Supplementary Table 1. Infectious virus validations.

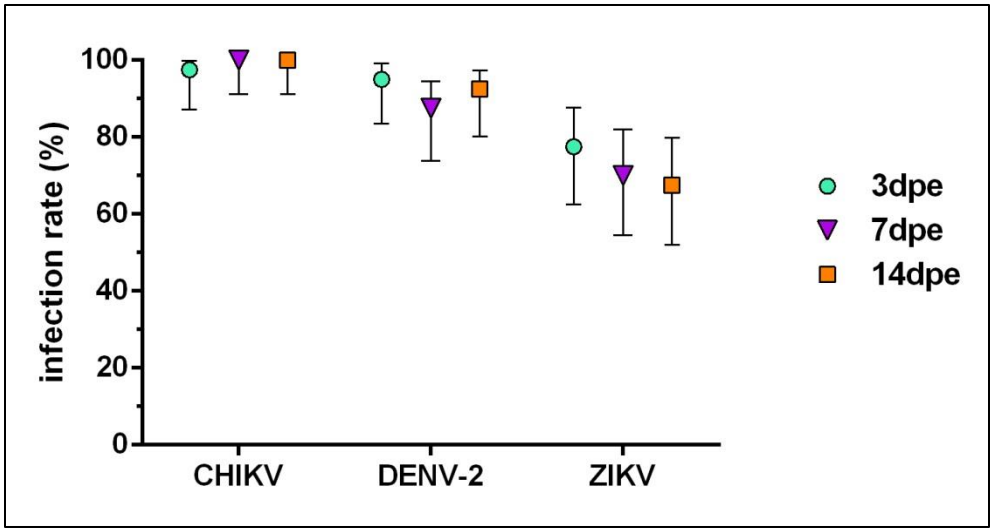
Experiment	Time	Condition	Sample #	PCR positive*	Infectious virus*
Coinfection 1	7dpi	CHIKV/ZIKV	62	C, Z	C
		CHIKV/ZIKV	42	C	C
		CHIKV/ZIKV	48	C	C
		CHIKV/ZIKV	49	Z	Z
		CHIKV/ZIKV	44	neg	neg
	14dpi	CHIKV/ZIKV	100	C, Z	C, Z
		CHIKV/ZIKV	102	C, Z	neg
		CHIKV/ZIKV	82	C	C
		CHIKV/ZIKV	103	C	C
		CHIKV/ZIKV	89	Z	Z
		CHIKV/ZIKV	96	neg	neg
Coinfection 2	14dpi	CHIKV/ZIKV	1	C, Z	neg
		CHIKV/ZIKV	19	C, Z	C, Z
		CHIKV/ZIKV	21	C, Z	C, Z
		CHIKV/ZIKV	7	C	C
		CHIKV/ZIKV	11	C	C
		CHIKV/ZIKV	38	Z	neg
		CHIKV/ZIKV	14	neg	neg
Coinfection 3	7dpi	CHIKV/DENV	37	C, D	C
		CHIKV/DENV	38	C, D	C,D
		CHIKV/DENV	1	C	C
		CHIKV/DENV	2	C	C
		CHIKV/DENV	30	D	neg
		CHIKV/DENV	10	neg	neg
	14dpi	CHIKV/DENV	41	C, D	C, D
		CHIKV/DENV	48	C	C
		CHIKV/DENV	80	C	C
		CHIKV/DENV	53	D	neg
		CHIKV/DENV	43	neg	neg
Coinfection 4	3dpi	CHIKV/ZIKV	18	C, Z	C,Z
		CHIKV/ZIKV	2	C	C
		CHIKV/ZIKV	5	C	C
		CHIKV/ZIKV	19	C	C
		CHIKV/ZIKV	1	neg	neg
	7dpi	CHIKV/DENV	42	C, D	C
		CHIKV/DENV	52	C, D	C, D
		CHIKV/DENV	54	C, D	C, D
		CHIKV/DENV	41	neg	neg
		CHIKV/ZIKV	53	C, Z	C, Z
		CHIKV/ZIKV	54	C, Z	C, Z
		CHIKV/ZIKV	44	C	C
		CHIKV/ZIKV	45	C	C
		CHIKV/ZIKV	41	neg	neg
	14dpi	CHIKV only	91	C	C
		CHIKV only	92	C	none
		CHIKV only	93	C	none
		DENV only	90	D	D
		DENV only	99	D	D

		DENV only	101	D	none
		ZIKV only	81	Z	Z
		ZIKV only	88	Z	Z
		ZIKV only	89	Z	Z
		CHIKV/DENV	82	C, D	D
		CHIKV/DENV	83	C, D	C, D
		CHIKV/DENV	90	C, D	C, D
		CHIKV/DENV	93	C, D	C, D
		CHIKV/DENV	98	C, D	D
		CHIKV/DENV	105	C, D	D
		CHIKV/DENV	116	C, D	D
		CHIKV/DENV	120	C, D	neg
		CHIKV/DENV	81	neg	neg
		CHIKV/ZIKV	88	C, Z	Z
		CHIKV/ZIKV	100	C, Z	neg
		CHIKV/ZIKV	87	C	C
		CHIKV/ZIKV	95	C	neg
		CHIKV/ZIKV	85	Z	Z
		CHIKV/ZIKV	107	Z	neg
		CHIKV/ZIKV	81	neg	neg
		DENV/ZIKV	81	D, Z	neg
		DENV/ZIKV	82	D, Z	D, Z
		DENV/ZIKV	85	D, Z	Z
		DENV/ZIKV	93	D, Z	D
		DENV/ZIKV	108	D, Z	D, Z
		DENV/ZIKV	116	D, Z	D, Z
		DENV/ZIKV	119	D, Z	D, Z
		DENV/ZIKV	90	D (Z ct>36.5)	D, Z
		DENV/ZIKV	106	D	D
		DENV/ZIKV	112	D	D
		DENV/ZIKV	120	D	D
		DENV/ZIKV	83	neg	neg
Triple infection	14dpi	Triple	2	C, D, Z	C, D, Z
		Triple	5	C, D, Z	C, D, Z
		Triple	14	C, D, Z	C, D, Z
		Triple	15	C, D, Z	C, Z
		Triple	16	C, D, Z	D, Z
		Triple	17	C, D, Z	C, D, Z
		Triple	32	C, D	none
		Triple	45	C, D	C
		Triple	48	D, Z	D, Z
		Triple	3	neg	neg
	21dpi	Triple	4	D, Z	Z
		Triple	22	D, Z	D, Z
		Triple	36	D, Z	D, Z
		Triple	43	C, Z	Z
		Triple	29	C, D, Z	Z
		Triple	48	C, D, Z	D, Z
		Triple	14	Neg	neg
		Triple	15	neg	neg

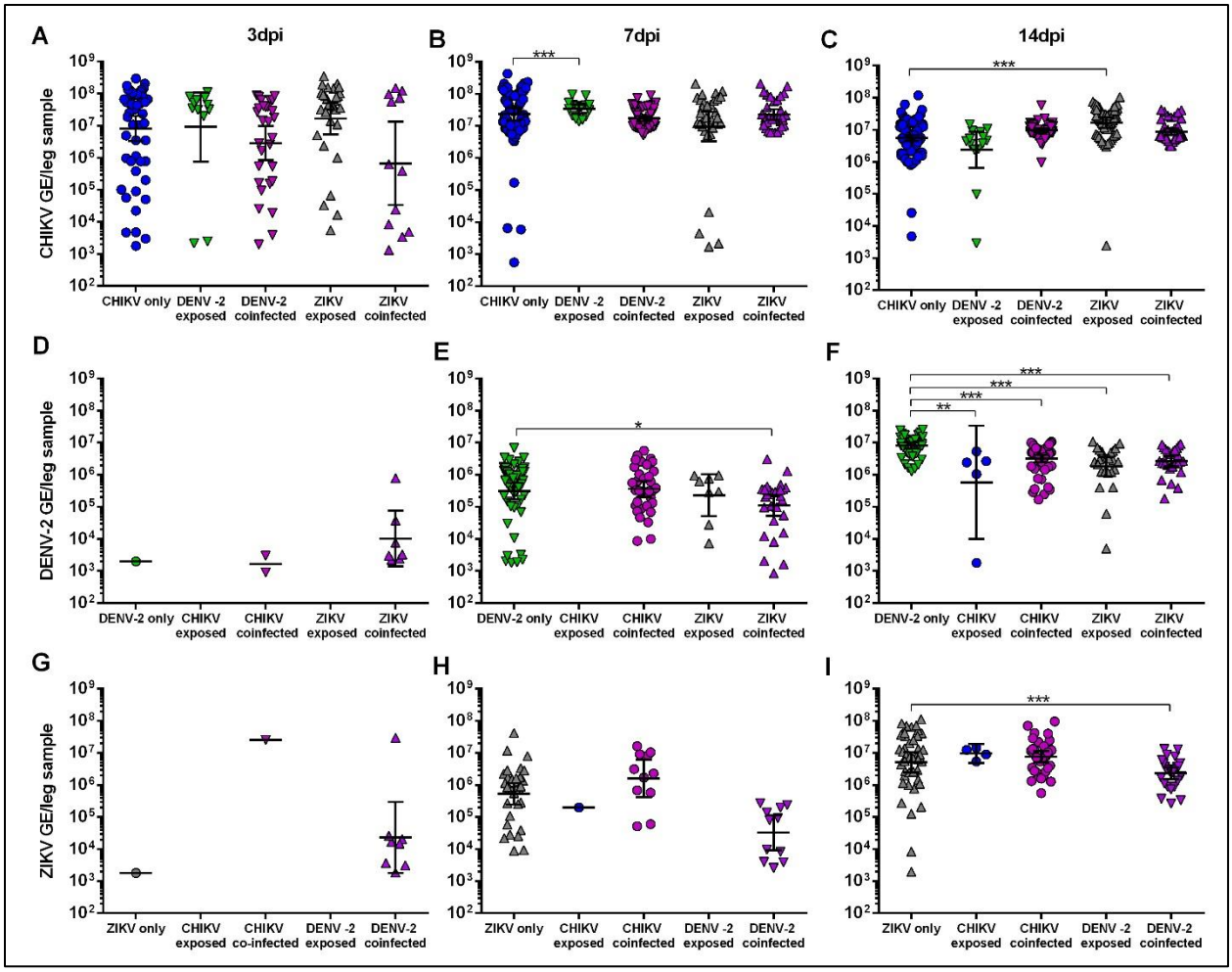
* virus abbreviations: C =CHIKV; D=DENV-2; Z=ZIKV

Supplementary Table 2. Primers and probes used for multiplex qRT-PCR.

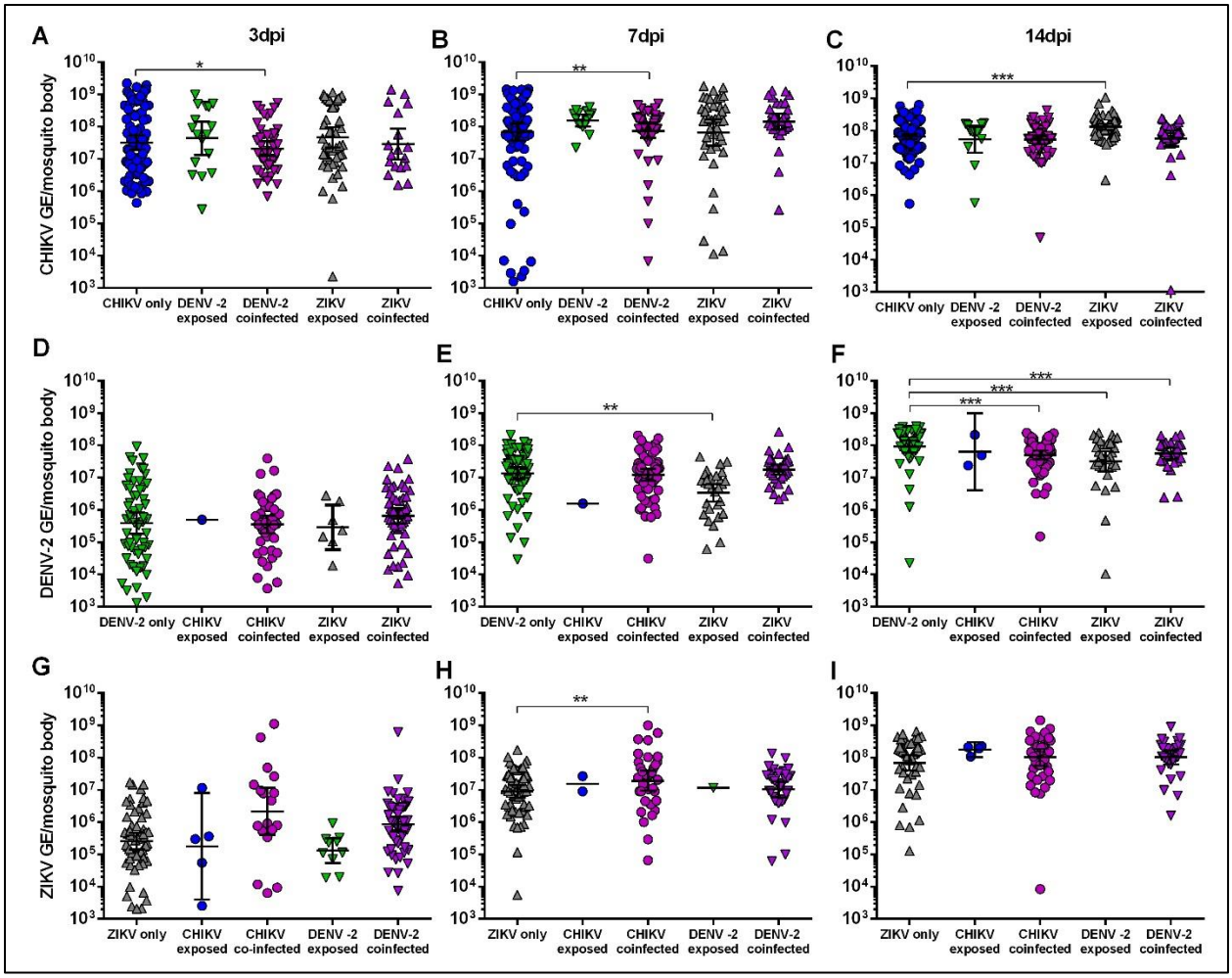
Primer name	Sequence
Zika1087_CDC_F[1]	CCGCTGCCCAACACAAG
Zika1108_CDC_FAM[1]	AGCCTACCTTGACAAGCAGTCAGACACTCAA
Zika1163_CDC_R[1]	CCACTAACGTTCTTTTGCAGACAT
CHIKV_qRT-PCR_JF_F[2]	CACCCGAAGTAGCCCTGAATG
CHIKV3288_Cy5[2]	GAGAATAGCCCGCTGTCTAGATCCAC
CHIKV_qRT-PCR_JF_R[2]	TCCGAACATCTTTCCTCCCG
DENV2-Merida_4198F	TCGTATGGAGGAGGCTGGAA
DENV2-Merida_4305R	GGGTTTTGTTTGGACGGCTC
DENV2-Merida_4219_HEX	CTAGAAGGAGAATGGAAGGAAGGAG
CHIKV_T7_2388F	TAATACGACTCACTATAGGGCGCTGCTCTTGAATGGATGC
CHIKV_T7_3505R	TAGTCTCCTGTTGGCCGGTA
DENV2-Merida_T7_3962F	TAATACGACTCACTATAGGGAACGGGCTGGAGTATTGTGG
DENV2-Merida_T7_4840R	CTGGGTCTGTGAAATGGGCT



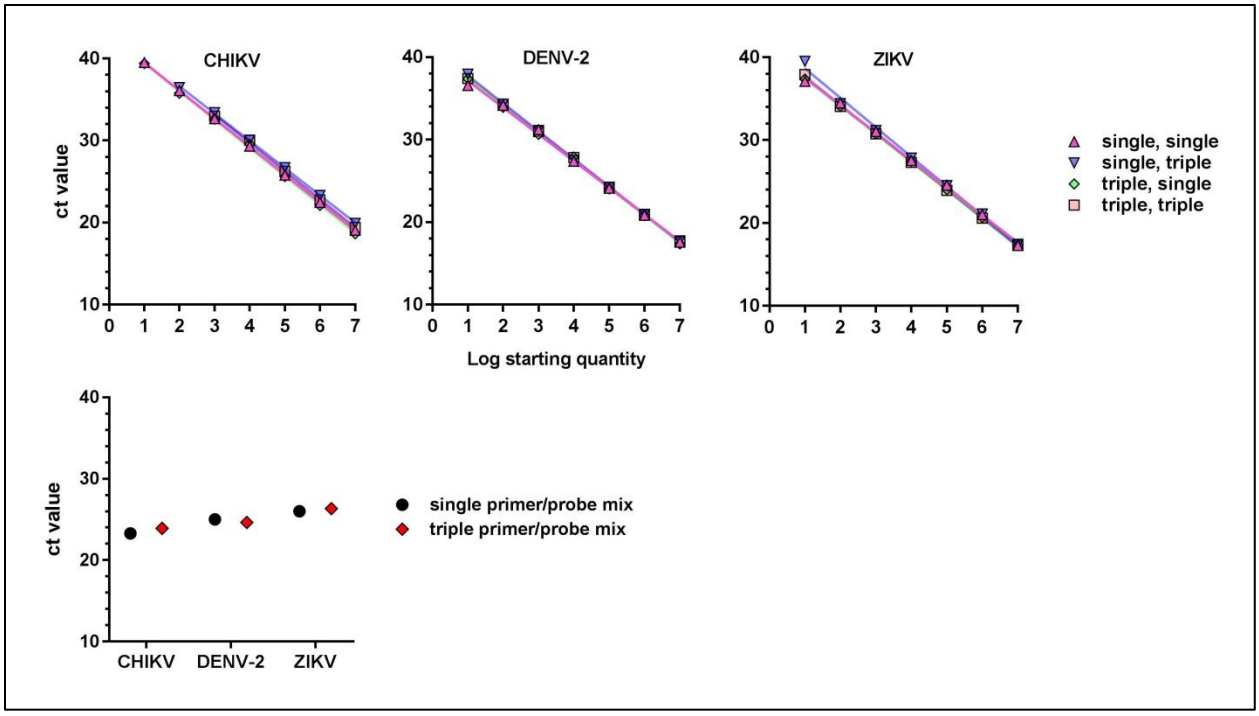
Supplementary Figure 1. Infection rates are comparable across time points. Infection rates for CHIKV, DENV-2 and ZIKV at all time points during single infection from one representative experiment (Experiment 4, see Table 3). Each data point indicates the percentage of infected mosquitoes (n=40 per condition/time point) and error bars indicate 95% CI. No significant differences were found between time points ($p>0.05$; Fisher's exact test).



Supplementary Figure 2. RNA levels in legs positive for CHIKV, DENV-2 and/or ZIKV. GE/legs are shown for samples positive for CHIKV (A, B, C), DENV-2 (D, E, F) and ZIKV (G, H, I) at 3dpi (A, D, G), 7dpi (B, E, H) and 14dpi (C, F, I). 'Exposed' indicates samples that were co-exposed with the named virus without resulting in coinfection. 'Coinfected' samples were positive for both viruses. The geometric mean is indicated and error bars represent the 95% confidence interval. Statistical significance was determined using one-way ANOVA with multiple comparisons (*p<0.05; **p<0.01; ***p<0.0001).



Supplementary Figure 3. RNA levels in bodies positive for CHIKV, DENV-2 and/or ZIKV. GE/body are shown for samples positive for CHIKV (A, B, C), DENV-2 (D, E, F) and ZIKV (G, H, I) at 3dpi (A, D, G), 7dpi (B, E, H) and 14dpi (C, F, I). 'Exposed' indicates samples that were co-exposed with the named virus without resulting in coinfection. 'Coinfected' samples were positive for both viruses. The geometric mean is indicated and error bars represent the 95% confidence interval. Statistical significance was determined using one-way ANOVA with multiple comparisons (*p<0.05; **p<0.01; ***p<0.0001).



Supplementary Figure 4. Multiplex qRT-PCR sensitivity and specificity. qRT-PCR was performed as described (see methods) to quantify in parallel CHIKV (A), DENV-2 (B) and ZIKV (C) RNA standards. The four different conditions were as follows: single virus standards with single primer/probe mix (single, single); single virus standards with triple primer/probe mix (single, triple); triple virus standards with single primer/probe mix (triple, single); triple standards with triple primer/probe mix (triple, triple). Panel D shows representative quantification using the three single primer/probe mixes as well as a multiplexed triple primer/probe mix from a triple positive leg sample . Single and triple primer/probe mix results for each virus were within a 0.65 ct value difference.

Supplementary References

1 Lanciotti, R. S. *et al.* Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerging infectious diseases* **14**, 1232-1239, doi:10.3201/eid1408.080287 (2008).

2 Grubaugh, N. D. *et al.* Xenosurveillance: a novel mosquito-based approach for examining the human-pathogen landscape. *PLoS neglected tropical diseases* **9**, e0003628, doi:10.1371/journal.pntd.0003628 (2015).