

Experimental studies of susceptibility of Italian *Aedes albopictus* to Zika virus

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We report a study on vector competence of an Italian population of *Aedes albopictus* for Zika virus (ZIKV). *Ae. albopictus* was susceptible to ZIKV infection (infection rate: 10%), and the virus could disseminate and was secreted in the mosquito's saliva (dissemination rate: 29%; transmission rate: 29%) after an extrinsic incubation period of 11 days. The observed vector competence was lower than that of an *Ae. aegypti* colony tested in parallel.

Zika virus (ZIKV) is an emerging mosquito-borne virus (Flaviviridae family) isolated from different *Aedes* species in the past. In the recent outbreaks that occurred in Latin America, *Aedes aegypti* is believed to be the main vector. The isolation of ZIKV from this mosquito species in Malaysia [1], and early experimental studies [2,3] appear to confirm this hypothesis. Recent vector competence studies have also shown that the American *Ae. albopictus* exhibits similar transmission potential as the American *Ae. aegypti* [4].

Ae. albopictus is widespread in Mediterranean countries, in particular in Italy where it caused an outbreak of Chikungunya virus (CHIKV) (*Togaviridae* family, *Alphavirus* genus) in 2007 [5]. To assess the risk of ZIKV transmission, we evaluated the vector competence of an Italian *Ae. albopictus* population for the virus. Potential vertical (transovarial) transmission of ZIKV was also evaluated.

Experimental infection by membrane feeding technique

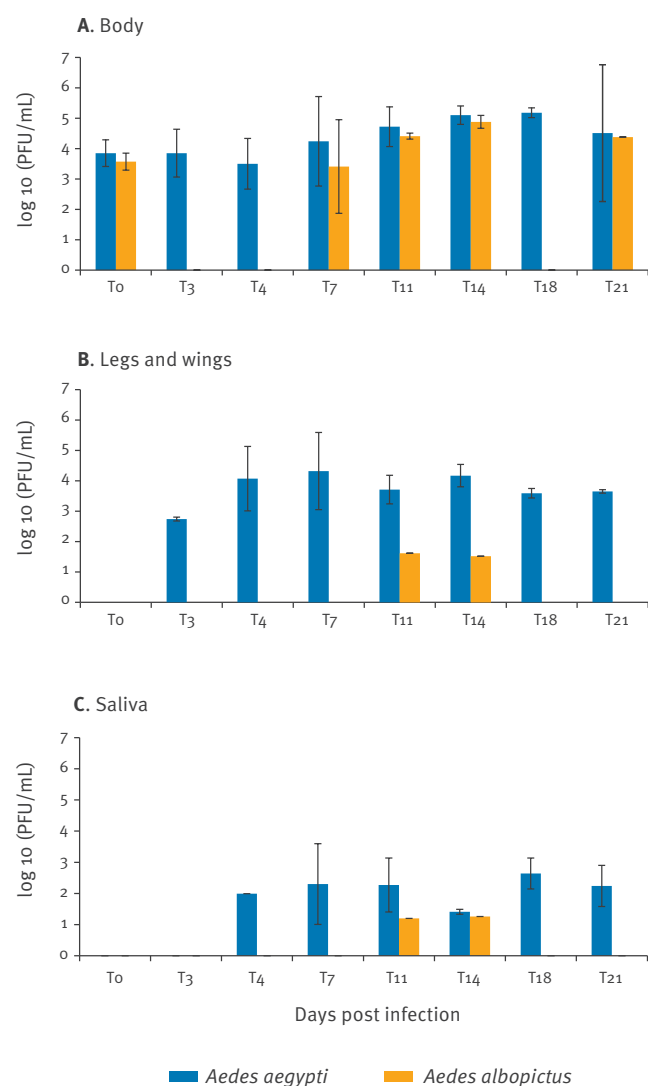
Oral infection was performed in a BSL-3 laboratory using a ZIKV strain of the Asian genotype (kindly provided by Dr Isabelle Leparc-Goffart of the French National Reference Centre for Arboviruses in Marseille)

isolated from a patient returning from French Polynesia in 2013 [6]. Ten-day-old mosquito females from an Italian *Ae. albopictus* population (collected in Scalea town, Calabria region, in the late summer of 2015) and from a long-established colony of *Ae. aegypti* (collected in Reynosa, Mexico, in 1998) were allowed to feed for 1 hour through a membrane feeding apparatus. The virus was diluted in rabbit blood (final virus concentration: 6.46 log₁₀ plaque-forming units (PFU)/mL) and maintained at 37°C by a warm water circulation system. After the blood meal, fully engorged females were transferred to cages and maintained on a 10% sucrose solution in a climatic chamber (26±1°C; 70% relative humidity; 14 h:10 h light/dark cycle) for 21 days. Ten mosquitoes from either species were individually processed at 0, 3, 4, 7, 11, 14, 18 and 21 days post infection (dpi). To evaluate viral infection, dissemination and transmission, body (head, thorax and abdomen), legs plus wings, and saliva were analysed, as previously described [7]. ZIKV titre was evaluated by quantitative reverse transcription PCR (qRT-PCR). Specific primers ZIKV 1086 and ZIKV 1162c were used, with 5-FAM as the reporter dye for the probe (ZIKV 1107-FAM) [8]. Crossing point values were compared with a standard curve obtained from 10-fold serial dilutions of virus stock of known concentration [8-10].

Mosquito bodies were analysed in order to evaluate the infection rate (IR), calculated as the number of ZIKV-positive bodies with respect to the total number of fed females. Legs plus wings were tested to assess the dissemination rate (DR), calculated as the number of the specimens with ZIKV-positive legs plus wings among the number of specimens with ZIKV-positive bodies. The saliva of the potentially infected females was processed to assess the transmission rate (TR), defined

FIGURE 1

Mean Zika virus titres in different body parts of *Aedes albopictus* and *Ae. aegypti* colonies, at different times post infection, Italy, 2016



PFU: plaque-forming units.

as the number of mosquitoes with ZIKV-positive saliva among the number of specimens with ZIKV-positive bodies [7]. The potential vector competence was expressed as population transmission rate (PTR), calculated as the number of specimens with ZIKV-positive saliva with respect to the total number of fed mosquitoes [9,11].

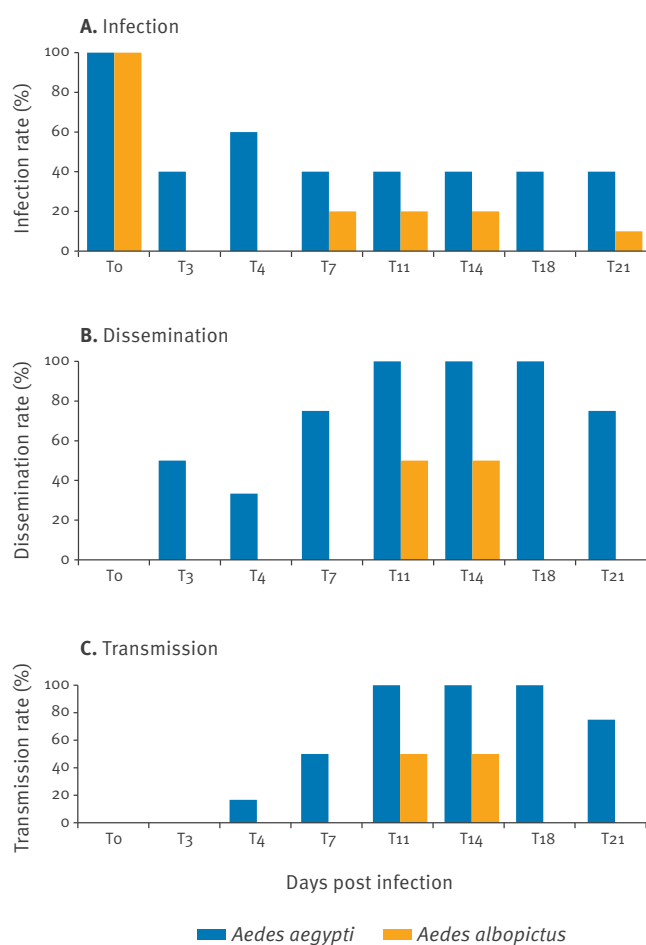
Vector competence analysis

Mean viral titres and IR, DR, and TR values are shown in Figures 1 and 2.

All of the *Ae. aegypti* and *Ae. albopictus* bodies analysed immediately after the infectious blood meal (day 0) showed positive results, with mean viral titres of $3.85 \pm 0.44 \log_{10}$ PFU/mL and $3.57 \pm 0.28 \log_{10}$ PFU/mL, respectively, confirming the ingestion of infectious viral particles. The viral titres detected in the

FIGURE 2

Infection, dissemination and transmission rates in *Aedes albopictus* and *Ae. aegypti* colonies, at different times post infection with Zika virus, Italy, 2016



Infection rate: number of positive bodies/number of tested fed females; dissemination rate: number of positive legs plus wings/number of positive bodies; transmission rate: number of positive saliva/number of positive bodies.

bodies increased gradually in both mosquito colonies, reaching $5.18 \pm 0.16 \log_{10}$ PFU/mL in *Ae. aegypti* and $4.88 \pm 0.21 \log_{10}$ PFU/mL in *Ae. albopictus* at 18 and 14 dpi (Figure 1A). As expected, differences in IR values between the two species were observed (Figure 2A). In particular, *Ae. albopictus* showed lower IR values than *Ae. aegypti* at all collection times. Whereas an IR of 40% was already detected at 3 dpi for *Ae. aegypti*, infected *Ae. albopictus* specimens were observed starting from 7 dpi, with an IR value of 20%. Cumulative IR values were 43% for *Ae. aegypti* and 10% for *Ae. albopictus* (Table).

Disseminated infection was observed in *Ae. aegypti* starting from 3 dpi, with a mean viral titre of $2.74 \pm 0.06 \log_{10}$ PFU/mL (DR 50%), while in *Ae. albopictus*, the presence of the virus in legs and wings was detected from 11 dpi, with a lower viral titre ($1.62 \log_{10}$ PFU/mL) and an equal value of DR (50%) (Figures 1B and 2B). Starting from 4 dpi, the saliva of *Ae. aegypti* showed ZIKV particles (titre of $1.99 \log_{10}$ PFU/mL and TR of

TABLE

Cumulative infection, dissemination, transmission and population transmission rates of *Aedes albopictus* and *Ae. aegypti* experimentally infected with Zika virus, Italy, 2016

	<i>Aedes aegypti</i>	<i>Aedes albopictus</i>
Infection rate	43%	10%
Dissemination rate	73%	29%
Transmission rate	60%	29%
Population transmission rate	26%	3%

Infection rate: number of positive bodies/number of tested fed females; dissemination rate: number of positive legs plus wings/number of positive bodies; transmission rate: number of positive saliva/number of positive bodies; population transmission rate: number of positive saliva/number of tested fed females.

17%) and remained positive throughout all collection times. In particular, the viral titres increased reaching the highest levels after 11 dpi ($2.64 \pm 0.50 \log_{10}$ PFU/mL). In contrast, virus was detected in the saliva of *Ae. albopictus* at 11 and 14 dpi, with TR values of 50% at both collection points, showing a longer extrinsic incubation period (EIP). Cumulative DR and TR values were 73% and 60% for *Ae. aegypti* and 29% and 29% for *Ae. albopictus*. Finally, PTR values were 26% for *Ae. aegypti* and 3% for *Ae. albopictus* (Table).

After the infectious blood meal, 40 to 50 engorged mosquitoes from each species were kept separate in different cages, under the same laboratory conditions as the ones analysed above, and were allowed to lay eggs (first gonotrophic cycle). Two weeks after the infectious blood meal, a second uninfected blood meal was provided to obtain a second gonotrophic cycle. Pools of 15 to 30 specimens (males and females) from the first and second gonotrophic cycles of both species were processed by qRT-PCR and were negative for ZIKV.

Discussion

Little is known on ZIKV despite its significant epidemic potential [12]. The introduction and dissemination of this previously neglected flavivirus in Latin America, raised concern in temperate climate countries with established *Ae. albopictus* populations [3]. In light of the spread of this mosquito species in Italy, proven vector in the 2007 outbreak of CHIK [5], it is particularly important to evaluate its vector competence for ZIKV and to assess the potential risk transmission in Italy as well as in other Mediterranean countries. Our study shows that the Italian *Ae. albopictus* population is susceptible to ZIKV, allowing viral replication and dissemination also in the salivary glands. The short persistence of the virus in the mosquito's saliva, the PTR value of 3% and the long EIP indicate a low transmission efficiency compared with that of *Ae. aegypti*. In addition, it should be noted that despite the use of a long-established mosquito colony, not representative of a wild population, the vector competence of *Ae. aegypti* for ZIKV was significant in our experiment. A

recent modelling study [13] that was based on parameters of susceptibility to infection of the *Ae. albopictus* derived from mosquito populations from the United States and Singapore [4,14], also estimated a low risk of sustained autochthonous transmission of ZIKV in northern Italy. Our results are similar to the above results on American *Ae. albopictus* and substantially confirm the low epidemic potential of ZIKV in Italy. However, the epidemic potential and the capacity to cause long chains of transmission depends on a series of factors such as the abundance of the mosquito population, the density of the human population, feeding host preferences, biting rates and environmental conditions. High mosquito density, day-biting activity, opportunistic feeding behaviour and climatic and environmental adaptability can affect the efficiency of *Ae. albopictus* as a vector, favouring its primary role in epidemics, also in the presence of a limited vector competence [15].

Our results also have important public health implications for preparedness. In fact, the extended EIP, which is consistent with the results of studies using American *Ae. albopictus* mosquitoes [4], would allow the implementation of mosquito control measures that are likely to be more efficient than those implemented in areas infested by the tropical mosquito *Ae. aegypti*. Moreover, our analysis of offspring of both species from the first and second gonotrophic cycle showed no evidence of transovarial transmission of ZIKV; this finding adds knowledge on the bionomics of this vector and may aid the optimisation of vector control management. Finally, ZIKV appears to be less well adapted to the Italian *Ae. albopictus* than the A226V variant of CHIKV (data not shown), which caused more than 250 cases in Italy after a single introduction from Kerala, India [5,16].

In conclusion, this experimentally infected Italian *Ae. albopictus* population appeared to be a competent vector for ZIKV, albeit less efficient than the primary vector *Ae. aegypti*. However, we should not forget the risk posed by CHIKV and dengue virus that remains high in southern European countries, where small outbreaks and clusters of autochthonous cases have been already documented [17,18].

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Conflict of interest

None declared.

Authors' contributions

DLM, SF, TL, BD, RME, SM, VG and FC performed the experiments; DLM, SF, TL, BD, VG and FC analysed the data; DLM, SF, TL, BD, VG, RR, RC, RG and FC wrote the manuscript.

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