Research Square

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First assessment of Aedes albopictus vector competence for chikungunya virus in the westernmost limit of its European range (Algarve, Portugal)

Manuel Silva

manuel.silva@insa.min-saude.pt

National Institute of Health Dr. Ricardo Jorge

Rita Fernandes

National Institute of Health Dr. Ricardo Jorge

Inês Campos Freitas

National Institute of Health Dr. Ricardo Jorge

Líbia Zé-Zé

National Institute of Health Dr. Ricardo Jorge

Ricardo Parreira

Instituto de Higiene e Medicina Tropical

Hugo Costa Osório

National Institute of Health Dr. Ricardo Jorge

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Abstract

Background: The global expansion of *Aedes albopictus* has facilitated autochthonous transmission of chikungunya virus (CHIKV) in temperate Europe. Despite the establishment of *Ae. albopictus* in the Algarve region of southern Portugal since 2017—a major tourist hub—the vector competence of local populations remains unknown. This absence of data represents a critical gap in assessing the potential for local outbreaks at the westernmost limit of the vector's European range.

Methods: Field-derived *Ae. albopictus* from Olhão (Algarve) were orally exposed to an infectious blood meal containing 2.5 x 10^7 PFU/mL of CHIKV (strain Réunion 2006). At 3, 7, 10, and 14 days post-infection (dpi), viral RNA copies in the abdomen and thorax were quantified via RT-qPCR to determine Infection Rates (IR) and Dissemination Rates (DR). Transmission Rates (TR) were evaluated through forced salivation and subsequent viral detection via cytopathic effect in Vero cells.

Results: The Olhão population demonstrated high susceptibility to CHIKV. IR increased progressively up to the 10^{th} dpi, peaking at 87.2%. Viral dissemination was remarkably rapid and efficient; DR reached 88.9% as early as 3 dpi and peaked at 92.7% at 10 dpi, supported by high viral RNA loads consistently exceeding 10^5 copies in secondary tissues. TR remained moderate throughout the study and reached its maximum value at 14 dpi (41.7%).

Conclusions: This first assessment confirms that the *Ae. albopictus* population from Olhão, southern Portugal, is competent for CHIKV strain LR2006-OPY1 under the experimental conditions used (constant 28ºC). The notably short extrinsic incubation period and robust systemic viral dissemination observed in this specific population indicate a risk for autochthonous outbreaks upon local mosquito exposure to a viraemic traveller. These findings provide critical baseline parameters for epidemiological modelling in the Algarve, underscoring the need for targeted vector surveillance.

Background

Chikungunya is an emerging arboviral disease of major public health concern, responsible for large epidemics in tropical regions and recurrent outbreaks in previously non-endemic areas (1). Since the early 2000s, the rapid global expansion of chikungunya virus (CHIKV), particularly the East/Central/South African (ECSA) lineage, has been driven by the spread of highly competent *Aedes* vectors and increasing human mobility. This synergy has led to explosive epidemics across the Indian Ocean, Asia, the Americas, and, more recently, Europe (2). Autochthonous transmission events documented in Italy and France demonstrated that temperate regions harbouring invasive *Aedes* populations can sustain local CHIKV transmission cycles under suitable ecological conditions (3, 4).

Aedes albopictus (Skuse), the Asian tiger mosquito, has undergone a remarkable global expansion over the last decades, establishing itself throughout much of Europe, the Americas, and parts of Africa (5, 6). Although traditionally regarded as a secondary vector for dengue, *Ae. albopictus* is now recognized as a highly efficient vector for CHIKV, particularly for viral strains carrying the alanine-to-valine substitution in

the E1 envelope glycoprotein gene (E1-A226V) adaptive mutation (7). Experimental infections of *Ae. albopictus* from southern Europe revealed high infection, dissemination, and transmission efficiencies, with infectious saliva detected as early as three days post-infection (8). Similarly, populations from the Americas have shown consistently high competence levels, underscoring the species' pivotal role in viral spread (9). These observations align with earlier assessments identifying *Ae. albopictus* not merely as a secondary actor, but as a primary driver of arboviral transmission in temperate climates.

However, vector competence for CHIKV is highly heterogeneous among *Ae. albopictus* populations and strongly modulated by environmental drivers, specifically temperature. Experimental and modelling studies indicate that temperature dictates key components of vectorial capacity, including viral replication kinetics, the extrinsic incubation period (EIP), and mosquito survival (10). Generally, vector competence follows a unimodal relationship with temperature; while intermediate values maximize transmission potential, extreme temperatures can inhibit viral dissemination. In temperate Europe, *Ae. albopictus* populations have demonstrated the capacity to transmit CHIKV efficiently even under cooler regimes representative of late spring and early autumn, reinforcing the need to incorporate local climatic suitability into risk assessments (11, 12).

Southern Europe is considered particularly vulnerable to *Aedes*-borne arboviruses due to the widespread establishment of *Ae. albopictus*, intense travel-related importation of viraemic cases, and increasingly favourable climatic trends (13). Models integrating empirical vector competence data with species distribution suggest that large areas of the Mediterranean basin, including the Iberian Peninsula, are suitable for CHIKV transmission. Yet, these risk assessments frequently rely on the extrapolation of competence parameters from a limited number of well-characterized populations (e.g., Italy, France) (10). Given the evidence that competence for CHIKV can vary markedly between populations, the lack of local data constitutes a significant blind spot in regional epidemiological surveillance.

In Portugal, *Ae. albopictus* was first detected in 2017 in the Algarve region and has since established stable populations across several southern municipalities (14). As a major tourist destination with high international connectivity, the Algarve creates a favourable interface between potential vectors and viraemic travellers. Despite this established presence, no experimental data currently exist regarding the vector competence of Portuguese *Ae. albopictus* populations. This absence of empirical evidence represents a critical gap in assessing the potential for autochthonous chikungunya transmission in Portugal and, broadly, at the south-western edge of the species' European range.

Here, we investigate the vector competence of an *Ae. albopictus* population from Olhão (Algarve, Portugal) for CHIKV under controlled laboratory conditions. By quantifying infection, dissemination, and transmission rates, and comparing these findings with data from other temperate populations, we provide the first empirical assessment of the competence of Portuguese *Ae. albopictus* to support CHIKV transmission. These data are intended to refine national risk assessments and inform evidence-based vector control strategies.

Methods

Mosquito Rearing:

Aedes albopictus eggs were collected in Olhão, in the Algarve region, from May 2025 to August 2026, under the Portuguese National Vector Surveillance Network (REVIVE). Mosquitoes were reared under controlled laboratory conditions, maintained at 28°C and 70% relative humidity, with a 12:12 h light:dark photoperiod. For the experiments, female mosquitoes with 7–9 days of age, from the second generation (F2) were used. This generation was used to reduce to near zero the chance of infected mosquitoes due to vertical transmission while at the same time reducing phenotypic changes from the wild populations.

Viral Strain and Preparation:

The CHIKV strain LR2006-OPY1 (La Réunion, 2006), ECSA lineage was used for oral infections, having a titer of 2.5 x 10^7 PFU/mL. The viral stock culture in Vero E6 cells (African green monkey, *Cercopithecus aethiops* kidney epithelial cells, ATCC® CRL 1586™) was preserved in Minimum Essential Medium (MEM 1X, Gibco®), supplemented with foetal bovine serum (FBS) at 10%, penicillin (100 units/ml) and streptomycin (100 µg/ml) + fungizone. The infectious titre of the viral stock was determined by endpoint dilution on Vero E6 cells. Ten-fold serial dilutions were inoculated in eight replicate wells per dilution (50 µL per well), and wells were scored for cytopathic effect after 3 days of incubation. The 50% tissue culture infectious dose (TCID50) was calculated with the Spearman-Kärber method, yielding 3.6×10^7 TCID50/mL. The corresponding titre in plaque-forming units (PFU) was estimated at 2.5×10^7 PFU/mL using the conversion 1 TCID50 ≈ 0.7 PFU (15).

Oral Infection:

To facilitate feeding, female mosquitoes were starved for 24 h prior to infection. The infectious blood meal was offered using a Hemotek membrane feeding system (Hemotek Ltd., Blackburn, UK). Females were allowed to feed for 30 to 45 minutes. Fully engorged females were selected after knockdown using CO_2, transferred to new containers, and maintained at 28ºC and 70% relative humidity, under a 12:12 h light:dark photoperiod with a 10% sucrose solution provided ad libitum.

Experimental Design and Sample Collection:

To assess viral kinetics, mosquitoes were analysed at 3, 7, 10, and 14 days post-infection (dpi). At each time point, a subset of 20 to 50 mosquitoes was processed to determine infection, dissemination, and transmission rates. This cohort size aligns with established sampling thresholds for controlled vector competence and forced salivation assays (16).

Salivation Assay:

Transmission potential was assessed via a forced salivation assay. Mosquitoes were anesthetized with CO_2 and immobilized. The proboscis of each female was inserted into a filter tip containing 5 µl of FBS for 30 min to collect saliva. After this time, the FBS containing the saliva is expelled into 45 µl of MEM.

Viral Detection and Processing:

Following salivation, mosquito bodies and saliva samples were stored at -80°C until processing.

- Viral RNA Detection: The abdomens and the thorax were macerated, in 500 µL of Minimum Essential Medium (MEM 1X, Gibco®), using individual plastic pestles. After maceration, total RNA was extracted, using QIAamp Viral RNA Mini Kit, from a subsample of 200 µL. Viral load was quantified using One-step NZYSpeedy RT-qPCR Probe kit (NZYtech, Portugal) following Pastorino et al protocol (17).
- Infectious Virus Detection: 45 µl of saliva samples inoculated on Vero E6 cells and incubated at 37^{o}C and 5% CO_2 in MEM (1X, Gibco®) supplemented with 10% FBS, penicillin (100 units/ml) and streptomycin (100 µg/ml) + fungizone, after viral adsorption for 1 h, in the same conditions until the detection of cytopathic effects (CPE) or 7 days, after which the presence of infectious viral particles was evaluated by RT-qPCR.

Data Analysis:

Indices were calculated as follows, adapted from Chouin-Carneiro et al.(16):

- Infection Rate (IR): Percentage of mosquitoes with virus-positive abdomens among blood-fed females.
- Dissemination Rate (DR): Percentage of mosquitoes with virus-positive thorax/head among the infected females.
- Transmission Rate (TR): Percentage of mosquitoes with infectious saliva among the females with viral dissemination.
- Transmission Efficiency (TE): Percentage of mosquitoes with infectious saliva among all blood-fed females.

Statistical analyses were conducted to evaluate temporal variations in vector competence parameters. The 95% confidence intervals (95% CI) for infection, dissemination, and transmission rates were calculated using the Wilson score interval method for binomial proportions. To determine statistical significance in IR, DR, and TR between the different extrinsic incubation periods (3, 7, 10, and 14 dpi), pairwise comparisons were performed using Fisher's exact test. To control the false discovery rate associated with multiple testing, raw p-values were adjusted using the Benjamini-Hochberg (BH)

procedure. An adjusted p-value of < 0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.5.3 2026-03-11 ucrt).

Results

1. Vector Competence: Infection, Dissemination, and Transmission Rates

Vector competence of the *Ae. albopictus* population from Olhão for CHIKV strain LR2006-OPY1 was evaluated over a 14-day EIP following the ingestion of an infectious blood meal containing 2.5 x 10^7 PFU/mL. The temporal dynamics of the physiological barriers are summarized in Table 1 and Fig. 1.

Table 1
Proportion of *Aedes albopictus* mosquitoes establishing infection, dissemination, transmission rates and efficiency at various dpi following an infectious blood meal containing 2.5 x 10^7 PFU/mL of CHIKV strain LR2006-OPY1.

CHIKV	Infection Rate	Dissemination Rate	Transmission Rate	Transmission Efficiency
3DPI	18/29 (62.1%)	16/18 (88.9%)	5/16 (31.2%)	5/29 (17.2%)
7DPI	33/44 (75%)	28/33 (84.8%)	9/28 (32.1%)	9/44 (20.5%)
10DPI	41/47 (87.2%)	38/41 (92.7%)	14/38 (36.8%)	14/47 (29.8%)
14DPI	16/30 (53.3%)	12/16 (75%)	5/12 (41.7%)	5/30 (16.7%)

The infection rate (IR) demonstrated a progressive increase during the first 10 dpi. At 3 dpi, 62.1% (95% CI: 44.0-77.3) of the mosquitoes had established midgut infections. This proportion rose steadily to 75.0% (95% CI: 60.6–85.4) at 7 dpi, peaking at 87.2% (95% CI: 74.8–94.0) at 10 dpi. A subsequent decline in the IR was observed at 14 dpi, dropping to 53.3% (95% CI: 36.1–69.8).

Viral dissemination from the midgut to secondary tissues occurred rapidly and highly efficiently. By 3 dpi, the DR had already reached 88.9% (95% CI: 67.2–96.9), maintaining robust levels at 7 dpi (84.8%; 95% CI: 69.1–93.3) and peaking at 10 dpi (92.7%; 95% CI: 80.6–97.5). At 14 dpi, the DR remained substantial at 75.0% (95% CI: 50.5–89.8).

The transmission rate (TR) incrementally rose from 31.2% (95% CI: 14.2–55.6) at 3 dpi to reach its maximum at 14 dpi, where 41.7% (95% CI: 19.3–68.0) of mosquitoes with disseminated infections were capable of transmission. Epidemiologically, the overall transmission efficiency (TE)—the proportion of all blood-fed mosquitoes capable of transmitting the virus—peaked at 10 dpi at 29.8% (95% CI: 18.4–44.1).

Pairwise statistical analysis was performed to evaluate the temporal variations of vector competence parameters across the 14-day extrinsic incubation period (Table 2). A statistically significant reduction in the Infection Rate (IR) was identified exclusively between 10 dpi and 14 dpi (adjusted p = 0.0083). All other temporal comparisons for IR yielded no significant differences. Furthermore, the fluctuations observed in both the Dissemination Rate (DR) and Transmission Rate (TR) were not statistically

significant across any of the evaluated time points (all adjusted $p > 0.05$). This confirms that while the prevalence of midgut infection experienced a late-stage decline, the systemic dissemination and transmission capacities within the infected mosquito cohort remained statistically stable throughout the duration of the experiment.

Table 2
The table presents the adjusted p-values for pairwise comparisons of Infection Rate (IR), Dissemination Rate (DR), and Transmission Rate (TR) across the 14-day extrinsic incubation period (3, 7, 10, and 14 dpi) following exposure to CHIKV strain LR2006-OPY1. Statistical significance was determined using Fisher's exact test. To control the false discovery rate associated with multiple testing, raw p-values were adjusted using the Benjamini-Hochberg (BH) procedure. An adjusted p-value < 0.05 is considered statistically significant.

Adjusted p-value numbers	3dpi vs 7dpi	3dpi vs 10dpi	3dpi vs 14dpi	7dpi vs 10dpi	7dpi vs 14dpi	10dpi vs 14dpi
Infection Rate	0.3601	0.064	0.601	0.2705	0.1586	0.0083
Dissemination Rate	1	0.7631	0.6806	0.6806	0.6806	0.5293
Transmission Rate	1	1	1	1	1	1

2. Viral Kinetics in Mosquito Tissues

Quantitative analysis of viral RNA copies in the abdomen (representing primarily the midgut) and thorax (representing disseminated infection) revealed rapid viral replication and systemic spread within the vector (Fig. 2).

Viral RNA loads in both tissue compartments were already detectable at high concentrations by 3 dpi. The abdomen exhibited median viral loads consistently exceeding 10^5 copies across all time points. The thorax compartment mirrored this intense viral replication; by 7 dpi, the median viral RNA copies in the thorax closely aligned with those in the abdomen, indicating that once the midgut escape barrier is breached, CHIKV

strain LR2006-OPY1 replicates aggressively in secondary target organs. The highest estimated viral RNA copies across all samples were recorded between 7 and 10 dpi, corroborating the peak infection and dissemination rates observed during the same window. Despite the drop in overall IR at 14 dpi, the mosquitoes that remained infected sustained high viral titers in both the abdomen and thorax, facilitating the peak transmission rates observed on that day.

Discussion

The present study demonstrates that the established *Ae. albopictus* population in the Algarve region (Olhão, southern Portugal) is highly susceptible to CHIKV strain LR2006-OPY1 infection and displays a robust competence for systemic viral dissemination and transmission. These findings indicate biological suitability for local CHIKV transmission in the region.

Our results reveal that the midgut infection barrier (MIB) and midgut escape barrier (MEB) in this population are weak against CHIKV strain LR2006-OPY1. The extremely high dissemination rate observed as early as 3 dpi (88.9%) indicates a remarkably short extrinsic incubation period (EIP) at the tested conditions (at 28ºC and 70% relative humidity). Rapid dissemination is a hallmark of highly competent vectors and is a critical parameter driving outbreak explosive potential, as it directly minimizes the time required for a mosquito to become infectious. Similar truncated EIPs for CHIKV in *Ae. albopictus* have been documented in populations responsible for major European outbreaks, such as the 2007 epidemic in Emilia-Romagna, Italy (18).

It is important to note that the cohort sizes utilized in this study (n = 29 to 47 per time point) reflect the standard operational thresholds for highly labor-intensive BSL-3 vector competence and forced salivation assays. While sample sizes of this magnitude might limit the statistical power to detect minor fluctuations, they are validated in the literature for establishing baseline vector competence profiles.

While the IR peaked at 10 dpi (87.2%), a reduction was observed at 14 dpi (53.3%). Because this experimental design relied on destructive sampling of distinct mosquito cohorts at each time point without parallel survival tracking, it is not possible to definitively attribute this fluctuation to specific biological mechanisms seen in other similar studies (19), such as active viral clearance by the mosquito's innate immune system or virus-induced mortality. The observed decrease may instead represent a sampling artifact resulting from natural variation between the specific mosquito batches tested at each day. Despite this drop in IR, the surviving infected mosquitoes maintained high systemic viral loads, as evidenced by the steady increase in the transmission rate, which peaked at 41.7% on day 14. This progressive increase in TR suggests that the salivary gland infection and escape barriers (SGIB/SGEB), while restrictive early in the infection timeline, are eventually overcome through sustained viral accumulation.

The viral kinetics data further support this epidemiological risk. The rapid attainment of high viral RNA copies in the thorax confirms that once CHIKV strain LR2006-OPY1 escapes the midgut, secondary tissue colonization is aggressive and unrestricted. High viral titers in secondary tissues are strongly correlated with elevated viral shedding into the saliva, directly influencing the transmission probability (b parameter in the vectorial capacity equation) (20).

The high vector competence demonstrated by the Olhão population aligns with findings from other Mediterranean *Ae. albopictus* populations (Table 3)(21). When combined with favourable ecological conditions, the observed vector competence suggests a potential for local CHIKV transmission. Consequently, the introduction of viraemic travellers during the vector's peak activity season (July-September) presents a tangible risk for local, sustained transmission (22).

Table 3

Comparison of Aedes albopictus vector competence parameters for CHIKV among different European populations. Max IR: Maximum Infection Rate; Max DR: Maximum Dissemination Rate; Max TR: Maximum Transmission Rate; EIP: Extrinsic Incubation Period (First Saliva Detection). The study by Vega-Rua et al. reported a 100% Disseminated Infection Rate at 6 dpi, which inherently requires a 100% baseline midgut Infection Rate. ** The 7 DPI and 14 DPI EIPs for the Italian and German populations represent the first-time points saliva was tested in their respective experimental designs, not necessarily the absolute biological minimum EIP. *** Lühken et al. did not report Dissemination Rates (NR = Not Reported); because their cohort Infection Rate was 100%, their maximum Transmission Efficiency (58.6%) is presented here as the closest equivalent to TR. The Italian maximum TR was calculated from the raw data provided by Mariconti et al., where 6 mosquitoes transmitted the virus out of 9 with disseminated infections in the Cesena 9 cohort at 7 dpi. Maximum Italian DR (83.3%) was observed in the Cesena 3 + 4 cohort at 14 dpi (20 disseminated out of 24 infected).

Population	Temp (ºC)	Max IR (%)	Max DR (%)	Max TR (%)	First Saliva Detection (EIP)	Reference
Olhão (Portugal)	28	87.2	92.7	41.7	3 DPI	This study
Cagnes-sur-Mer (France)	28	100*	100	84.2	3 DPI	Vega-Rua et al. (21)
Emilia-Romagna (Italy)	28	100	83.3	66.7	7 DPI**	Mariconti et al. (8)
Freidburg (Germany)	15–24	100	NR***	58.6***	14 DPI**	Luhken et al. (12)

These entomological and virological indices provide critical empirical data for regional epidemiological modelling. Future entomological surveillance in the Algarve should prioritize early detection of viral circulation and rapid implementation of vector control measures to mitigate the risk of explosive CHIKV outbreaks.

Conclusions

This study provides the first experimental assessment of the vector competence of Aedes albopictus from the Algarve for chikungunya virus. The Olhão population was susceptible to CHIKV strain LR2006-OPY1, with infection rates of up to 87.2% and dissemination rates of 75.0–92.7% across all time points. Infectious saliva was detected from 3 days post-infection, with transmission rates of 31.2–41.7% among mosquitoes with disseminated infection and a maximum transmission efficiency of 29.8% at 10 days post-infection, under a constant temperature of 28°C. These results show that Portuguese Ae. albopictus is competent to transmit CHIKV and provide baseline parameters for epidemiological modelling and risk assessment in southern Portugal. Further studies with other viral strains, fluctuating temperatures and additional mosquito populations, combined with vector surveillance, are needed to refine the risk of local transmission.

Abbreviations

ATCC: American Type Culture Collection

BH: Benjamini-Hochberg

BSL-3: Biosafety level 3

CHIKV: Chikungunya virus

CI: Confidence interval

CPE: Cytopathic effect

dpi: Days post-infection

DR: Dissemination rate

E1-A226V: Alanine-to-valine substitution at position 226 of the E1 envelope glycoprotein

ECSA: East/Central/South African

EIP: Extrinsic incubation period

F2: Second filial generation

FBS: Foetal bovine serum

IR: Infection rate

MEB: Midgut escape barrier

MEM: Minimum Essential Medium

MIB: Midgut infection barrier

NR: Not reported

PFU: Plaque-forming units

REVIVE: Portuguese National Vector Surveillance Network

RT-qPCR: Reverse transcription quantitative polymerase chain reaction

SGEB: Salivary gland escape barrier

SGIB: Salivary gland infection barrier

TCID50: 50% Tissue Culture Infectious Dose

TE: Transmission efficiency

TR: Transmission rate

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MS conceived the study, performed the laboratory work, analysed the data and wrote the manuscript. RF, ICF and LZZ assisted with the laboratory work and revised the manuscript. RP contributed to work planning, funding acquisition and manuscript revision. HO contributed to the laboratory work, work planning, funding acquisition and manuscript revision. All authors read and approved the final manuscript.

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Figures

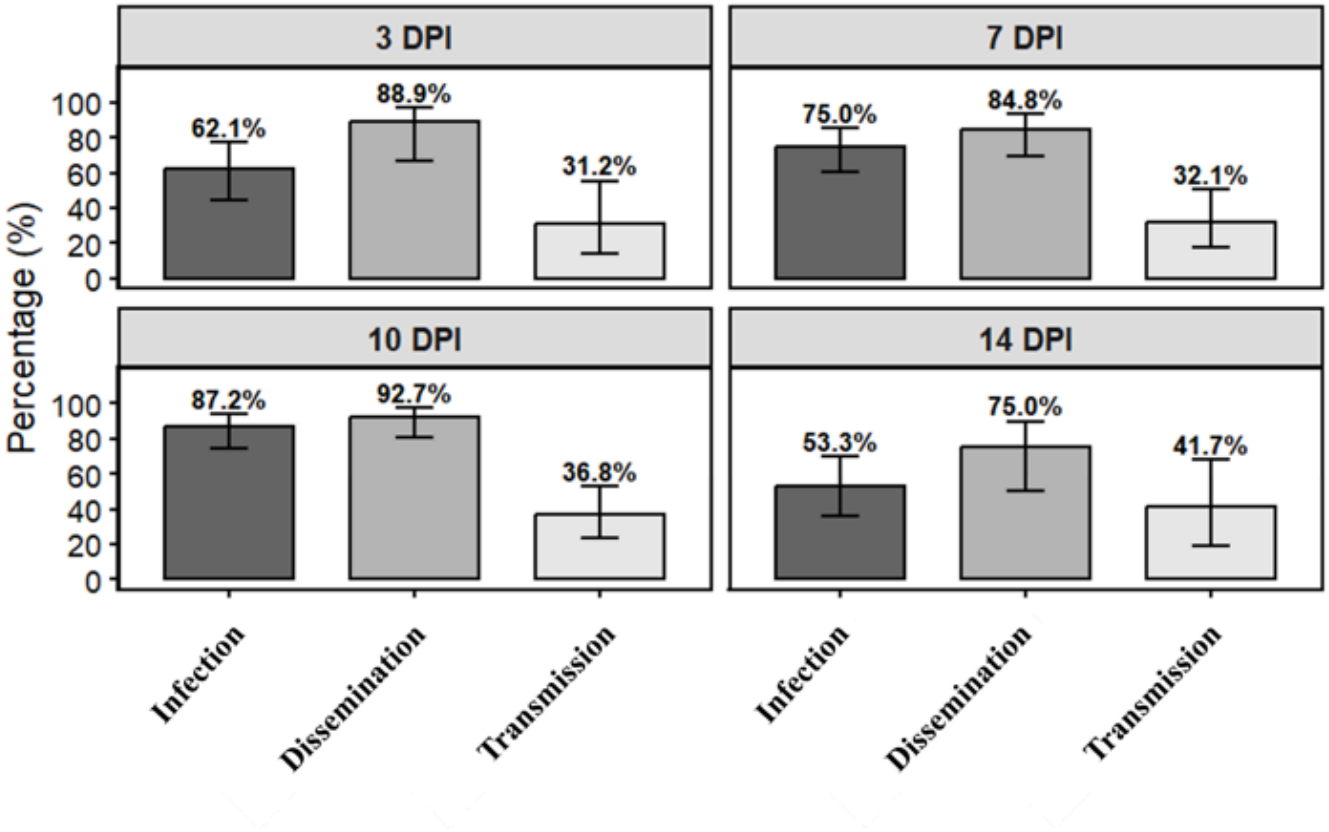


Figure 1 - Vector competence of *Aedes albopictus* for CHIKV strain LR2006-OPY1. Bar charts illustrate the progression of infection (dark grey), dissemination (medium grey), and transmission (light grey) rates at 3, 7, 10, and 14 days post-infection (dpi) following an infectious blood meal. Percentages are indicated above each bar; error bars denote 95% confidence intervals.

Figure 1

See image above for figure legend.

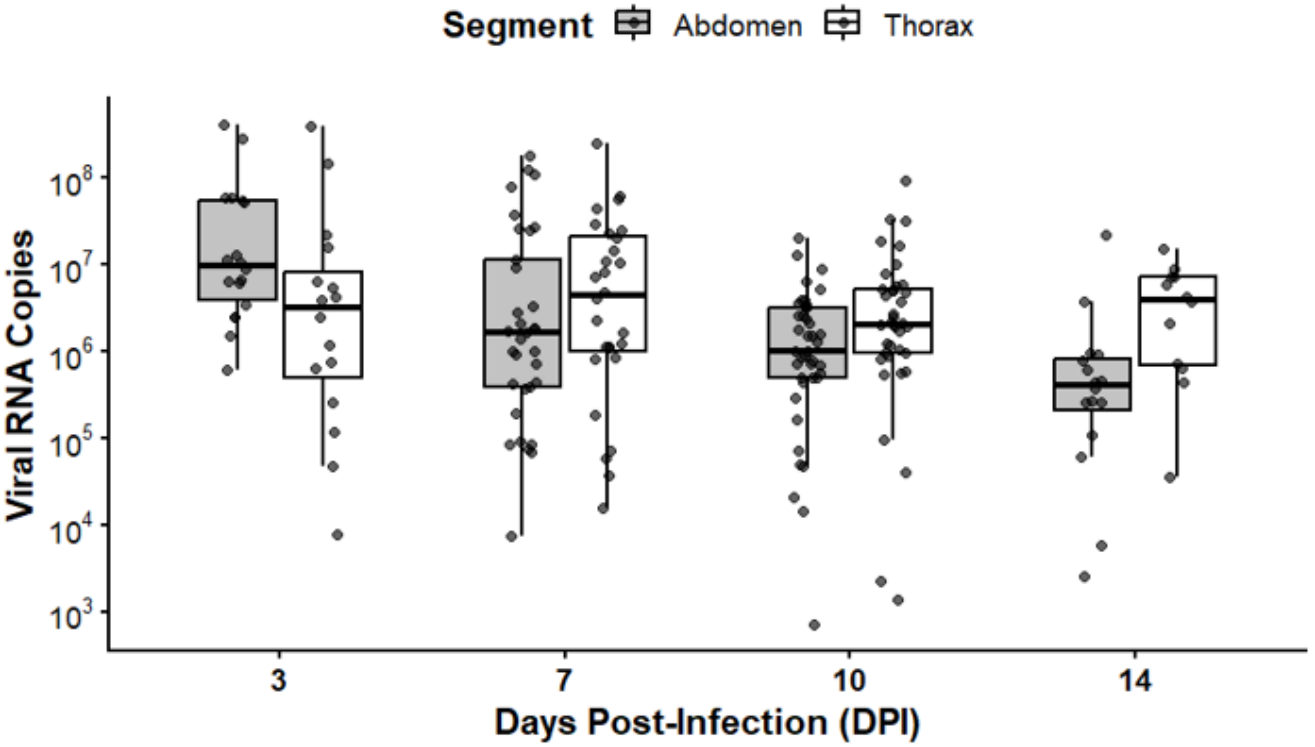


Figure 2 - Quantitative assessment of CHIKV strain LR2006-OPY1 RNA loads in *Aedes albopictus* tissue compartments. Box and whisker plots depict the median and interquartile ranges of viral RNA copies in the abdomen (representing midgut infection) and thorax (representing disseminated infection) across a 14-day extrinsic incubation period. Individual points denote the viral load of individual mosquitoes.

Figure 2

See image above for figure legend.