-infection. In our study, the infection and the dissemination rates were lower than those of CHIKV confirming that *Ae. albopictus* is more efficient vector for CHIKV (mutated strain) than for DEN II. The risk of outbreak calculated in the Greek localities, based on the vector competence estimated for the Greek *Ae. albopictus* strain was relatively low, but we were able to test relatively low virus titers, which are likely to be higher under field conditions. This consideration, together with the likely increase of the mosquito population due to the global warming effects, and to the arboviruses' mutation capacity [27], make the quantitative ovitraps-based monitoring a necessary and useful tool to estimate the risk of outbreaks. Differences among virus titers, suspension preparation protocol, body parts used to estimate the dissemination rate, the day post-infection chosen for the tests may influence vector competence study results.

Table 1: CHIKV and DENV II titers, preparation details and number of tested females (C=mutated CHIKV, and D=DENV II). The virus suspension was obtained by diluting the initial virus titer with the D-MEM substrate.

| Treatment | Initial virus titer TCID ₅₀ /mL | Volume employed (mL) | D-MEM added (mL) | Virus suspension employed (mL) | Blood added (mL) | Virus titer obtained TCID ₅₀ /mL | No. blood fed females |
|-----------|--|----------------------|------------------|--------------------------------|------------------|---|-----------------------|
| C1 | 1.0×10 ⁶ | 4 | 0 | - | 8 | 0.3×10 ⁶ | 24 |
| D1 | 1.8×10 ⁶ | 4 | 0 | - | 8 | 0.6×10 ⁶ | 38 |
| C2 | 1.0×10 ⁶ | 2 | 18 | 4 | 8 | 0.3×10 ⁵ | 26 |
| D2 | 1.8×10 ⁶ | 2 | 18 | 4 | 8 | 0.6×10 ⁵ | 24 |
| C3 | 1.0×10 ⁵ | 2 | 18 | 4 | 8 | 0.3×10 ⁴ | 29 |
| D3 | 1.8×10 ⁵ | 2 | 18 | 4 | 8 | 0.6×10 ⁴ | 33 |
| Control | 0 | 0 | 4 | 0 | 8 | 0 | 36 |

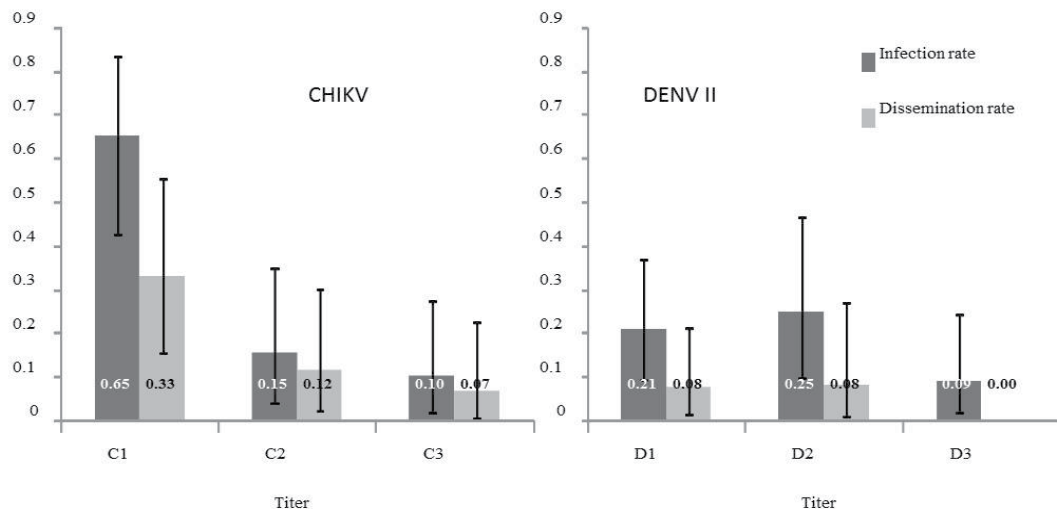


Figure 1: . CHIKV and DENV II infection and dissemination rates in virus-exposed *Ae. albopictus* females (bars represents 95% confidence limits).

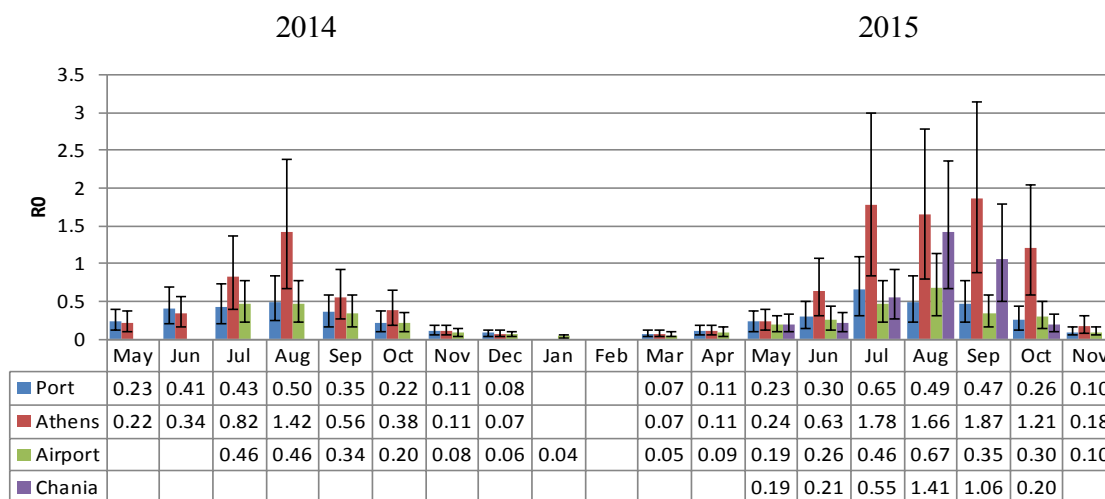


Figure 2: Risk of outbreak for the mutated CHIK virus in four Greek localities (bars represent 95% confidence limits).

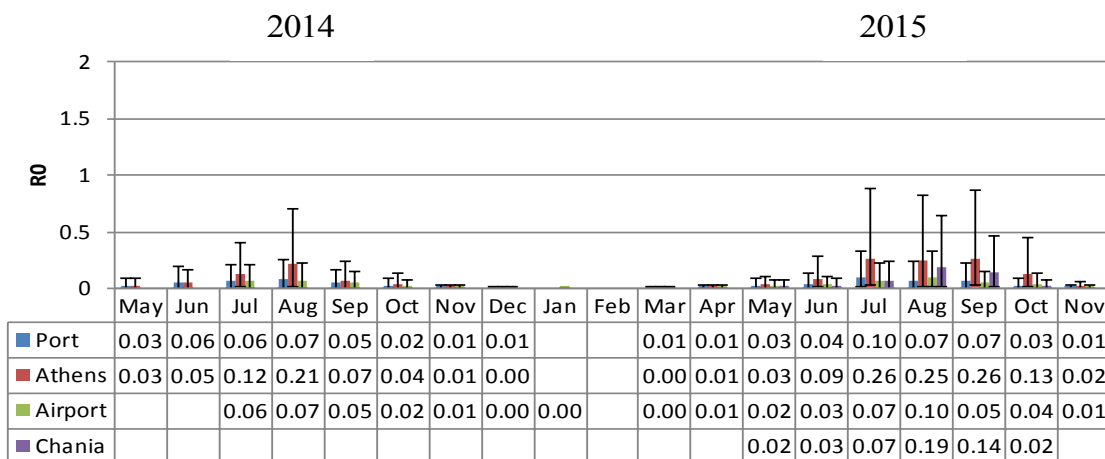


Figure 3: Risk of outbreak for the DEN II virus in four Greek localities (bars represent 95% confidence limits).

Competing Interest

The authors declare no competing interests.

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