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Successive blood meals enhance virus dissemination within mosquitoes and increase transmission potential

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Supplementary Information

SUPPLEMENTARY TABLES

Table S1. Characteristics of Published Studies Included in Meta-Analysis

Study	ZIKV Strain	Sites virus collected	Number of days sampled
Li et al. 2012 ¹	Ugandan	Salivary glands	1
Li et al. 2017 ²	Asian	Salivary glands	2
Liu et al. 2017 ³	Asian	Salivary glands; heads	3
Ruckert et al. 2017 4	American	Salivary secretions; legs	4
Roundy et al. 2017 5	American	Legs	5
Richard et al. 2016 6	Polynesian	Salivary secretions; legs	6
Boccolini et al. 2016 ⁷	Asian	Salivary secretions; legs, wings	7
Hall-Mendelin et al. 2016 ⁸	Ugandan	Salivary secretions; legs, wings	8
Costa-da-Silva et al. 2017 ⁹	Brazilian	Salivary secretions; heads; bodies	9

Table S2. Model results summarized by dataset.

Dataset	Gamma distribution parameter estimates $\alpha; \beta$	μ_{EIP} (σ_{EIP})	μ_{R_0} (95% CI)
Single-feed DI (meta-analysis)*	3.89; 0.39	10.17 (5.19)	2.97 (1.84-4.29)
Single-feed DI (this study)**	9.72; 1.13	8.88 (2.94)	2.96 (2.58-3.39)
Double-feed DI (this study)**	1.76; 0.24	7.33 (5.96)	4.05 (3.22-5.17)
Single-feed SGI (meta-analysis)*	3.78; 0.37	10.82 (5.60)	--*
Single-feed SGI (this study)**	5.88; 0.62	9.65 (4.58)	--*
Double-feed SGI (this study)**	5.74; 0.74	7.84 (3.65)	--*

*The single-feed DI meta-analysis included data from 7 published studies and our own experimental results, or 38 total observations over 15 time points with n=8-40 mosquitos per study per time point. The single-feed SGI meta-analysis included data from 8 published studies and our own experimental results, or 48 total observations over 17 time points with n=8-78 mosquitos per study per time point.

**This study assessed DI at 7 time points, with n=16-18 mosquitos per time point, and SGI at 3 time points, with 53-78 mosquitos per time point.

***The basic reproductive number (R_0) was only estimated based on the dissemination data due to the limited experimental time points and large variability in EIP estimates based on the salivary gland data, as noted above.

Table S3. Parameter Values, Ranges, and Distributions Used to Estimate R_0

Symbol	Description	Parameter value (range)	PDF	Reference
m	Mosquito density	4 (1-10)	Uniform	10-12
a	Human biting rate (per mosquito per day)	0.67 (0.3-1)	Triangular	12-16
p	Probability of daily survival (for mosquitoes)	0.83 (0.73-0.91)	Uniform	10,17
N	Extrinsic incubation period	SF: 8.9 (8-10)* DF: 7.3 (6-9)*	Gamma	
b	Vector competence	0.4 (0.10-0.75)	Uniform	15,18,19
$\frac{c}{r}$	Human-to-mosquito transmission probability (c); human recovery rate (r).	3.5	Constant	15
h	Proportion of human to animal blood feeds	0.85 (0.83-0.99)	Uniform	20-22

*We determined the value and range for the extrinsic incubation period (EIP) by calculating the mean and 95% CI around the mean of the two respective EIP distributions derived from a posterior subset of 10,000 iterations of our models based on our experimental results (SF: single-feed, DF: double-feed). Note: To calculate R_0 , we sampled directly from the posterior distribution of the respective EIP, and we used the EIP values in this table only for sensitivity analyses.

Table S4. Estimates of the basic reproductive number (R_0) of Zika virus in the published literature. Estimates of the generation interval or extrinsic incubation period (EIP) used in the estimate of R_0 are also presented, if applicable.

Location	Outbreak Year	R_0 (estimate and 95% CI)	Generation Interval (estimate and range, days)	EIP (estimate and range, days)	Reference
Yap Island	2007	3.2 (2.4, 4.1)		10.6 (8.7, 12.5)	23
Yap Island	2007	5.8 (4.4, 7.7)	20-22		12
Yap Island	2007	5.05 (2.8, 12.5)		10 (6, 23)	24
French Polynesia	2013-2014	1.9 (1.5, 3.1)		10 (6, 23)	24
French Polynesia	2013-2014	6 (.06, 11.95)	16 (10, 23)		25
French Polynesia	2013-2014	1.61 (1.53, 1.69)	11		26
Moorea	2013-2014	2.6 (2.2, 3.3)		10.5 (8.6, 12.4)	23
Moorea	2013-2014	4.8 (3.2, 8.4)		10.5 (SD=0.5)	27
Tahiti	2013-2014	2.4 (2.0, 3.2)		10.5 (8.6, 12.6)	23
Tahiti	2013-2014	3.5 (2.6, 5.3)		10.5 (SD=0.5)	27
Sous-le-vent	2013-2014	4.1 (3.1, 5.7)		10.5 (SD=0.5)	27
Tuamotu-Gambier	2013-2014	3 (2.2, 6.1)		10.5 (SD=0.5)	27
Marquises	2013-2014	2.6 (1.7, 5.3)		10.5 (SD=0.5)	27
Australes	2013-2014	3.1 (2.2, 4.6)		10.5 (SD=0.5)	27
New Caledonia	2014	2 (1.8, 2.2)		10.7 (8.9, 12.5)	23
Colombia	2015-2016	4.8 (2.2, 14.8)		10 (6, 23)	28
Colombia	2015-2016	4.82 (2.34, 8.32)	16 (10, 23)		29
Colombia	2015-2016	2.56 (1.42, 3.83)	16 (10, 23)		29
Colombia	2015	1.75 (1.34, 2.16)	16 (10, 23)		25
San Andres, Colombia	2015-2016	1.41 (1.15, 1.74)	22		30
Girardot, Colombia	2015-2016	4.61 (4.11, 5.16)	22		30
Barranquilla, Colombia	2015	3.8 (2.4, 5.6)	16 (10, 23)		31

Nechi, Antioquia, Colombia	2016	2.2 (1.54, 2.86)	NA*	32
Antioquia, Colombia	2016	10.3 (8.3, 12.4)	14 (SD=2)	33
Antioquia, Colombia	2016	2.8 (2.4, 3.1)	14 (SD=2)	33
Cucuta, Colombia	2015-2016	(2.68, 4.57)	(18, 27)	34
Salvador, Brazil	2015	2 (1.9, 2.1)	15 (SD=3)	35
Salvador, Brazil	2015-2016	2.1 (1.8, 2.5)	17.8 (12.8, 24.8) [#]	36
Rio de Janeiro, Brazil	2015	2.33 (1.97, 2.97)	10.7 (4.4, 17)	37
Salvador, Brazil	2015	1.8 (1.2, 2.1)	NA*	35
Dominican Republic	2015-2016	1.8 (1.78, 1.82)	20-22	38
El Salvador	2015-2016	2.2 (1.5, 3.2)	8.4 (4.5, 17)	39
Costa Rica	2016-2017	1.52 (1.51, 1.53)	10	40
Suriname	2015-2016	2.4 (1.6, 3.5)	8.3 (4.5, 17)	39
Suriname	2015-2016	1.68 (1.32, 2.04)	16 (10, 23)	25
Guatemala	2015-2016	1.59 (1.28, 1.9)	16 (10, 23)	25
Saint Martin	2015-2016	5.7 (0, 11.75)	16 (10, 23)	25
Puerto Rico	2016	6.89 (0, 16.24)	16 (10, 23)	25
Cabo Verde Islands	2015-2016	1.85 (1.5, 2.2)	10.8 (SD=3.9)	41
Martinique	2016	1.36 (1.3, 1.42)	11	26
Singapore	2016	3.62 (3.48, 3.77)	7.4 (4.6, 10.2)	42
Brazil, Colombia, El Salvador	2015-2016	2.055 (0.523, 6.3)	10 (8, 12)	18

*Not applicable: Ospina et al. (ref. 41) derived an approximate equation for R_0 that did not depend on the generation interval or EIP; Rodriguez-Barraquer et al. (ref. 43) estimated R_0 based on the final size of the epidemic, as determined from serological data.

[#]Estimate includes the mosquito-to-human generation interval and intrinsic incubation period; does not include human infectious period

Table S5. Experimental determination of the correlation between ZIKV-induced cytopathic effect (CPE) in Vero cells and RT-qPCR cycle thresholds.

Zika (PRVABC59)	Virus Titer (PFU/mL)	Amount of virus used to inoculate flask (PFU)	Days Post-Inoculation that CPE Appeared	C _t values
no dilution	4.8x10 ⁶	4.8x10 ⁵	3	16.14
10 ⁻¹	4.8x10 ⁵	4.8x10 ⁴	3	19.3
10 ⁻²	4.8x10 ⁴	4.8x10 ³	4	22.89
10 ⁻³	4.8x10 ³	4.8x10 ²	5	25.3
10 ⁻⁴	4.8x10 ²	48	6	28.34
10 ⁻⁵	48	4.8	7	32.03
10 ⁻⁶	4.8	0.48	8	36.52

Table S6. Representative comparison of ZIKV detection rates using RT-qPCR and virus isolation on Vero cells.

	RT-qPCR (%)	Isolation on Cell Culture (%)
Single Feed	8/20 (40%)	2/20 (10%)
Double Feed	13/20 (64%)	9/20 (45%)

*Samples used for this study were from *Ae. aegypti* salivary glands 8 dpi.

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