1. **Risk of dengue in Central Africa: vector competence studies with *Aedes aegypti* and *Aedes***

# *albopictus* (Diptera: Culicidae) populations and dengue 2 virus.

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# Abstract

1. **Introduction:** Dengue is the most important mosquito-borne diseases worldwide but was
2. considered scarce in West-Central Africa. During the last decade, dengue outbreaks have
3. increasingly been reported in urban foci in this region suggesting major epidemiological changes.
4. However, in Central Africa where both vectors, *Aedes aegypti* and *Aedes albopictus* are well
5. established, the role of each species in dengue transmission remains poorly investigated.
6. **Methodology/Principal findings:** Field-collected strains of *Ae. aegypti* and *Ae. albopictus* from
7. different ecological settings in Central Africa were experimentally challenged with dengue 2
8. virus (DENV-2). Mosquitoes were analysed at 14- and 21-days post-infection. Analysis provide
9. evidence that both *Ae. aegypti* and *Ae. albopictus* in Central Africa were able to transmit dengue
10. virus with *Ae. aegypti* exhibiting a higher transmission rate. Unexpectedly, two *Ae. aegypti*
11. populations from Bénoué and Maroua, in northern Cameroon, were not able to transmit DENV-2.
12. **Conclusions/Significance:** We conclude that both *Ae. aegypti* and *Ae. albopictus* are susceptible
13. to DENV-2 and may intervene as active dengue vectors. These findings highlight the urgent need
14. to plan a vector surveillance program and control methods against dengue vectors in Central
15. Africa in order to prevent future outbreaks.
16. **Key words:** *Aedes aegypti*, *Aedes albopictus*, dengue virus, vector competence, Central Africa.

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# Author summary

1. Dengue virus (DENV) is a flavivirus mainly transmitted to humans through the bite of infected
2. mosquitoes notably *Aedes aegypti* and *Aedes albopictus*. In Central Africa where both vectors,
3. *Ae. aegypti* and *Ae. albopictus* are well established, the role of each species in dengue
4. transmission remains poorly investigated. Here, we assessed the vector competence of *Ae.*
5. *aegypti* and *Ae. albopictus* collected in different ecological settings in Central Africa to transmit
6. dengue 2 virus (DENV-2). We provide evidence that both *Ae. aegypti* and *Ae. albopictus* in
7. Central Africa were able to transmit dengue virus with *Ae. aegypti* exhibiting a higher
8. transmission rate. These findings could increase the risk of dengue outbreak in the region and
9. emphasize the need for a comprehensive vector surveillance program to prevent and preparedness
10. for an intervention in case of outbreaks.

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# Introduction

1. Dengue is one of the most important arboviral diseases in the world with nearly 390 million
2. annual dengue infections and 96 million (67–136 million) clinical cases [[1](#_bookmark0)]. Dengue is caused by
3. a dengue virus (DENV) belonging to the genus *Flavivirus* (family *Flaviviridae*). There are four
4. distinct, but closely related serotypes of dengue (DENV-1, DENV-2, DENV-3 and DENV-4).
5. DENV is transmitted to humans through the bite of infected *Aedes* mosquitoes primarily *Aedes*
6. *aegypti* Linneaus 1772 and *Aedes albopictus* (Skuse 1894).
7. In Africa, the situation of dengue was less critical as human cases were mainly associated with
8. mild symptoms [[2,](#_bookmark1)[3](#_bookmark2)]. Haemorrhagic syndromes were only reported in East Africa [[4,](#_bookmark3)[5](#_bookmark4)].
9. However, dengue outbreaks have been reported recently in some West-Central African countries
10. [[6-10](#_bookmark5)] suggesting a switch in the epidemiological dynamics of dengue. The two invasive species,
11. *Ae. aegypti* and *Ae. albopictus* are well established in Africa. While *Ae. aegypti* native from
12. Africa took 400-500 years to invade the tropical belt [[11](#_bookmark8)[,12](#_bookmark9)], *Ae. albopictus* originated from
13. Asian forests has colonized all five continents in less than four decades [[13](#_bookmark10)[,14](#_bookmark11)]. *Aedes albopictus*
14. has been first reported in Central Africa in early 2000s in Cameroon [[15](#_bookmark12)], and since then, this
15. species has invaded almost all countries of the region including the Republic of Congo [[16-18](#_bookmark13)].
16. In sympatric areas, *Ae. albopictus* outcompetes with the native species *Ae. aegypti* [[18-21](#_bookmark14)].
17. Coincidentally, the emergence of arboviral diseases such as dengue and chikungunya in Central
18. Africa has coincided with the establishment of *Ae. albopictus* in this region. Indeed, *Ae.*
19. *albopictus* was identified as the main vector during concurrent dengue/chikungunya outbreak in
20. Gabon in 2007 [[8](#_bookmark6)[,22](#_bookmark16)], and in Cameroon in 2006 [[23](#_bookmark17)]. During the last two decades, DENV-1 and
21. DENV-2 mainly, were circulating in Cameroon [[24-29](#_bookmark18)]. Nationwide surveillance of dengue in
22. 2006/2007 only revealed that seroprevalence (IgG and IgM antibodies) was higher in Douala
23. [[29](#_bookmark20)]. In the neighbouring country of Republic of Congo, only little information is known about
24. dengue circulation. The vector competence (which refers to the potential of an arthropod to ingest
25. the pathogen, ensure replication, dissemination and transmission) which is one of the main
26. factors required to establish the epidemiological role of mosquitoes in transmission is poorly
27. studied in Central Africa. Previous studies only focused on infection and dissemination rates
28. [[8](#_bookmark6)[,30](#_bookmark21)[,31](#_bookmark22)] and not transmission potential (i.e. virus detection in mosquito saliva). To fill this
29. important gap, we performed a comparative analysis aiming to assess the ability of *Ae. aegypti*
30. and *Ae. albopictus* collected in different ecological settings in Central Africa to transmit DENV-

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# 101 Materials and Methods

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# Ethics statement

1. This study was approved by the Cameroonian national ethics committee for human health
2. research N˚2017/05/911/CE/CNERSH/SP. Oral consent to inspect the potential breeding sites
3. was obtained in the field in household or garage owners. The Institut Pasteur animal facility has
4. received accreditation from the French Ministry of Agriculture to perform experiments on live
5. animals in compliance with the French and European regulations on care and protection of
6. laboratory animals (EC Directive 2010/63, French Law 2013-118, February 6th, 2013). All
7. experiments were approved by the Ethics Committee and registered under the reference

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# Mosquito sampling

1. Larvae and pupae were collected from August 2017 to April 2018 in several locations in Central
2. Africa including Brazzaville (Republic of the Congo), Yaoundé, Douala, Tibati and Bénoué
3. National Park (Cameroon, Fig. 1). Each of these locations have been previously characterised
4. [[18](#_bookmark14)[,19](#_bookmark15)]. In each location, mosquitoes were collected in peri-urban (i.e. peripheral area of the city*)*
5. and downtown (i.e. city centre with high building density) from a minimum of 20 containers per
6. environment. Immature stages of *Aedes* were transported in the insectary and pooled together
7. according to the city. Larvae were raised until adults and identified morphologically. Adults from
8. same location and species were reared at 28°±1°C under 12h dark:12h light cycle and 80%
9. relative humidity . Eggs obtained (Table 1) were transported to the Institut Pasteur Paris, reared
10. to adult stage and used to challenge with DENV-2.

# Virus strain

1. The dengue 2 virus (DENV-2) strain provided by Leon Rosen (Institut Pasteur, Paris, France)
2. was isolated in 1974 from a human sera from Bangkok, Thailand [[32](#_bookmark23)]. This virus had been
3. passed only in different mosquito species (*Toxorhynchites amboinensis*, *Ae. albopictus*, and *Ae.*
4. *aegypti*) by intrathoracic inoculation. Viral stocks were produced by inoculating *Ae. albopictus*
5. cells (C6/36 clone) with triturated infected mosquitoes.

# Challenging mosquitoes with DENV-2

1. For each sample, six batches of 60 7-10 day old females were challenged with an infectious blood
2. meal (Institut Pasteur, Paris, France) containing 1.4 mL of washed rabbit erythrocytes and 700 μL
3. of viral suspension. The blood meal was supplemented with adenosine 5’-triphosphate (ATP) as a
4. phagostimulant at a final concentration of 1 mM and provided to mosquitoes at a viral titre of 107
5. focus-forming unit (ffu)/mL using a Hemotek membrane feeding system (Hemotek Ltd,
6. Blackburn, United Kingdom**)**. Mosquitoes were allowed to feed for 20 min through a piece of
7. pork intestine (Institut Pasteur, Paris, France) covering the base of a Hemotek feeder maintained
8. at 37°C. Fully engorged females were transferred in cardboard containers and maintained with
9. 10% sucrose under controlled conditions (28±1°C, relative humidity of 80%, light: dark cycle of
10. 12 h: 12 h) for up to 21 days with mosquito analysis 14 and 21 days post-infection (dpi). 21–32
11. mosquitoes were examined at each dpi.

# Infection, dissemination and transmission assays

1. For each mosquito examined, body (abdomen and thorax) and head were tested respectively for
2. infection and dissemination rates at 14 and 21 dpi. For this, each part was ground individually in
3. 300 μL of L15 medium (Invitrogen, CA, USA) supplemented with 2% fetal serum bovine (FBS),
4. and centrifuged at 10,000×g for 5 min at +4°C. The supernatant was processed for viral titration.
5. Saliva was collected from individual mosquitoes using technique of forced salivation as
6. described previously [[33](#_bookmark24)]. Briefly, mosquitoes were cool anesthetized, wings and legs of each
7. mosquito were removed and the proboscis inserted into a tip of 20 µL containing 5 µL of FBS.
8. After 30 min, FBS containing saliva was mixed in 45 µL of L15 medium for titration.
9. Infection rate (IR) refers to the proportion of mosquitoes with infected body (i.e. abdomen and
10. thorax) among tested mosquitoes. Disseminated infection rate (DIR) corresponds to the
11. proportion of mosquitoes with infected head among the previously detected infected mosquitoes
12. (i.e. virus positive abdomen/thorax). Transmission rate (TR) represents the proportion of
13. mosquitoes with infectious saliva among mosquitoes with disseminated infection. Vector
14. competence can be summarized by the transmission efficiency (TE) which was calculated as the
15. proportion of mosquitoes with infectious saliva among total of mosquitoes tested [[34](#_bookmark25)].

# Viral titration by focus forming assay

1. Samples were titrated by focus fluorescent assay on *Ae*. *albopictus* C6/36 cells [[35](#_bookmark26)]. Body, head
2. and saliva suspensions were serially diluted in L15 medium supplemented with 2% of FBS and
3. inoculated onto cells in 96-well plates. After incubation of 5 days at 28°C, samples were fixed
4. with 0.1mL/well of formaldehyde 3.6% in phosphate buffer saline (PBS) during 20 min at room
5. temperature. Then, plates were stained using antibodies specific to DENV as the primary
6. antibody, and conjugated goat anti-mouse immunoglobulin G (Alexa Fluor 488) as the second
7. antibody (Life Technologies, CA, USA). Titres were expressed as ffu/mL.

# Statistical analysis

1. All statistical analyses were performed with R software v 3.5.2 (R Core Team, Vienna, Austria).
2. Qualitative variables were expressed as proportion and compared using Fisher’s exact test
3. (RVAideMemoire package). While quantitative variables were described as mean and compared
4. using non-parametric test of Kruskal-Wallis because of non-normal distribution. Pairwise
5. comparison were performed using Fisher’s exact test for proportions and Kruskal-wallis test for
6. means. *P-value* <0.05 was considered as statistically different.

# Result

1. **Infection and disseminated infection rates in *Ae. albopictus* and *Ae. aegypti***
2. To determine if *Ae. aegypti* (six populations) or *Ae. albopictus* (four populations) were more
3. likely to sustain DENV outbreak in Central Africa, the ability of the virus to replicate and
4. disseminate in both species was examined at 14 and 21 dpi as well as DENV particles excreted in
5. saliva (only at 21 dpi) (Fig. 2 and 3). At 14 dpi, *Ae. albopictus* infection rate (IR) ranged from
6. 33.3% in Douala population to 68.4% in Yaoundé urban population but no statistical difference
7. was detected (Fig. 2A, Fisher’s Exact test: *P*=0.16). For DIRs, similar trend was observed with
8. lowest rate in Douala population (14.3%) and highest in Brazzaville population (41.6%) (Fig. 2A,
9. Fisher’s Exact test: *P*=0.47). While for *Ae. aegypti,* results exhibited higher IRs ranging from
10. 70.83% for Maroua to 100% for Douala populations and DIRs varying from 58.82% for Maroua
11. to 100% for Douala populations. When considering all populations of same species, IRs for *Ae.*
12. *aegypti* (mean=76.61%) was significantly higher than for *Ae. albopictus* (mean=51.76%)
13. (Fisher’s exact test: *P*=0.0003). Similar pattern was found for DIRs (*Ae. aegypti:* mean=83.15%
14. and *Ae. albopictus:* mean=27.27%) (Fig. 3A, Fisher’s exact test: *P*<10-6).
15. At 21 dpi, *Ae. albopictus* displayed higher IRs ranging from 50% for Douala population to 83.3%
16. for Yaoundé urban and were not significantly different (Fisher exact test: *P*=0.06). But pairwise
17. comparisons showed that significant difference was found between Douala and Yaoundé urban
18. (Fisher’s exact test: *P*=0.03), Tibati and Yaoundé urban (Fisher’s exact test: *P*=0.03). Higher
19. DIRs was also reported: it varied from 70% for Yaoundé urban population to 91.66% for Douala
20. population but no significant difference was found according to population (Fisher’s exact test:
21. *P*=0.52). For *Ae. aegypti,* IRs ranged from 70.83% for Bénoué population to 95.83% for
22. Brazzaville and Douala populations and were not statistically different (Fisher exact test:
23. *P*=0.06). In contrast, a higher significant variation of DIRs was reported: it ranged from 41.17%
24. for Bénoué population to 95.65% for Brazzaville population (Fisher exact test: *P*<10-6). Overall,
25. IRs for *Ae. aegypti* (mean= 81.94%) were significantly higher than for *Ae. albopictus*
26. (mean=61.05%) (Fisher exact test: *P=*0.0005). For DIR, no significant difference was found
27. between *Ae. aegypti* and *Ae. albopictus* population (Fisher exact test: *P*=0.45)

# Transmission rate and efficiency

1. Transmission rate (TR) and Transmission efficiency (TE) were assessed at 21 dpi in four *Ae.*
2. *albopictus* and six *Ae. aegypti* populations (Fig. 2B and 3B). In *Ae. albopictus*, DENV was
3. detected in saliva of four populations with TRs ranging from 9.1% (1/11) for Douala to 50%
4. (5/10) for Tibati populations; TRs were not statistically different (Fig. 2B, Fisher exact test:
5. *P*=0.2). In contrast, for *Ae. aegypti,* DENV was not detected in saliva of Maroua and Bénoué
6. populations, both located in northern Cameroon suggesting a low vector competence of these
7. populations. For the other *Ae. aegypti* populations, TR ranged from 21.42% for Yaoundé urban
8. population to 50% for Douala population (Fig 3B, Fisher exact test: *P*=0.4). Overall, no
9. significant difference was reported among *Ae. aegypti* and *Ae. albopictus* regarding TRs and TEs
10. (Fisher exact test: *P*>0.05). When comparing populations from sympatric areas, TRs were
11. significantly higher for *Ae. aegypti* (mean=50%) than for *Ae. albopictus* (mean=27.7%) (Fisher
12. exact test: *P*=0.007) while for viral load, no significant difference was reported between both
13. species (Chi-squared=0.14, df=1, *P*=0.70). For *Ae. aegypti,* no significant variation of viral loads
14. was reported according to population (Fig.4; Chi-squared=0.29, df=3, *P*=0.96) while for *Ae.*
15. *albopictus,* a significant difference of viral loads was detected between Tibati and Brazzaville
16. samples (Fig.4; Chi-squared=2.31, df=1, *P*=0.018).

# Discussion

1. During the past decade, there has been a rise of dengue cases in urban foci in Central Africa
2. notably in Cameroon [[26-29](#_bookmark19)]. Even suspected, vectors were not well identified and characterised.
3. In this study, we assessed for the first time, the ability of *Ae. aegypti* and *Ae. albopictus* collected
4. in different ecological settings (Fig. 1) in Central Africa to transmit DENV-2, a serotype
5. repeatedly reported in the region [[9,](#_bookmark7)[29](#_bookmark20)]. We demonstrated that DENV-2 was able to replicate,
6. disseminate and be secreted in saliva of both *Ae. aegypti* and *Ae. albopictus* populations from
7. Central Africa, thus enable to transmit DENV. However, infection rates were significantly higher
8. for *Ae. aegypti* than for *Ae. albopictus* at 14 and 21 dpi. Disseminated infection rates followed the
9. same trend at 14 dpi. Nevertheless, DENV was detected in saliva of all *Ae. albopictus*
10. populations tested while for *Ae. aegypti*, virus was not detected in both populations (2/6) from
11. northern Cameroon, Bénoué and Maroua. These results suggest that vector competence of *Ae.*
12. *aegypti* to DENV-2 in Central Africa vary significantly according to geographical population as
13. previously suggested elsewhere [[36,](#_bookmark27)[37](#_bookmark28)]. This may due to the fact that populations from Bénoué
14. and Maroua exhibited an extrinsic incubation period longer than 21 days; to note, the extrinsic
15. incubation period refers to the duration between the ingestion of an infectious blood meal and the
16. excretion of virus in saliva when the mosquito bites [[38](#_bookmark29)]. It depends on the three-way
17. combination of mosquito, virus and environment described under genotype-by-genotype-by-
18. environment (GxGxE) interactions [[39](#_bookmark30)]. In addition, low vector competence in these populations
19. would be due to presence of specific refractory genes [[40](#_bookmark31)[,41](#_bookmark32)]. Indeed, refractoriness of mosquito
20. to dengue virus may be caused by different parameters like microbiome composition as bacterial
21. symbionts of mosquitoes have been shown to alter the vector competence to arboviruses [[42](#_bookmark33)] and
22. immune system of mosquito since it was demonstrated that anti-viral immunity in mosquito
23. vectors is critical to prevent virus replication and transmission [[43](#_bookmark34)]. Further investigations in this
24. regard are needed to elucidate.
25. Moreover, the seroprevalence of dengue examined in 2006/2007 in three main cities of
26. Cameroon located in different ecological settings revealed that anti-DENV IgG and IgM
27. antibodies varied significantly with a higher prevalence reported in Douala [[29](#_bookmark20)], location where
28. the highest transmission rate and viral load were also detected in *Ae. aegypti* in this study. Beside
29. the mosquito genetic background, mosquito microbiome can modulate arbovirus transmission
30. [[42](#_bookmark33)[,44](#_bookmark35)[,45](#_bookmark36)]. The transmission rate was higher for *Ae. aegypti* compared to *Ae. albopictus* in
31. locations where both species are sympatric. This result is in agreement with the fact that *Ae.*
32. *aegypti* is considered as a major dengue vector, and *Ae. albopictus,* the secondary one [[46](#_bookmark37)].
33. Meanwhile, it would be interesting to highlight that *Ae. albopictus* can become a major dengue
34. vector in the absence of *Ae. aegypti* as reported previously in China, the Seychelles, Japan,
35. Hawaii and on La Réunion [[47](#_bookmark38)] or when *Ae. albopictus* becomes the most prevalent species as
36. reported in Gabon [[8](#_bookmark6)]. Nevertheless, infection and disseminated infection rates assessed for *Ae.*
37. *albopictus* in this study are similar to those reported in previous studies in Africa [[8,](#_bookmark6)[31](#_bookmark22)] and in
38. Southeast Asia [[48](#_bookmark39)]. For *Ae. aegypti*, infection and disseminated infection rates are higher
39. compared to that previously reported in Cameroon (17.2% to 59.7%) but similar to that often
40. reported outside Africa [[37](#_bookmark28)[,48](#_bookmark39)]. Albeit *Ae. aegypti* is more competent than *Ae. albopictus* to
41. transmit DENV, some parameters can influence DENV transmission in nature, such as vector
42. densities, host preference, virus evolution and proportion of immunologically naive people [[49](#_bookmark40)].
43. Additional studies using a local strain of DENV circulating in Central Africa are needed to
44. validate these results. Regarding vector densities, recent studies in Cameroon and Republic of
45. Congo revealed that *Ae. albopictus* tends to replace *Ae. aegypti* in most areas where both species
46. are sympatric [[18,](#_bookmark14)[19](#_bookmark15)]. It was also demonstrated that in Yaoundé (Cameroon) *Ae. albopictus*
47. preferentially fed on humans rather than on available domestic animals [[50](#_bookmark41)] Data generated in
48. our study demonstrated that both *Ae. aegypti* and *Ae. albopictus* can sustain dengue transmission
49. in Central Africa. This could increase the risk of dengue outbreak in the region and urge the need
50. of a vector surveillance program to prevent and preparedness for an intervention in case of
51. outbreaks.

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# References

1. 1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global distribution
2. and burden of dengue. Nature 496: 504-507.
3. 2. Gonzalez JP, Fiset P, Georges AJ, Saluzzo JF, Wisseman CL, Jr. (1985) [Serological approach
4. to the occurrence of rickettsioses in the Central African Republic]. Bull Soc Pathol Exot

281 Filiales 78: 153-156.

1. 3. Saluzzo JF, Cornet M, Adam C, Eyraud M, Digoutte JP (1986) [Dengue 2 in eastern Senegal:
2. serologic survey in simian and human populations. 1974-85]. Bull Soc Pathol Exot

284 Filiales 79: 313-322.

1. 4. Kanesa-thasan N, Chang GJ, Smoak BL, Magill A, Burrous MJ, et al. (1998) Molecular and
2. epidemiologic analysis of dengue virus isolates from Somalia. Emerg Infect Dis 4: 299-

287 303.

1. 5. Rodier GR, Gubler DJ, Cope SE, Cropp CB, Soliman AK, et al. (1996) Epidemic dengue 2 in
2. the city of Djibouti 1991-1992. Trans R Soc Trop Med Hyg 90: 237-240.
3. 6. Schwartz E, Meltzer E, Mendelson M, Tooke A, Steiner F, et al. (2013) Detection on four
4. continents of dengue fever cases related to an ongoing outbreak in Luanda, Angola,
5. March to May 2013. Euro Surveill 18.
6. 7. Franco L, Di Caro A, Carletti F, Vapalahti O, Renaudat C, et al. (2010) Recent expansion of
7. dengue virus serotype 3 in West Africa. Euro Surveill 15.
8. 8. Paupy C, Ollomo B, Kamgang B, Moutailler S, Rousset D, et al. (2010) Comparative role of
9. *Aedes albopictus* and *Aedes aegypti* in the emergence of Dengue and Chikungunya in
10. central Africa. Vector Borne Zoonotic Dis 10: 259-266.
11. 9. Leroy EM, Nkoghe D, Ollomo B, Nze-Nkogue C, Becquart P, et al. (2009) Concurrent
12. chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007.
13. Emerg Infect Dis 15: 591-593.
14. 10. Tarnagda Z, Cisse A, Bicaba BW, Diagbouga S, Sagna T, et al. (2018) Dengue Fever in
15. Burkina Faso, 2016. Emerg Infect Dis 24: 170-172.
16. 11. Powell JR, Tabachnick WJ (2013) History of domestication and spread of *Aedes aegypti*--a
17. review. Mem Inst Oswaldo Cruz 108 Suppl 1: 11-17.
18. 12. Powell JR (2016) Mosquitoes on the move. Science 354: 971-972.
19. 13. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009) *Aedes albopictus*, an arbovirus
20. vector: from the darkness to the light. Microbes Infect 11: 1177-1185.
21. 14. Benedict MQ, Levine RS, Hawley WA, Lounibos LP (2007) Spread of the tiger: global risk
22. of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonotic Dis 7: 76-85.
23. 15. Fontenille D, Toto JC (2001) *Aedes (Stegomyia) albopictus* (Skuse), a potential new Dengue
24. vector in southern Cameroon. Emerg Infect Dis 7: 1066-1067.
25. 16. Ngoagouni C, Kamgang B, Nakoune E, Paupy C, Kazanji M (2015) Invasion of *Aedes*
26. *albopictus* (Diptera: Culicidae) into central Africa: what consequences for emerging
27. diseases? Parasit Vectors 8: 191.
28. 17. Weetman D, Kamgang B, Badolo A, Moyes CL, Shearer FM, et al. (2018) *Aedes* Mosquitoes
29. and *Aedes*-Borne Arboviruses in Africa: Current and Future Threats. Int J Environ Res
30. Public Health 15.
31. 18. Kamgang B, Wilson-Bahun TA, Irving H, Kusimo MO, Lenga A, et al. (2018) Geographical
32. distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and genetic
33. diversity of invading population of *Ae. albopictus* in the Republic of the Congo.
34. Wellcome Open Res 3: 79.
35. 19. Tedjou AN, Kamgang B, Yougang AP, Njiokou F, Wondji CS (2019) Update on the
36. geographical distribution and prevalence of *Aedes aegypti* and *Aedes albopictus* (Diptera:
37. Culicidae), two major arbovirus vectors in Cameroon. PLoS Negl Trop Dis 13: e0007137.
38. 20. Kamgang B, Yougang AP, Tchoupo M, Riveron JM, Wondji C (2017) Temporal distribution
39. and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes*
40. *albopictus* in Yaounde, the capital city of Cameroon. Parasit Vectors 10: 469.
41. 21. Kamgang B, Ngoagouni C, Manirakiza A, Nakoune E, Paupy C, et al. (2013) Temporal
42. patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and
43. mitochondrial DNA analysis of *Ae. albopictus* in the Central African Republic. PLoS
44. Negl Trop Dis 7: e2590.
45. 22. Pages F, Peyrefitte CN, Mve MT, Jarjaval F, Brisse S, et al. (2009) Aedes albopictus
46. mosquito: the main vector of the 2007 Chikungunya outbreak in Gabon. PLoS One 4:

334 e4691.

1. 23. Peyrefitte CN, Rousset D, Pastorino BA, Pouillot R, Bessaud M, et al. (2007) Chikungunya
2. virus, Cameroon, 2006. Emerg Infect Dis 13: 768-771.
3. 24. Ndip LM, Bouyer DH, Travassos Da Rosa AP, Titanji VP, Tesh RB, et al. (2004) Acute
4. spotted fever rickettsiosis among febrile patients, Cameroon. Emerg Infect Dis 10: 432-

339 437.

1. 25. Kuniholm MH, Wolfe ND, Huang CY, Mpoudi-Ngole E, Tamoufe U, et al. (2006)
2. Seroprevalence and distribution of *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* arboviral
3. infections in rural Cameroonian adults. Am J Trop Med Hyg 74: 1078-1083.
4. 26. Yousseu FBS, Nemg FBS, Ngouanet SA, Mekanda FMO, Demanou M (2018) Detection and
5. serotyping of dengue viruses in febrile patients consulting at the New-Bell District
6. Hospital in Douala, Cameroon. PLoS One 13: e0204143.
7. 27. Nemg Simo FB, Sado Yousseu FB, Evouna Mbarga A, Bigna JJ, Melong A, et al. (2018)
8. Investigation of an Outbreak of Dengue Virus Serotype 1 in a Rural Area of Kribi, South
9. Cameroon: A Cross-Sectional Study. Intervirology 61: 265-271.
10. 28. Monamele GC, Demanou M (2018) First documented evidence of dengue and malaria co-
11. infection in children attending two health centers in Yaounde, Cameroon. Pan Afr Med J

351 29: 227.

1. 29. Demanou M, Pouillot R, Grandadam M, Boisier P, Kamgang B, et al. (2014) Evidence of
2. dengue virus transmission and factors associated with the presence of anti-dengue virus
3. antibodies in humans in three major towns in Cameroon. PLoS Negl Trop Dis 8: e2950.
4. 30. Vazeille-Falcoz M, Failloux AB, Mousson L, Elissa N, Rodhain F (1999) [Oral receptivity of
5. *Aedes aegypti formosus* from Franceville (Gabon, central Africa) for type 2 dengue virus].
6. Bull Soc Pathol Exot 92: 341-342.
7. 31. Vazeille M, Moutailler S, Pages F, Jarjaval F, Failloux AB (2008) Introduction of *Aedes*
8. *albopictus* in Gabon: what consequences for dengue and chikungunya transmission? Trop

360 Med Int Health 13: 1176-1179.

1. 32. Vazeille-Falcoz M, Mousson L, Rodhain F, Chungue E, Failloux AB (1999) Variation in oral
2. susceptibility to dengue type 2 virus of populations of *Aedes aegypti* from the islands of
3. Tahiti and Moorea, French Polynesia. Am J Trop Med Hyg 60: 292-299.
4. 33. Dubrulle M, Mousson L, Moutailler S, Vazeille M, Failloux AB (2009) Chikungunya virus
5. and *Aedes* mosquitoes: saliva is infectious as soon as two days after oral infection. PloS

366 one 4: e5895.

1. 34. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, et al. (2016)
2. Differential Susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to
3. Zika Virus. PLoS Negl Trop Dis 10: e0004543.
4. 35. Payne AF, Binduga-Gajewska I, Kauffman EB, Kramer LD (2006) Quantitation of
5. flaviviruses by fluorescent focus assay. J Virol Methods 134: 183-189.
6. 36. Failloux AB, Vazeille M, Rodhain F (2002) Geographic genetic variation in populations of
7. the dengue virus vector *Aedes aegypti*. J Mol Evol 55: 653-663.
8. 37. Lourenco-de-Oliveira R, Vazeille M, de Filippis AM, Failloux AB (2004) *Aedes aegypti* in
9. Brazil: genetically differentiated populations with high susceptibility to dengue and
10. yellow fever viruses. Trans R Soc Trop Med Hyg 98: 43-54.
11. 38. Kramer LD, Ebel GD (2003) Dynamics of flavivirus infection in mosquitoes. Adv Virus Res

378 60: 187-232.

1. 39. Zouache K, Fontaine A, Vega-Rua A, Mousson L, Thiberge JM, et al. (2014) Three-way
2. interactions between mosquito population, viral strain and temperature underlying
3. chikungunya virus transmission potential. Proc Biol Sci 281.
4. 40. Bosio CF, Fulton RE, Salasek ML, Beaty BJ, Black WCt (2000) Quantitative trait loci that
5. control vector competence for dengue-2 virus in the mosquito Aedes aegypti. Genetics

384 156: 687-698.

1. 41. Jupatanakul N, Sim S, Anglero-Rodriguez YI, Souza-Neto J, Das S, et al. (2017) Engineered
2. *Aedes aegypti* JAK/STAT Pathway-Mediated Immunity to Dengue Virus. PLoS Negl

387 Trop Dis 11: e0005187.

1. 42. Jupatanakul N, Sim S, Dimopoulos G (2014) The insect microbiome modulates vector
2. competence for arboviruses. Viruses 6: 4294-4313.
3. 43. Houé V, Bonizzoni M, Failloux A-B (2019) Endogenous non-retroviral elements in genomes
4. of *Aedes* mosquitoes and vector competence. Emerging microbes & infections 8: 542-555.
5. 44. Souza-Neto JA, Powell JR, Bonizzoni M (2019) *Aedes aegypti* vector competence studies: A
6. review. Infect Genet Evol 67: 191-209.
7. 45. Hegde S, Rasgon JL, Hughes GL (2015) The microbiome modulates arbovirus transmission
8. in mosquitoes. Curr Opin Virol 15: 97-102.
9. 46. Carrington LB, Simmons CP (2014) Human to mosquito transmission of dengue viruses.
10. Front Immunol 5: 290.
11. 47. Gratz NG (2004) Critical review of the vector status of *Aedes albopictus*. Med Vet Entomol

399 18: 215-227.

1. 48. Vazeille M, Rosen L, Mousson L, Failloux AB (2003) Low oral receptivity for dengue type 2
2. viruses of Aedes albopictus from Southeast Asia compared with that of *Aedes aegypti*.
3. Am J Trop Med Hyg 68: 203-208.
4. 49. Huang YS, Higgs S, Vanlandingham DL (2019) Arbovirus-Mosquito Vector-Host
5. Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses.
6. Front Microbiol 10: 22.
7. 50. Kamgang B, Nchoutpouen E, Simard F, Paupy C (2012) Notes on the blood-feeding behavior
8. of *Aedes albopictus* (Diptera: Culicidae) in Cameroon. Parasit Vectors 5: 57.

# Figure Legends

1. **Figure 1. Map of Cameroon vegetation showing the sampling sites.**
2. **Figure 2. Infection, disseminated infection, transmission rates and transmission efficiency**
3. **of *Ae. albopictus* from Central Africa to dengue virus.** A) Infection and disseminated infection
4. rates at 14 days post-infection (dpi). B) Infection, disseminated infection, transmission rates and
5. transmission efficiency at 21 dpi. Error bars show the 95% confidence interval. In brackets, the
6. number of mosquitoes examined. IR: the proportion of mosquitoes with infected body among
7. engorged mosquitoes; DIR: the proportion of mosquitoes with infected head among mosquitoes
8. with infected body; TR: the proportion of mosquitoes with infectious saliva among mosquitoes
9. with infected head. TE: the proportion of mosquitoes with infectious saliva among all analysed
10. ones.

# Figure 3. Infection, disseminated infection, transmission rates and transmission efficiency

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8. ones.
9. **Figure 4. Dengue virus titres in saliva of *Ae. aegypti* and *Ae. albopictus* at 21 days post-**
10. **infection.** The bars indicate the confidence interval of the mean for viral load in each population.
11. **Table 1: Origin of *Ae. aegypti* and *Ae. albopictus* used for vector competence**

|  |  |  |
| --- | --- | --- |
| **Location** | **Species** | **Generation** |
| Yaoundé urban | *Ae. albopictus* | G2 |
| Tibati | *Ae. albopictus* | G2 |
| Douala | *Ae. albopictus* | G2 |
| Brazzaville | *Ae. albopictus* | G5 |
| Yaoundé urban | *Ae. aegypti* | G2 |
| Yaoundé rural | *Ae. aegypti* | G2 |
| Bénoué Parc | *Ae. aegypti* | G4 |
| Brazzaville | *Ae. aegypti* | G2 |
| Maroua | *Ae. aegypti* | G2 |
| Douala | *Ae. aegypti* | G2 |