

Table S1. Demographic data of the 253 patients included in this study.

Population Data	N (%)	DENV (%)	CHIK (%)	ZIKA (%)	CHIK/ZIKA (%)	Leptospira (%)	Total Positives (%)
April–June 2015							
Age Group							
0–20	19 (7.51)	1 (0.40)	16 (6.32)	0 (0)	0 (0)	0 (0)	17 (6.71)
21–30	30 (11.86)	0 (0)	27 (10.67)	1 (0.40)	0 (0)	0 (0)	28 (11.07)
31–40	45 (17.79)	0 (0)	40 (15.81)	5 (1.98)	0 (0)	0 (0)	45 (17.79)
41–50	64 (25.30)	0 (0)	58 (22.92)	4 (1.58)	0 (0)	2 (0.79)	64 (25.30)
>50	48 (18.97)	0 (0)	42 (16.60)	2 (0.79)	0 (0)	1 (0.40)	45 (17.79)
Subtotal	206 (81.42)	1 (0.40)	184 (72.73)	12 (4.74)	0 (0)	3 (1.19)	200 (79.05)
Gender							
Female	138 (54.55)	1 (0.40)	125 (49.41)	7 (2.77)	0 (0)	3 (1.19)	136 (53.75)
Male	68 (26.88)	0 (0)	58 (22.92)	5 (1.98)	0 (0)	0 (0)	63 (24.90)
February–March 2016							
Age Group							
0–20	7 (2.77)	0 (0)	2 (0.79)	3 (1.19)	0 (0)	2 (0.79)	7 (2.77)
21–30	13 (5.14)	0 (0)	2 (0.79)	5 (1.98)	1 (0.40)	2 (0.79)	10 (3.95)
31–40	11 (4.35)	0 (0)	2 (0.79)	4 (1.58)	1 (0.40)	0 (0)	7 (2.77)
41–50	8 (3.16)	0 (0)	0 (0)	4 (1.58)	2 (0.79)	0 (0)	6 (2.37)
>50	8 (3.16)	0 (0)	0 (0)	6 (2.37)	1 (0.40)	1 (0.40)	8 (3.16)
Subtotal	47 (18.58)	0 (0)	6 (2.37)	22 (8.69)	5 (1.98)	5 (1.98)	38 (15.01)
Gender							
Female	31 (12.25)	0 (0)	5 (1.98)	13 (5.13)	3 (1.19)	3 (1.19)	24 (9.48)
Male	16 (6.32)	0 (0)	1 (0.40)	9 (3.56)	2 (0.79)	2 (0.79)	14 (5.53)
Total Women, 169 (66.8); Total Mens, 84 (33.2); Global Total, 253 (100).							

Table S2. Demographic information of pathogen localities in febrile patients.

Collection Site	Samples Collected, No.					
	Samples Collected, No.	Positive for CHIKV	Positive for ZIKV	co-Infection Positive for CHIKV/ZIKV	Positive for Leptospira	Positive for DENV
Acacoyagua	1	1	–	–	–	–
Cacahoatan	23	18	4	–	–	–
Huehuetan	24	20	3	–	1	–
Huixtla	23	20	1	–	–	–
Mazatan	2	2	0	–	–	–
Metapa de Dominguez	1	1	0	–	–	–
Suchiate	4	4	0	–	–	–
Tapachula	132	91	22	3	6	1
Tuxtla Chico	39	30	3	2	1	–
Tuzantan	2	1	1	–	–	–
Villa Comaltitlan	2	1	0	–	–	–
<b>Gender</b>						
Female	169	130	20	3	6	1
Male	84	59	14	2	2	
<b>Total</b>	<b>253</b>	<b>189</b>	<b>34</b>	<b>5</b>	<b>8</b>	<b>1</b>

Table S3. Comparison of clinical variables between the CHIKV and ZIKV groups.

<b>SYMPTOM</b>	<b>ZIKV+ / n=35 (%)</b>	<b>CHIKV+ / n=189 (%)</b>	<b><sup>a</sup> <i>p</i>-Value</b>
Headache	33 (94.3)	173 (91.5)	0.746
Myalgia *	23 (65.7)	22 (11.6)	<0.01
Mild-Moderate Arthralgia *	25 (71.4)	76 (40.2)	<0.01
Severe Polyarthralgia *	10 (28.6)	112 (59.3)	<0.01
Arthritis *	6 (17.1)	103 (54.5)	<0.01
Retroocular Pain *	18 (51.4)	30 (15.9)	<0.01
Exanthema *	21 (60.0)	151 (79.9)	<0.01
Pruritus	22 (62.9)	138 (73.0)	0.227
Vomit	3 (8.6)	27 (14.3)	0.588
Sickness	23 (65.7)	116 (61.4)	0.707
Shivers	32 (91.4)	175 (92.6)	0.734
Photophobia	1 (2.9)	2 (1.1)	0.401
Abdominal Pain	11 (31.4)	81 (42.9)	0.262
Diarrhea	9 (25.7)	73 (38.6)	0.182
Conjunctivitis *	25 (71.4)	20 (10.6)	<0.01
Nasal Congestion	8 (22.9)	24 (12.7)	0.12
Cough *	15 (42.9)	34 (18.0)	<0.01
Pharyngitis	11 (31.4)	70 (37.0)	0.571
Taste Alteration *	15 (42.9)	150 (79.4)	<0.01
Adenomegaly *	9 (25.7)	85 (45.0)	<0.05
Inflammation Of The Eyelid	3 (8.6)	8 (4.2)	0.384

Dyspnoea	3 (8.6)	6 (3.2)	0.151
Cardiac Alteration	0 (0)	2 (1.1)	1
Disorientation	0 (0)	1 (0.5)	1
Muscular Weakness	4 (11.4)	30 (15.9)	0.615
Otitis	0 (0)	1 (0.5)	1
Petequias	1 (2.9)	2 (1.1)	0.401
Edema *	5 (14.3)	70 (37.0)	<0.05

Lanciotti et al (2008)	Primer sentido (ZIKV 1086)										Sonda (ZIKV 1107)										Primer anti-sentido (ZIKV 1162c)																																															
	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
BrazilAmniotic2015	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
BrazilBrain2015	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Brazil2015_1	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Brazil2015_2	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Brazil2015_3	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Brazil2015_4	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Brazil2015_5	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Cambodia2010	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
CAR1968	T	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	T	G	C	A	A	A	G	A	A	C	A	T	T	G	G	T	G	G		
CAR1976	T	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	T	A	A	G	C	A	A	T	C	A	G	A	C	A	C	C	C	A	A	A	T	G	T	G	T	G	C	A	A	A	G	A	A	C	A	T	T	G	G	T	G	G	
CAR1979	T	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	T	A	A	G	C	A	A	T	C	A	G	A	C	A	C	C	C	A	A	A	T	G	T	G	T	G	C	A	A	A	G	A	A	C	A	T	T	G	G	T	G	G	
CAR1980	T	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	A	T	G	C	A	A	A	G	A	A	C	A	T	T	G	G	T	G	G	
FrenchPolynesia2013	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Guatemala2015_1	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Guatemala2015_2	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Haiti2014	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Malaysia1966	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Martinique2015	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Nigeria1968	T	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	T	A	A	A	G	A	A	C	A	T	T	G	G	T	G	G
Philippines2012	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G
PuertoRico2015	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
SaoPaulo2015	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Senegal1968	C	C	G	C	T	G	T	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	G	A	A	C	A	T	T	G	G	T	G	G	
Senegal1984	C	C	G	T	T	G	T	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	A	T	T	G	G	T	G	G
Senegal1997	C	C	G	C	T	G	T	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	A	T	T	A	G	T	G	G	
Senegal2001	T	C	G	T	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	A	T	T	A	G	T	G	G	
Suriname2015	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Thailand2013	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Thailand2014	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Uganda1947_1	T	C	G	T	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	A	T	T	A	G	T	G	G	
Uganda1947_2	T	C	G	T	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	A	T	T	A	G	T	G	G	
Yap2007	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	G	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G
Mexico2016_1	C	C	G	C	T	G	C	C	C	A	A	C	A	C	A	A	G	A	G	C	C	T	T	G	A	C	A	A	G	C	A	A	T	C	A	G	A	C	A	C	T	C	A	A	A	T	G	T	C	T	G	C	A	A	A	G	A	A	C	G	T	T	A	G	T	G	G	
Mexico2016_2	C	C	G	C	T																																																															

Figure S1. **Alignment primers/probes for ZIKV.** All primers/probes are in the 5' to 3' direction. Mismatches are highlighted with blue boxes. The antisense primer sequence is inversely complemented. This primer/probe set targets the envelope gene that is capable of detecting all isolates from Asia, South America, and Mexico. However, there are 1–7 mismatches that can alter the efficiency of the assay.





Figure S2. **Multiple alignment of leptospira species.** Multiple alignment of partial sequence of the 16S ribosomal RNA gene of leptospira species reported in GenBank. Identical bases are shaded the same color, asterisks show 100% identity in the different sequences. The BLAST match shows 87.38% identity with *L. Kmetyi* (KY411405.1) and ~ 80% for the other leptospira species.