

Methodological procedures explain observed differences in the competence of European populations of *Aedes albopictus* for the transmission of Zika virus

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ABSTRACT

The risk of autochthonous transmission of Zika virus (ZIKV) in Europe greatly depends on the capacity of *Aedes albopictus* to transmit ZIKV. Experimental studies of the vector competence of European populations of this invasive mosquito species provided contrasting results, that may suggest differences between mosquito populations in ZIKV competence and/or differences in the methodological procedures used on the experimental studies. We systematically reviewed published studies of experimental mosquito infections and show that differences detected in the infection and transmission rates of *Ae. albopictus* are mostly due to differences between studies in the temperature, the viral load used to inoculate mosquitoes, and the number of days post-exposure (dpe) to the virus at which mosquitoes were analysed. In particular, the proportion of infected mosquitoes was affected by the viral load inoculated and the dpe, whereas the transmission rate was influenced by the viral load and temperature. Thus, the methodological differences used in these studies greatly affect the conclusions obtained regarding the role of *Ae. albopictus* in Europe as a competent vector for ZIKV and its epidemiological relevance.

1. Introduction

Since the outbreaks caused by Zika virus (ZIKV) in the Pacific Islands and the Americas in 2013–2016, numerous studies have quantified the vector competence of certain species of mosquitoes for ZIKV transmission (Diagne et al., 2015; Gendernalik et al., 2017). Invasive *Aedes* species act as vectors of ZIKV worldwide and *Aedes aegypti* is considered to be its main vector. In America, ZIKV is mainly transmitted by *Ae. aegypti* (Guerbois et al., 2016), although other species such as *Ae. albopictus* and *Ae. (Ochlerotatus) vexans* probably also play an important role as vectors (Weger-Lucarelli et al., 2016; Gendernalik et al., 2017).

In Europe, *Ae. aegypti* has a limited range as an established invasive species in Georgia, Russia and eastern Turkey (ECDC, 2021). However, the similarly invasive *Ae. albopictus* is widespread in many European countries where it can create novel epidemiological scenarios favouring the transmission of certain pathogens. Numerous studies support the

role that *Ae. albopictus* plays in the transmission of ZIKV (Weger-Lucarelli et al., 2016; Li et al., 2012) and, for example, this species has been shown to be involved in the local transmission of ZIKV in southern France and may also play a key role in the local transmission in Europe of flavivirus such as dengue and chikungunya virus (Tomasello and Schlagenhauf, 2013; Brady and Hay, 2019; Giron et al., 2019). However, studies analyzing the vector competence for ZIKV of *Ae. albopictus* from western and central Europe have reported important differences in the ability of mosquitoes from different regions to transmit the virus (Table 1). Therefore, the role played by *Ae. albopictus* in the transmission of ZIKV in these regions is still uncertain, as are the reasons for the contrasting results of the experimental studies published to date.

Experiments to estimate the vectorial competence of *Ae. albopictus* have used different viral loads for inoculation and different temperatures, and have analysed competence at a different number of days post exposure (dpe) to the virus. This variability in experimental conditions

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Table 1
Summary of the results of the studies on the ZIKV vector competence included in this article. Mosquito infection and transmission rates are shown for the different combinations of mosquito populations, temperatures, virus loads and dpe analysed here.

Reference	Mosquito origin	Colony / wild population	Virus strain	Temperature °C	DPE	Virus load (Log10 PFU/ml)	Mosquitoes analyzed	Mosquitoes infected	Infection rate	Mosquitoes with positive saliva	Transmission rate	Transmission efficiency
[Jupille et al. 2016]	France	Wild population (F7-8)	New Caledonia (NC-2014-5132)	28	3	6.85	40	2	5.00	0	0.00	0.00
					6	6.85	40	15	37.50	0	0.00	
					9	6.85	48	16	33.33	0	0.00	
					14	6.85	48	18	37.50	1	5.56	2.08
[Vazeille et al. 2019]	France	Wild population (F0)	Cambodia (FSS 13,025) and Martinique (MRS_OPY_Martinique_PaRi_2015)	28	7	6.85	96	70	72.92	0	0.00	0.00
					14	6.85	95	85	89.47	4	4.71	4.21
					21	6.85	93	81	87.10	8	9.88	8.60
[Di Luca et al. 2016]	Italy	Wild population (Colony with 1 year old)	French Polynesia (H/PF/2013)	26	3	6.46	10	0	0.00	0	0.00	0.00
					4	6.46	10	0	0.00	0	0.00	
					7	6.46	10	2	20.00	0	0.00	
					11	6.46	10	2	20.00	1	50.00	10.00
					14	6.46	10	2	20.00	1	50.00	10.00
					18	6.46	10	0	0.00	0	0.00	
[González et al. 2019]	Spain	Wild population (F1)	Brazil (ZIKV-PE243)	27	7	6.26	125	51	40.80	0	0.00	0.00
					14	6.26	125	69	55.20	0	0.00	0.00
[Gutiérrez-López et al. 2019]	Spain	Wild population (F2)	Cambodia (FSS13025) and Puerto Rico (PRCABC59)	27	7	7.6	54	51	94.44	2	3.92	3.70
					14	7.6	57	50	87.22	2	4.00	3.51
					21	7.6	44	42	95.45	13	30.95	29.55
[Hernández-Triana et al. 2019]	Spain	Colony from 2009	French Polynesia (H/PF/2013)	20	7	7.2	9	4	44.44	0	0.00	0.00
					14	7.2	16	5	31.25	0	0.00	0.00
					21	7.2	15	2	13.33	0	0.00	0.00
				25	7	7.2	7	7	100.00	0	0.00	0.00
					14	7.2	10	6	60.00	0	0.00	0.00
					21	7.2	16	0	0.00	0	0.00	0.00
[Nuñez et al. 2020]	Spain	Wild population (F0)	Martinique (MRS_OPY_Martinique_PaRi_2015)	28	7	6.85	48	26	54.17	0	0.00	0.00
					14	6.85	47	24	51.06	1	4.17	2.13
					21	6.85	39	15	38.46	1	6.67	2.56
[Heitmann et al. 2017]	Germany	Wild population (F7)	Guatemala (ZIKV_FB-GWUH-2016)	18	14	7	32	4	12.50	0	0.00	0.00
					21	7	32	11	34.38	0	0.00	0.00
					27	14	7	31	20	64.52	4	20.00
	Italy	Wild population (F7)		18	21	7	34	18	52.94	6	33.33	17.65
					14	7	30	19	63.33	0	0.00	0.00
					27	14	7	39	14	35.90	0	0.00
[Mariconti et al. 2019]	Greece	Wild population (F2)	Brazil (ZIKV-PE243)	28	14	7	24	0	0.00	0	0.00	0.00
					21	7	24	1	4.17	0	0.00	0.00
					21	7	24	1	4.17	0	0.00	0.00
	Montenegro	Wild population (F2)		28	7	7	24	0	0.00	0	0.00	0.00
					14	7	24	2	8.33	0	0.00	0.00
					21	7	24	2	8.33	0	0.00	0.00
	Croatia	Wild population (F2-F3)		28	7	7	54	3	5.56	0	0.00	0.00
					14	7	48	1	2.08	0	0.00	0.00
					21	7	48	0	0.00	0	0.00	0.00
	Italy	Wild population (F3)		28	7	7	48	5	10.42	0	0.00	0.00
					14	7	48	1	2.08	1	100.00	2.08
					21	7	48	2	4.17	0	0.00	0.00
Switzerland	Wild population (F2-F3-F4)		28	7	7	24	1	4.17	0	0.00	0.00	
				14	7	24	1	4.17	0	0.00	0.00	
				21	7	38	0	0.00	0	0.00	0.00	

(temperature, viral load and dpe) may well explain the contrasting results of studies undertaken on the rates of infection and transmission of ZIKV by *Ae. albopictus* in Europe. For example, studies of flavivirus have demonstrated that temperature can affect virus infection and transmission rates in mosquitoes (Tesla et al., 2018a; Folly et al., 2021). Although *Culex pipiens* is susceptible to Japanese Encephalitis Virus (JEV) infection at 20 °C and 25 °C, this virus was only detected in the saliva of mosquitoes kept at 25 °C (Folly et al. 2021). High temperatures may also have a negative impact on the infection and transmission rates of flavivirus in mosquitoes. Tesla et al. (2018a) measured experimentally the thermal performance of Zika virus in *Aedes aegypti* at eight different temperatures (from 16 to 38 °C). They found that temperature affected the rate of ZIKV infection, dissemination and detection in saliva, and that ZIKV detection was highest at 29 °C but lower at lower and higher temperatures.

In addition, the viral load used for inoculation may also affect the subsequent infection and transmission rate by mosquitoes. For example, Ciota et al. (2017) found differences in the infection and transmission rate of *Ae. albopictus* to ZIKV. They find a lower infection and transmission rates when mosquitoes were inoculated with low viral loads. Chouin-Carneiro et al. (2020) report similar results for *Ae. aegypti* and *Ae. Albopictus*, which were unable to transmit ZIKV if virus loads were lower than 10^3 PFU/mL.

Finally, ZIKV, like other viruses, requires several days to develop and reach the salivary glands and so it is expected that transmission rates will increase with the number of days post-exposure (Gutiérrez-López et al., 2019).

Therefore, the main aim of this study was to assess the factors potentially explaining the wide range of results reported by the many studies that have experimentally measured ZIKV transmission rates by *Ae. albopictus* in Europe. To do so, we tested for the effects of the main methodological approaches used in published studies and analysed how differences between studies regarding these three variables affect the conclusions obtained in analyses of the competence for ZIKV of European populations of *Ae. albopictus*.

2. Materials and methods

2.1. Data collection

We undertook a systematic review of the literature published on the vector competence of *Ae. albopictus* for ZIKV in Europe. The factors that we analyzed in our study were: i) the effect of temperature in the experimental chambers used to keep the mosquitoes (hereafter temperature), ii) the viral load used for ZIKV inoculation (hereafter viral load) and, iii) the number of days post exposure when the mosquitoes were tested (hereafter dpe). The range of dates used in the search in Scopus was for articles published from 2012 up to the date of the search (22th July 2022). The languages considered were English, Spanish, French, Italian, German, Hungarian and Serbo-Croat. We used the keywords: ("zika" and "albopictus") AND ("albania" OR "bosnia & herzegovina" OR "bulgaria" OR "croatia" OR "france" OR "georgia" OR "germany" OR "greece" OR "hungary" OR "italy" OR "malta" OR "monaco" OR "montenegro" OR "romania" OR "russia" OR "slovenia" OR "spain" OR "switzerland" OR "turkey"), with the option 'all fields' to recover articles in which the search terms appeared in the title, abstracts or keywords. Titles and abstracts were read in order to produce the final list of publications to be read in full-text. The final list of articles was assembled after reading the full text and applying the following exclusion criteria:

1. Studies which were based in previously published data.
2. Studies that did not report transmission rates.
3. Studies that analysed another *Aedes* species but without including *Ae. albopictus* as a control.
4. Studies where the raw data was not available and for which we failed to obtain the data from the authors.

A flow diagram was created to outline all the steps taken and the number of articles analysed at each step, and to show why articles were included or excluded (see Fig. 1) (Page et al., 2021). Finally, nine articles that fulfilled all the criteria were selected (Jupille et al., 2016; Di Luca et al., 2016; Heitmann et al., 2017; González et al., 2019; Gutiérrez-López et al., 2019; Hernández-Triana et al., 2019; Mariconti et al., 2019; Vazeille et al., 2019; Nuñez et al., 2020; Table 1). We obtained from these published studies the following information: i) the study identity, ii) the virus strain, iii) the number of mosquitoes analysed in each study, iv) the viral load inoculated in each experiment, v) the temperature at which mosquitoes were kept during the experimental procedures, and vi) the number of days post exposure (dpe) to the virus when mosquitoes were analysed. For analyses of infection rate, vii) we quantified the number of mosquitoes positive/negative for ZIKV in their bodies considering the total number of mosquitoes analyzed, viii) for transmission rate, we quantified the number of mosquitoes with saliva positive/negative for ZIKV in relation to all mosquitoes with a positive amplification of ZIKV in the body. Finally, ix) for the transmission efficiency, we quantified the number of mosquitoes with saliva positive/negative for ZIKV out of the total number of mosquitoes analysed. Jupille et al. (2016) used TCID₅₀ for the calculation of the viral load that the mosquitoes were exposed to, which we converted to Plaque forming units (PFU) following Cell Biology Protocols (<https://www.sciencegateway.org/protocols/cellbio/cell/moipfu.htm>). Vazeille et al. (2019) and Nuñez et al. (2020) also studied the vector competence for strains of the ZIKV African lineage but we only analysed data from mosquitoes exposed to the Asian lineage of ZIKV (Lanciotti et al. 2016; Weger-Lucarelli et al., 2016).

2.2. Statistical analyses

Three independent generalized mixed linear models (GLMM) with binomial error and logit link functions were used to assess the effects of (i) temperature during the experimental infection, (ii) the viral load used for the ZIKV inoculation, and (iii) the number of days post-exposure when the mosquitoes were analyzed, on either the infection rate, transmission rate and transmission efficiency (dependent variables) of *Ae. albopictus*. The two-way interactions between the three variables were also included in the models. The variable viral load was log transformed to attain normality. Temperature, viral load (log transformed) and dpe were standardized by subtracting the mean and dividing by the standard deviation. The study identity was included as a random factor in the analyses because *Ae. albopictus* populations from France, Italy and Spain were studied more than once in the different articles we reviewed (Fig. 2). The virus strain was also included as random factor because the strains from Cambodia (FSS13025), Brazil (ZIKV-PE243), and Martinique (MRS_OPY_Martinique_PaRi_2015), were studied more than once in at least two independent studies (Table 1). The low number of mosquitoes analyzed ($N < 25$) in the studies for the populations of Italy and Switzerland (Di Luca et al., 2016; Mariconti et al., 2019) prevented us from testing statistically for the effect of *Ae. albopictus* country of origin on ZIKV vector competence since the statistical models did not converge. Statistical analyses were performed in R software 3.2.5 (R Core Development Team, 2016) using the package *lme4* (Bates et al., 2011).

3. Results

We obtained information from nine studies reporting the experimental exposure of 1924 mosquito specimens to ZIKV. Of these, 789 showed infections after exposure to ZIKV but only 51 of them had ZIKV in their saliva (Table 1). The findings of these infection experiments differed widely between studies. At 14 dpe, Jupille et al. (2016) and Vazeille et al. (2019) found that 2–4% of *Ae. albopictus* were able to transmit ZIKV. By contrast, Di Luca et al. (2016) and Heitmann et al. (2017) found greater transmission efficiency in mosquitoes from Italy,

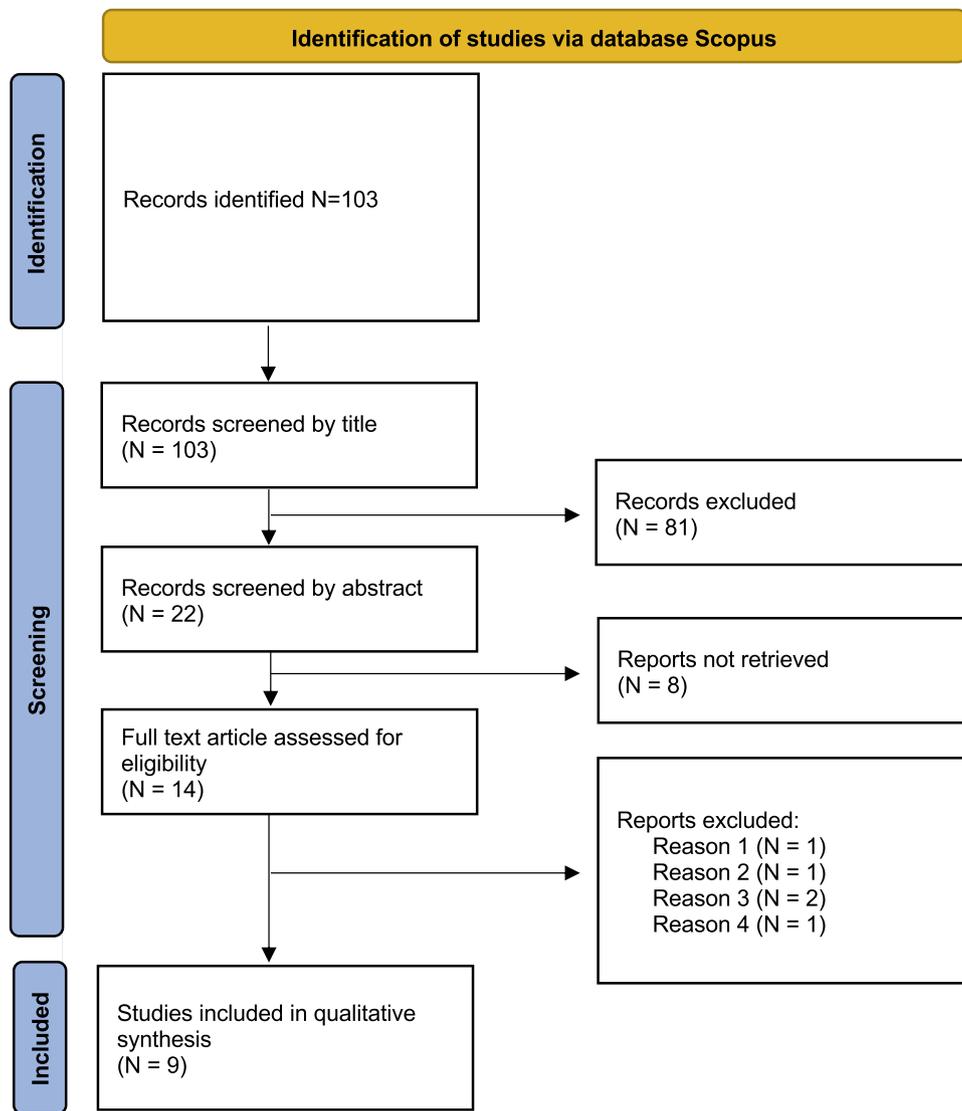


Fig. 1. Flowchart describing the selection of articles.

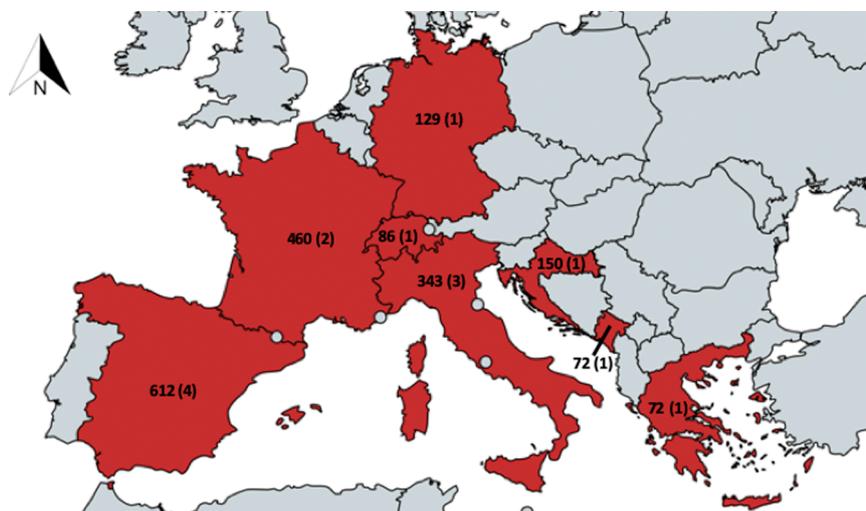


Fig. 2. Countries with *Aedes albopictus* populations studied here are shown in red. The number of mosquitoes analyzed and the number of studies conducted in these populations (in brackets) are shown.

with 10% and 12,6%, respectively, at 14 dpe. Although both Gutiérrez-López et al. (2019) and Nuñez et al. (2020) found that *Ae. albopictus* was able to transmit ZIKV, this was not the case according to the results published by González et al. (2019) and Hernández-Triana et al. (2019) (Table 1).

The results from the models are summarized in Table 2. Although the variables, temperature, viral loads and dpe were not significantly related to the infection rate, we did find that the infection rate was significantly associated to the interaction between dpe and viral load. Our results showed that the infection rate varied in mosquitoes exposed to different viral loads: mosquitoes exposed to a lower viral load increased their infection rate as the dpe increased, although variation in terms of the dpe was less important when mosquitoes were exposed to higher viral loads. The ZIKV transmission rate were not significantly related to temperature and dpe. However, we did find a significant effect on transmission rate for both viral load and the interaction between temperature and viral load (Table 2). According to the significant effect of this interaction, in mosquitoes exposed to higher viral loads the rate of transmission rises as the temperature increases. This effect was observed in the study by Heitmann et al. (2017), who showed that using a virus load of 7 Log₁₀ PFU/mL, at 18 °C, the transmission rate is null, but at 27 °C the transmission rate was 33% at 21 dpe. However, all studies performed with low viral loads to date have tested only a small range of temperatures (from 26 °C to 28 °C) and consequently it is not possible to infer any relationship with temperature. For example, Gonzalez et al. (2019) did not find transmission rate using 6.26 Log₁₀ PFU/mL at 27 °C, which is thus evidence that the transmission rate is greatly affected by the viral load.

No significant association between transmission efficiency and any of the analysed variables, temperature, virus load (Fig. 3) or dpe, or any two-way interactions, was found (Table 2).

4. Discussion

The results of this review support the affirmation that differences in the infection and transmission rates reported in experimental studies on the competence of European *Ae. albopictus* for ZIKV may be due to

Table 2

Results of GLMMs on the effects of temperature, virus load and dpe and their two-way interactions on the infection and transmission rates of ZIKV by *Aedes albopictus* mosquitoes. Significant effects are highlighted in bold.

Infection rate			
	Estimate	Std. Error	P-value
Temperature	0.205	0.253	0.417
Dpe	-0.051	0.083	0.537
Virus load	0.708	0.580	0.222
Temperature*dpe	0.148	0.101	0.142
Temperature*virus load	0.179	0.689	0.795
Dpe*virus load	-0.309	0.101	0.002
Transmission rate			
	Estimate	Std. Error	P-value
Temperature	-0.746	1267	0.556
Dpe	0.536	0.822	0.514
Virus load	-2.644	1052	0.012
Temperature*dpe	0.814	1401	0.561
Temperature*virus load	8182	2811	0.004
Dpe*virus load	0.158	0.248	0.525
Transmission efficiency			
	Estimate	Std. Error	P-value
Temperature	-0.056	1416	0.969
Dpe	0.144	0.607	0.812
Virus load	-1.096	1391	0.431
Temperature*dpe	1194	1121	0.287
Temperature*virus load	5628	3831	0.142
Dpe*virus load	0.305	0.213	0.153

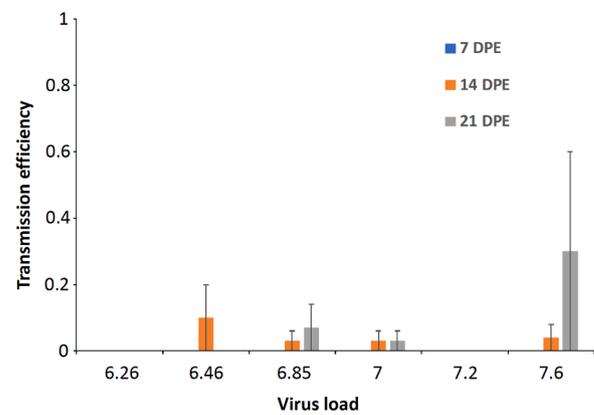


Fig. 3. Transmission efficiency (proportion) of the mosquitoes analyzed in the different experiments based on the virus load used. Bars = SE.

differences in the experimental conditions used. Viral load was a major factor affecting both the infection and transmission rates of ZIKV in studies on European *Ae. albopictus*, although their effects were also affected by their interactions with other factors including dpe and temperature.

The effects of viral load on experimental infections designed to determine the competence of mosquitoes for the transmission of virus is well studied (Tesla et al., 2018b; Chouin-Carneiro et al., 2020). After feeding on infected blood, the virus spreads from the stomach through the body of the mosquito. The experimental infection of *Ae. aegypti* with dengue virus (DENV) indicated that virus concentration in the infectious dose was a significant predictor of the proportion of infected mosquitoes (Pongsiri et al., 2014). For example, Novelo et al. (2019) demonstrated that mosquitoes inoculated with a high viral dose had higher infection rates immediately after the first days post-inoculation, while mosquitoes inoculated with lower viral dose required more time to reach similar infection rates. Different viral loads have been used in the experiment to determine the competence of mosquitoes for the transmission of ZIKV. For example, previous studies exposing *Ae. aegypti* and *Ae. albopictus* to ZIKV in USA used a minimum dose of 4 and 5.3 Log₁₀ PFU/mL, respectively (Ciota et al., 2017; Tesla et al., 2018b). For the studies considered here, virus concentration ranged between 6.26 and 7.6 Log₁₀ PFU/mL (Table 1), which may have affected the conclusions obtained. In fact, these values are much higher than those found in ZIKV-infected patients which usually vary from 2.9 to 5.86 Log₁₀ PFU/mL (Lanciotti et al., 2008). The aim of most of the reviewed studies was to test the capacity of *Ae. albopictus* to transmit ZIKV in Europe. Thus, an appropriate response to this question should use virus concentrations in the range occurring in human infections as, otherwise, the results will be of little practical use and, according to our findings, may overestimate the capacity of *Ae. albopictus* for ZIKV transmission. Although Lequime et al. (2020) found that the mosquito infection rate increased as a function of the infectious blood meal dose independently of the time post-exposure, the analyses conducted here support the importance of the effects of viral load together with dpe on infection rates in *Ae. albopictus* mosquitoes exposed to ZIKV. Mosquitoes exposed to a higher viral load had a higher infection rate at lower dpe, while the infection rates of mosquitoes exposed to a lower viral load increased as the dpe increased. If a mosquito is exposed to a higher viral load, the probability that it will develop viraemia earlier is higher than if the mosquito is exposed to a lower virus load. However, although Tesla et al. (2018b) found no significant effects of ZIKV dose on *Ae. aegypti* mortality, studies with other mosquito-borne pathogens, including other flaviviruses, strongly support the detrimental effects of infections on mosquito survival (Lambrechts and Scott, 2009; Kramer and Ciota, 2015; Gutiérrez-López et al., 2019).

This potential negative effect on survival suggests that mosquitoes

receiving a higher viral load may show virus development earlier (dpe), which will have long-term deleterious effects on mosquito survival and reduce their lifespan, resulting ultimately in a reduced percentage of infected individuals in the samples collected more than two weeks post-exposure. The same reason may explain - at least in part - the negative association between the viral load and the transmission rate. Consequently, it is advisable to monitor the mortality rate of mosquitoes in studies that attempt to analyze the vectorial competence for different pathogens or that use differing inoculation doses or mosquito growing conditions.

We found that the interaction between viral load and temperature determined the transmission rate of ZIKV by *Ae. albopictus*. Temperature can have a limiting effect on the development of ZIKV in mosquitoes through its effects on the mosquito metabolism and/or on the virus cycle (Watts et al., 1987). As evidence for this possibility, none of the studies analysed here that used temperatures equal or lower than 25 °C found ZIKV in the saliva of mosquitoes, regardless of their origin (see Table 1, Heitmann et al., 2017; Hernández-Triana et al. 2019). Considering this limiting effect of temperature, our results suggest that an increase in temperature increases the transmission rate in mosquitoes exposed to higher viral load but also reduces the transmission rate in mosquitoes exposed to lower viral load, although this latter result may be an artifact related to the relative low number of data points and how the interactions between continuous variables are fitted in the models. Temperature largely affects vector competence as various studies of different mosquito-virus systems have shown (Heitmann et al., 2017; Tesla et al., 2018a; Chouin-Carneiro et al., 2020). In fact, the median time from ZIKV ingestion to transmission by *Ae. aegypti* at a temperature of 20 °C is 24.2 days, while, this time gets reduced to 9.6 and 5.1 days when the temperature is 26 °C and 30 °C, respectively (Winekur et al., 2020). In addition, Tesla et al. (2018a), estimated 30.6 °C as the optimum temperature for the vector competence of *Ae. aegypti* for the transmission of ZIKV, with an estimated thermal minimum and maximum of 22.9 °C and 38.4 °C, respectively. Nevertheless, studies with *Ae. albopictus* have found that ZIKV needs a higher temperature if it is to be transmitted by this mosquito species. Thus, differences between these two mosquito species in their vector competence have been observed to depend on factors such as temperature or virus load. Chouin-Carneiro et al. (2020) found that both virus load and temperature affect the transmission rate of ZIKV by Brazilian *Ae. aegypti* and *Ae. albopictus*. *Aedes aegypti* was able to transmit ZIKV at a temperature of 22 °C with a virus load of 4 Log₁₀ PFU/mL, while *Ae. albopictus* only was able to transmit ZIKV at temperatures over 28 °C with higher virus loads (6 Log₁₀ PFU/mL). In addition, the transmission rate obtained by Chouin-Carneiro et al. (2020) in *Ae. albopictus* was only 18.75%, similar to the transmission rates obtained for *Ae. albopictus* in Europe (Heitmann et al., 2017; Gutiérrez-López et al., 2019). Although all European studies used virus loads above 6 Log₁₀ PFU/mL, the temperatures used during the development of the experiment varied from 18 °C to 28 °C. This factor could explain why studies such as Hernández-Triana et al. (2019) – who used temperatures below 26 °C - did not find evidence of virus transmission despite using a high virus load (7.2 Log₁₀ PFU/mL). Thus, when analyzing the effects of other variables on *Ae. albopictus* competence for ZIKV, we suggest using a temperature range between 27–29 °C and monitoring infection parameters until at least 21 dpe, when more mosquitoes have virus in their saliva. Our results show that both virus load and temperature should be considered together when studying the transmission rate of ZIKV by mosquitoes. In particular more studies controlling for temperature need to be undertaken to study the vectorial competence at doses below 6 Log₁₀ PFU/mL. In addition, more research is required to clarify the effects of temperature with specific attention paid to the upper range given the upwards trends and variations in summer temperature in Europe. Temperatures up to 28 °C maybe relevant for mosquitoes in northern Europe but for understanding virus amplification in southern Europe experiments temperatures well over this value may be necessary, especially given the often-assumed

negative effects of high temperature on viral replication and the lack of experiments in high temperature ranges (Shocket et al., 2020). In addition, other factors not considered in our study, including variation in the gene expression or microbiota alteration due to the virus load and/or temperature, may also affect vector competence (Ferreira et al., 2020; do Nascimento et al., 2022), although the mechanism affecting these associations are still unclear. These results may have epidemiological consequences and should be considered with care in the development of predictive models of ZIKV transmission in Europe (Guzzeta et al., 2016; Solimini et al., 2018; Rocklov et al., 2016), as has previously been the case for other mosquito-borne viruses (Shocket et al., 2020).

Although our results provide valuable information on the potential factors determining reported discrepancies in the role played by European *Ae. albopictus* in ZIKV transmission, we cannot rule out the possibility that conclusions obtained here were partially affected by the low sample size of some published studies. Specifically, the number of mosquito specimens analyzed in the studies reviewed here ranged from 7 to 125 individuals for the different treatments. However, to obtain reliable estimates of transmission rate it is advisable to use at least 25 engorged mosquitoes per treatment, thereby reducing the error in estimates and increasing the statistical power of the analyses (Jovani and Tella, 2006). A low sample size may compromise the estimates obtained for infection and transmission rates. As well, although we only focused on the Asian ZIKV lineages, previous studies on *Ae. albopictus* suggest the existence of large-scale geographical variation in the competence of this species for ZIKV transmission, with East Asian/Oceanic mosquito populations showing the highest transmission rate for ZIKV (Ciota et al., 2017; McKenzie et al., 2019). This pattern could be due to the greater length of time ZIKV and *Ae. albopictus* populations have been interacting in East Asia and Oceania, which has allowed ZIKV to adapt to this vector species (Weaver et al., 2016). European populations of *Ae. albopictus* originated from at least three independent introduction events, one in Albania, and two in Italy, from where the species subsequently spread to countries in western and central Europe (Sherpa et al., 2019). It seems unlikely that the rapid spread of *Ae. albopictus* in Europe has resulted in genetic differences in vectorial competence to ZIKV and further studies should be bear in mind that small sample size may limit the capacity for statistically testing for such geographical differences in ZIKV susceptibility. Temperature appears to be an important factor affecting vectorial competence and so the impact of temperature-related effects on vectorial competence must be clarified – along with its consequences for ZIKV transmission in Europe – in light of ongoing climate change.

5. Conclusions

European populations of *Ae. albopictus* are competent vectors for ZIKV as demonstrated by multiple experimental studies and by the three cases of autochthonous transmission of ZIKV in southern France (Giron et al., 2019). However, based on results from this study, future research should use standardized conditions to compare the results obtained in different laboratories. The analyses conducted here based on published information revealed that methodological differences (i.e., viral load and temperature) may explain, at least in part, the different competences for the transmission of ZIKV by European *Ae. albopictus* mosquitoes. Although statistically replicated experimental infections are necessary to clarify the impact of temperature and infection dose on vectorial competence parameters, existing experimental evidence does indicate that European populations of *Ae. albopictus* are competent vectors of ZIKV. In addition, more assays are required with doses closer to the range found in humans and at temperatures covering the full range of average summer temperatures in Europe to gain a full understanding of the epidemic potential of *Ae. albopictus* for ZIKV transmission in areas where *Ae. aegypti* is absent.

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CRedit authorship contribution statement

Rafael Gutiérrez-López: Conceptualization, Methodology, Data curation, Writing – original draft. **Jordi Figuerola:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Josué Martínez-de la Puente:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability statement

No original research data.

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