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Re-evaluation of routine dengue virus serology in travelers in the era of Zika virus emergence

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ABSTRACT

Background: Diagnostic requests for both Zika virus (ZIKV) and dengue virus (DENV) infections in returning travelers have significantly increased during the recent ZIKV outbreak in the Americás. These flaviviruses have overlapping clinical syndromes and geographical distribution, but diagnostic differentiation is important because of different clinical consequences. As flaviviruses are known to have a short viremic period, diagnostics often rely on serological methods, which are challenging due to extensive cross-reactive antibodies.

Objective: To re-evaluate the performance of DENV serological assays in laboratory confirmed ZIKV-infected travelers.

Study design: The extent of cross-reactivity of the DENV NS1 antigen, IgM and IgG ELISA was analyzed in 152 clinical blood samples collected from 69 qRT-PCR and 24 virus neutralization titer (VNT) confirmed ZIKV-infected travelers.

Results: The majority of travelers in the presented cohort returned to the Netherlands from Suriname and presented with symptoms of fever and rash. Twenty-three percent of the female travelers were pregnant. None of the 39 ZIKV RNA positive blood samples were cross-reactive in the DENV NS1 antigen ELISA. The rates of cross-reactivity of the DENV IgM and IgG ELISÁs were 31% and 54%, respectively, after excluding travelers with (potential) previous DENV exposure.

Conclusions: Although the DENV NS1 antigen assay was highly specific in this cohort of laboratory confirmed ZIKV-infected travelers, we demonstrate high percentages of cross-reactivity of DENV IgM and IgG ELISÁs of which diagnostic laboratories should be aware. In addition, the high rate of DENV IgG background of > 25% complicates a proper serological diagnosis in this group.

1. Background

The recent outbreak of Zika virus (ZIKV) in the America's has caused a significant increase in diagnostic requests for both ZIKV and dengue virus (DENV) infections in travelers returning from endemic regions to Europe. Both flaviviruses typically cause overlapping symptoms while co-circulating in large parts of the world which often results in a combined diagnostic request [1,2].

Serology is an important diagnostic tool to confirm or rule out DENV and/or ZIKV infection. In most patients, molecular and/or antigen testing on serum will not suffice because of the short duration of viremia (typically about 3–7 days post onset of symptoms and for ZIKV possibly longer in pregnant women) [1,3,4]. Reverse-transcriptase polymerase chain reaction (RT-PCR) on urine or whole blood can lengthen the detection window of both DENV and ZIKV. Detection times of up to 15 days post onset of disease in urine have been reported for both flaviviruses [5–7]. In daily practice however, there is often no urine available or the delay of sampling is more than 2 weeks post onset of symptoms. Similarly as has been described for West Nile virus [8], longer detection times of up to 81 days could be obtained for ZIKV RNA in whole blood [9], but routine processing of whole blood samples is often absent in diagnostic work flows of clinical virology laboratories.

Because of the teratogenicity of ZIKV [10], confirming or ruling out a ZIKV infection is important especially in pregnant women. With an estimated 80% of ZIKV infections being asymptomatic [11], ZIKV diagnostics are nowadays often part of routine work-up in healthy

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pregnant women or couples with a desire to have children who have visited an endemic area. The diagnosis of a ZIKV infection then often relies on comparative ZIKV and DENV serological methods as the interpretation of flavivirus serology is complex [12]. Flaviviruses are known for their antigenic similarity, e.g. the amino acid sequence of the envelope protein of the DENV serocomplex shows a similarity of 54–59% with that of ZIKV [13]. In humans this is known to trigger a cross-reactive antibody response, which makes discrimination between the respective infections by routine serological assays, such as immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA), difficult. Furthermore, a proper interpretation of serology results is complicated by original antigenic sin in patients with a secondary flavivirus infection or a previous flavivirus vaccination, as the highest titer is often found for antibodies directed to the virus or vaccine of primary exposure [14–16]. Additionally, the close relationship between ZIKV and DENV was highlighted by the observation of antibody-dependent enhancement of ZIKV replication in *in vitro* studies with pooled convalescent serum collected from subjects who recovered from infection with DENV [17].

Since the start of the ZIKV outbreak in the Americás in May 2015 much attention has been paid to the evaluation of the specificity and sensitivity of different ZIKV serological assays [18–21] while reassessment of routine DENV serological tests has been neglected. In the current era it is therefore necessary to re-evaluate the previously implemented serodiagnostics for DENV. We reassessed our routine DENV serology in the scope of potentially cross-reactive ZIKV antigen and antibodies using an anonymized cohort of 93 ZIKV-infected travelers returning to the Netherlands.

2. Objectives

We re-evaluated i) the specificity of the Platelia™ DENV NS1 antigen ELISA in ZIKV viremic travelers and ii) the specificity of the Euroimmun DENV IgM and IgG ELISA by assessing the extent of cross-reactivity in blood samples of laboratory confirmed ZIKV-infected travelers.

3. Study design

From December 2015 to October 2016 the Viroscience laboratory at Erasmus University Medical Center (Rotterdam, the Netherlands) diagnosed ZIKV infection in 93 returning travelers who had visited a ZIKV endemic region. For analysis in this study, all patient samples were anonymized according to medical ethical regulations. ZIKV infection was confirmed in 69 patients by an ISO15189:2012 validated in-house real-time semi-quantitative (q)RT-PCR using the E-set (1086–1162) of Lanciotti et al. [22] on EDTA-plasma, serum or urine. Forty-seven patients had ZIKV viremia, and in 24 cases a follow-up serum was available (Fig. 1A). Twenty-two patients were diagnosed by a positive qRT-PCR on urine. For all of them a serum sample of the same collection date as the urine sample was available, while for 11 of them a follow-up serum was available as well (Fig. 1A). Additionally, ZIKV infection was diagnosed in 24 travelers with acute-convalescent phase serum pairs, based on the presence of ZIKV neutralizing antibodies in convalescent serum samples (Fig. 1B). ZIKV virus neutralization was carried out as follows: two-fold serum dilutions were incubated with 100 TCID50 of Zika virus Suriname strain 2016 (Genbank reference KU937936) at 37 °C and then used to inoculate Vero cells for 5 days at 37 °C. ZIKV infection was read out under the microscope by cytopathic effect. A reciprocal VNT ZIKV immunoglobulins (Ig) titer of ≥1/32 was considered positive.

In total, 152 blood samples from 93 patients were available for reassessment of DENV serology tests (Fig. 1A and B). Specificity of the DENV NS1 antigen ELISA (Platelia™ Enzym Immunoassay, Biorad, Hercules, CA, USA) was assessed using 39 out of 47 viremic samples (i.e., plasma or serum ZIKV qRT-PCR positive) of which sufficient volume was left (Fig. 1A). The specificity of the DENV IgM and IgG ELISÁs (Euroimmun, Lübeck, Germany) was re-evaluated by assessing the extent of cross-reactivity in relation to the Euroimmun ZIKV IgM and IgG ELISA results on first and follow-up samples of qRT-PCR or VNT confirmed ZIKV-infected travelers. IgM and IgG cross-reactivity were defined as a positive ZIKV IgM/G result in combination with a positive or equivalent DENV IgM/G result. We did not include any ZIKV IgM/IgG equivalent sera in our cross-reactivity analysis, since we would like to measure all possible DENV IgM and IgG cross-reactive antibodies (i.e., both equivalent and positive DENV signals) only in samples with clearly positive ZIKV IgM/IgG signals. An equivalent IgM/G ELISA result was defined as 0.8 ≥ signal to cut-off ratio < 1.1. All ELISÁs were performed as described by the manufactureŕs instructions guide.

4. Results

4.1. Cohort description

Table 1 describes the clinical characteristics of the 93 laboratory confirmed ZIKV-infected returning travelers. The majority of them returned from Suriname (50%) and Dominican Republic (16%). The most common reported symptoms were fever (60%) and rash (70%), sometimes in combination with arthralgia (41%) and/or conjunctivitis (25%). Symptoms were unknown in 13% and 9% of the travelers were asymptomatic. A previous history of dengue fever or other flavivirus infection and/or vaccination was unknown for all travelers. Flavivirus infections, besides DENV, were not systematically ruled out. The median time of known days between onset of disease and diagnostics was 4 days (range of 1–36 days) in 46 travelers (Table 1).

4.1.1. Specificity of DENV NS1 antigen ELISA

The Platelia™ DENV NS1 antigen ELISA appeared to be 100% specific using a cohort of 39 ZIKV-RNA positive samples. All of these viremic samples tested negative. As far as known, these samples were taken ≤11 days post onset of disease (< 5 days: N = 17; 5–10 days: N = 3; 11 days: N = 1; and unknown number of days post onset of disease: N = 18).

4.1.2. Specificity of DENV IgM and IgG ELISA

To exclude potential co-infections with DENV, first of all the Platelia™ DENV NS1 antigen ELISA was performed in all first samples where possible (N = 84, in 9 samples there was insufficient volume left for this test). None of these 84 first samples were NS1 antigen positive. The sensitivity and specificity of the Platelia™ NS1 antigen ELISA vs. our in-house DENV type 1–4 qRT-PCR [23,24] in a retrospective cohort of 141 returning travelers clinically suspected for DENV infection were 100% (95% Confidence Interval (CI): 94.4–100%) and 90.8% (95% CI: 82.2–95.5%), respectively. Finally, all first 93 and 59 available follow-up blood samples were used for calculation of the extent of DENV IgM cross-reactivity (Fig. 1A and B).

To define IgG cross-reactivity we excluded patients in whom previous DENV exposure could not be ruled out. Twenty-five out of 93 (27%) travelers already had DENV IgG antibodies in their first serum while the ZIKV IgG result was negative. Forty-five patients out of 93 (48%) presented with both ZIKV and DENV IgG antibodies in their first serum. In these 70 travelers it is thus impossible to discriminate if reactivity in the DENV ELISA is detected due to the actual presence of pre-existing DENV specific antibodies or due to cross-reactive ZIKV IgG antibodies of the present infection. To prevent bias from these detectable DENV IgG antibodies all these cases (i.e., with a positive or equivalent DENV IgG result in their first serum) were excluded from the IgG cross-reactivity analysis leaving 23 patients with 35 sera to consider (Fig. 1A and B).

Table 2A summarizes the DENV IgM and IgG results vs. ZIKV IgM and IgG results from qRT-PCR confirmed ZIKV-infected patients, divided in subcategories of samples drawn < 5 days, 5–10 days, > 10 days and an unknown number of days post onset of disease. These

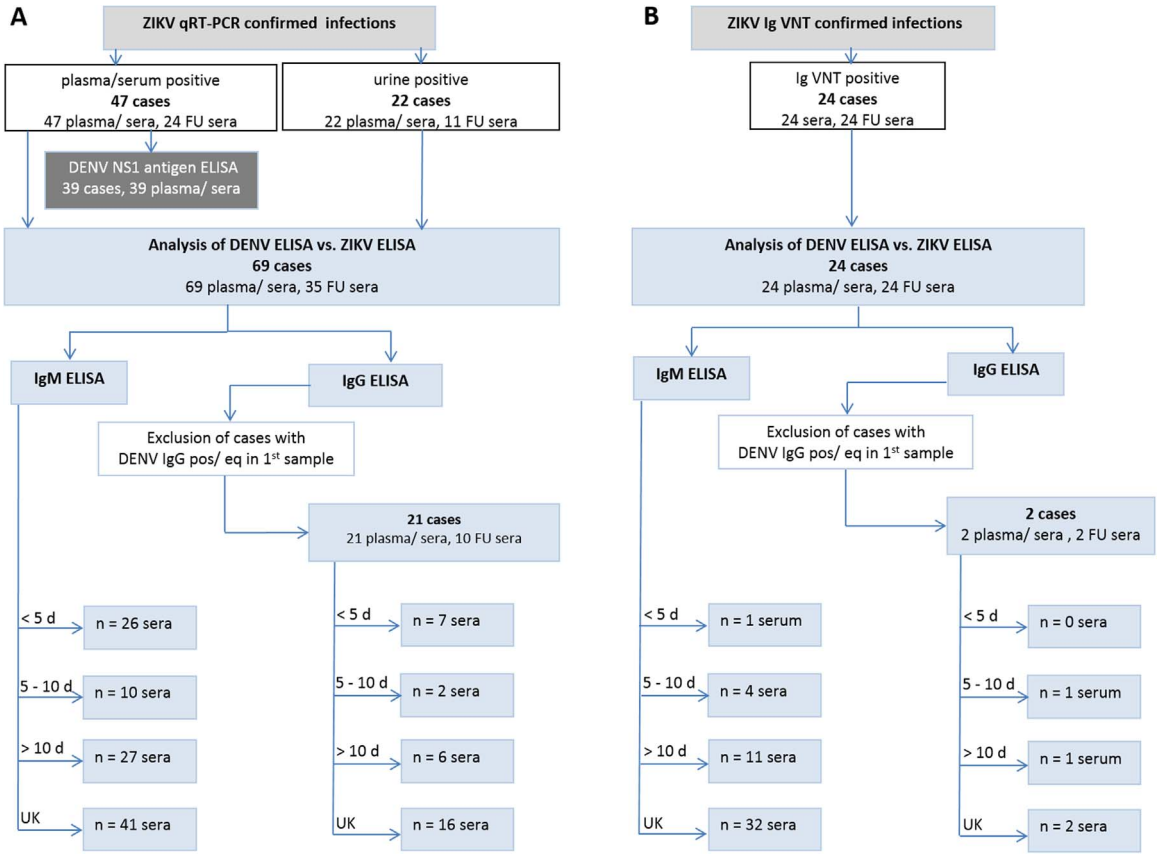

Fig. 1. Overview of performed DENV and ZIKV serology tests on blood samples, drawn < 5 days, 5–10 days, > 10 days and an unknown (UK) number of days post onset of disease, from 69 qRT-PCR (A) and 24 Ig VNT (B) confirmed ZIKV-infected travelers. FU: follow-up; pos: positive; eq: equivalent.

Table 1
Characteristics of 93 travelers with a laboratory confirmed ZIKV infection.

	qRT-PCR positive (N = 69)	Ig VNT positive (N = 24)
Male	22 (32%)	5 (21%)
Female	47 (68%)	19 (79%)
Mean age ± SD (years)	46.9 ± 14.6	37.0 ± 11.4
Pregnant? (N) (% of female)	4 (9%)	11 (58%)
Travel history (N)		
Suriname	36 (52%)	10 (42%)
Dominican Republic	11 (16%)	4 (17%)
Nicaragua	3 (4%)	0 (0%)
Unknown	6 (9%)	4 (17%)
Other	13 (19%)[b]	6 (25%)[c]
Symptomatic (N)[a]	60 (87%)	13 (54%)
fever (% of symptomatic)	34 (57%)	10 (77%)
rash	45 (75%)	6 (46%)
arthralgia	27 (45%)	3 (23%)
conjunctivitis	17 (28%)	1 (8%)
Asymptomatic (N)	2 (3%)	6 (25%)
Symptoms unknown (N)	7 (10%)	5 (21%)
Median time in days (range) between onset of disease and first serum (number of patients)	3 (1–24) (N = 38)	10 (3–36) (N = 8)
Follow-up serum available? (N)	35 (51%)	24 (100%)
Median interval time in days (range) between first and follow-up serum	19 (6–67)	20.5 (10–70)

[a] Less frequently reported symptoms in all travelers were: myalgia, headache, malaise, lymphadenopathy, nausea, diarrhoea, sore throat, oedema and pruritus.
[b] Other countries: N = 2: Barbados and St. Maarten; N = 1: Aruba, Brazil, Cape Verde, Cayman Islands, Costa Rica, Curaçao, Martinique, St. Lucia and Venezuela.
[c] Other countries: N = 1: Brazil, Curaçao, El Salvador, Panama, Thailand and Venezuela.

subcategories were based on known kinetics of antibodies in other flaviviruses like DENV [25] and West Nile virus [26]. The percentages of ZIKV IgM and IgG antibodies that were cross-reactive in the DENV IgM and IgG ELISA were 33% (13/39) and 50% (5/10), respectively. Table 2B shows the DENV IgM and IgG results vs. ZIKV IgM and IgG results in VNT confirmed travelers. Percentages of DENV IgM and IgG cross-reactivity in this group were comparable, i.e., 20% (2/10) and 67% (2/3), respectively. After combining the samples from ZIKV qRT-PCR and VNT confirmed infected travelers the total rates of DENV IgM and IgG cross-reactivity were 31% (15/49) and 54% (7/13), respectively.

After excluding the 70 cases with a DENV positive or equivalent result in their first serum, there remained 12 out of 23 cases (N = 10 ZIKV qRT-PCR and N = 2 VNT confirmed) from whom a follow-up serum was available (Fig. 1) which allowed more detailed analysis. Their clinical characteristics together with the performed ZIKV qRT-PCR and VNT diagnostics are summarized in the context of the ZIKV and DENV ELISA results (Table 3). In these DENV naive patients, IgG cross-reactivity in the DENV ELISA was observed in seven out of 13 sera (54%) in which ZIKV IgG antibodies were detected, representing 11 travelers. ZIKV VNT was positive in two patients (case 2 and case 12) with equivalent and in one patient (case 6) with negative ZIKV IgG ELISA results, while corresponding DENV IgG results were negative (Table 3). These three sera were not included in the IgG cross-reactivity analysis, since the percentage of cross-reacting IgG antibodies in the DENV ELISA was solely calculated with respect to the ZIKV IgG ELISA positive samples.

5. Discussion

The presented data in this study demonstrated a 100% specificity of DENV NS1 antigen ELISA testing in 39 ZIKV viremic blood samples. A

Table 2
A. DENV and corresponding ZIKV IgM (left) and IgG (right) ELISA results in 104 and 31 samples from 69 and 21 ZIKV qRT-PCR confirmed infected travelers, respectively. Samples with cross-reactive ZIKV antibodies in the DENV ELISA are highlighted in red. B. DENV and corresponding ZIKV IgM (left) and IgG (right) ELISA results in 48 and 4 samples from 24 and 2 ZIKV Ig VNT confirmed infected travelers, respectively. Samples with cross-reactive ZIKV antibodies in the DENV ELISA are highlighted in red.

A.

			69 ZIKV qRT-PCR confirmed cases						21 ZIKV qRT-PCR confirmed cases			
			DENV IgM (N= 104)						DENV IgG (N= 31)			
days p.o.d.			pos	eq	neg	Total			pos	eq	neg	Total
< 5	ZIKV IgM	pos	0	0	5	5	ZIKV IgG	pos	0	0	0	0
		eq	1	0	0	1		eq	0	0	0	0
		neg	1	1	18	20		neg	0	0	7	7
5 - 10	ZIKV IgM	pos	0	2	3	5	ZIKV IgG	pos	0	0	0	0
		eq	0	0	0	0		eq	0	0	0	0
		neg	0	1	4	5		neg	0	0	2	2
> 10	ZIKV IgM	pos	2	1	11	14	ZIKV IgG	pos	3	0	2	5
		eq	1	0	4	5		eq	0	0	1	1
		neg	0	0	8	8		neg	0	0	0	0
Unknown	ZIKV IgM	pos	7	1	7	15	ZIKV IgG	pos	2	0	3	5
		eq	0	0	1	1		eq	0	0	0	0
		neg	1	0	24	25		neg	0	0	11	11
Total	ZIKV IgM	pos	9	4	26	39	ZIKV IgG	pos	5	0	5	10
		eq	2	0	5	7		eq	0	0	1	1
		neg	2	2	54	58		neg	0	0	20	20
		total	13	6	85	104		total	5	0	26	31

B.

			24 ZIKV Ig VNT confirmed cases						2 ZIKV Ig VNT confirmed cases			
			DENV IgM (N= 48)						DENV IgG (N= 4)			
days p.o.d.			pos	eq	neg	Total			pos	eq	neg	Total
< 5	ZIKV IgM	pos	0	0	0	0	ZIKV IgG	pos	0	0	0	0
		eq	0	0	0	0		eq	0	0	0	0
		neg	0	0	1	1		neg	0	0	0	0
5 - 10	ZIKV IgM	pos	2	0	1	3	ZIKV IgG	pos	0	0	0	0
		eq	0	0	0	0		eq	0	0	1	1
		neg	0	0	1	1		neg	0	0	0	0
> 10	ZIKV IgM	pos	0	0	3	3	ZIKV IgG	pos	1	0	0	1
		eq	0	0	0	0		eq	0	0	0	0
		neg	0	1	7	8		neg	0	0	0	0
Unknown	ZIKV IgM	pos	0	0	4	4	ZIKV IgG	pos	0	1	1	2
		eq	0	0	2	2		eq	0	0	0	0
		neg	2	1	23	26		neg	0	0	0	0
Total	ZIKV IgM	pos	2	0	8	10	ZIKV IgG	pos	1	1	1	3
		eq	0	0	2	2		eq	0	0	1	1
		neg	2	2	32	36		neg	0	0	0	0
		total	4	2	42	48		total	1	1	2	4

p.o.d.: post onset of disease; pos: positive; eq: equivalent; neg: negative.

Table 3
Characteristics of 12 DENV naive travelers with ZIKV infection and corresponding results of ZIKV and DENV ELISÁs. Sera with cross-reactive ZIKV IgG antibodies in the DENV ELISA are highlighted in red.

Case	M/F	1st serum	Days post onset of disease	Days 2nd – 1st serum	Travel history	Clinical symptoms	qRT-PCR ZIKV	ELISA				
	pregnant?	2nd serum						VNT ZIKV Ig	ZIKV IgM	ZIKV IgG	DENV IgM	DENV IgG
1	M	1st	N.A.	54	unknown	none	Pos (plasma)	insufficient material	Neg	Neg	Neg	Neg
	N.A.	2nd	N.A.					1/101	Neg	Pos	Neg	**Pos**
2	F	1st	2	25	unknown	fever, rash, arthralgia,	Pos (plasma)	Neg	Neg	Neg	Neg	Neg
	No	2nd	27			headache, conjunctivitis		1/40	Equiv	Equiv	Neg	Neg
3	M	1st	unknown	11	Suriname	rash, swollen hand	Pos (plasma)	insufficient material	Pos	Neg	Neg	Neg
	N.A.	2nd	unknown					1/203	Pos	Pos	Neg	Neg
4	F	1st	2	36	Suriname	fever, malaise, myalgia, rash	Pos (plasma)	Neg	Neg	Neg	Neg	Neg
	No	2nd	38					1/128	Equiv	Pos	Neg	**Pos**
5	M	1st	1	67	Venezuela	fever, rash, arthralgia, headache, cough, retro-orbital pain	Pos (plasma)	Neg	Neg	Neg	Neg	Neg
	N.A.	2nd	68					1/406	Neg	Pos	Neg	**Pos**
6	F	1st	unknown	15	St. Maarten	arthralgia, rash	Pos (plasma)	1/64	Neg	Neg	Neg	Neg
	No	2nd	unknown					1/201	Pos	Pos	Pos	**Pos**
7	F	1st	5	6	St. Maarten	itching rash, periorbital headache, conjunctivitis	Pos (urine)	Neg	Neg	Neg	Neg	Neg
	No	2nd	11					1/128	Pos	Pos	Neg	Neg
8	M	1st	unknown	14	Barbados	fever, maculopapular rash, conjunctivitis	Pos (urine)	1/256	Pos	Pos	Pos	Neg
	N.A.	2nd	unknown					1/128	Neg	Pos	Pos	Neg
9	F	1st	2	17	Aruba	rash, lymphadenopathy	Pos (urine)	Neg	Neg	Neg	Neg	Neg
	No	2nd	19					1/203	Pos	Pos	Neg	Neg
10	M	1st	8	25	Dominican Republic	fever, rash, arthralgia, myalgia, conjunctivitis	Pos (urine)	Neg	Pos	Neg	Equiv	Neg
	N.A.	2nd	33					1/256	Pos	Pos	Pos	**Pos**
11	F	1st	N.A.	21	Brazil	none	Neg (plasma + urine)	1/32	Neg	Pos	Neg	Neg
	Yes	2nd	N.A.					1/50	Neg	Pos	Neg	**Equiv**
12	F	1st	10	14	unknown	fever, rash, arthralgia, retro-orbital pain	Neg (plasma + urine)	1/101	Pos	Equiv	Pos	Neg
	No	2nd	24					1/203	Neg	Pos	Equiv	**Pos**

Neg: negative; Pos: positive; Equiv: equivalent; M: male; F: female; N.A.: non applicable.

recent case report by Gyurech et al. [27] described a false positive DENV NS1 antigen test result in a patient with an acute ZIKV infection, who was initially suspected for acute dengue fever. Notably, the false positive DENV NS1 result was only found when applying the SD Bioline Dengue Duo rapid immunochromatographic NS1 antigen test (ICT) (Standard Diagnostics, South Korea) and not when applying the Biorad NS1 antigen strip or Biorad Platelia™ NS1 antigen ELISA that was assessed here. In accordance, a recent retrospective study by Matheus et al. [28] did not show any DENV NS1 antigen cross-reactivity in 65 qRT-PCR confirmed acute ZIKV samples of French Guiana in neither the SD Bioline Dengue Duo ICT nor the Biorad Platelia™ ELISA.

The total rate of DENV IgM cross-reactivity after combining the samples from qRT-PCR and VNT confirmed ZIKV-infected travelers was 31% (15/49). Although it has been reported that ZIKV-specific-IgM generally appears around 5 days post onset of disease [21], the majority of ZIKV IgM positive and equivalent samples in this cohort were drawn > 10 days post onset of disease. A specific period post onset of disease for occurrence of cross-reactive IgM antibodies could therefore not be derived. Furthermore, it should be noted that for 7 of the 15 DENV IgM positive sera a ZIKV − DENV co-infection could not be excluded by DENV NS1 antigen ELISA as either the sampling day post onset of symptoms was unknown and/or a the ZIKV infection was confirmed based on solely a qRT-PCR positive urine (n = 5) or positive VNT (n = 2). Nevertheless, DENV RNA type 1–4 was not detected in these five urine samples (n = 1 taken 8 days p.o.d. and in n = 4 unknown). In the 2 VNT positive cases first sera were drawn at 9 and 10 days post onset of symptoms which is too late for testing for acute DENV infection by NS1 antigen ELISA or DENV qRT-PCR. Although

ZIKV-DENV co-infections have only been occasionally reported [29–31] the prevalence could be underestimated due to the high rate of asymptomatic infections with both flaviviruses, similar clinical symptoms and/or lack of concurrent testing.

Within the presented cohort 27% of the travelers has IgG antibodies against DENV before they develop IgG antibodies against ZIKV, indicating that a substantial part of the potential cross-reactive ZIKV IgG antibodies in the DENV ELISA can likely be attributed to background DENV IgG antibodies. After eliminating these samples, the Euroimmun IgG DENV ELISA was cross-reactive in 54% (7 out of 13) of blood samples of confirmed ZIKV-infected patients, who were DENV IgG naive at their first presentation. As expected, all DENV IgG cross-reactive samples occurred > 10 days in the group of travelers with known date of onset of symptoms. This emphasizes the importance of molecular diagnostics on preferably acute samples in ZIKV diagnostics, in particular because original antigenic sin may boost the (neutralizing antibody) titer against other flaviviruses to which the person previously was exposed even to a factor four-fold in paired serum samples [32]. Current developments indicate that the sensitivity of ZIKV diagnostics can be increased by molecular testing in urine, saliva, semen and whole blood in combination with analysis of serum.

To discriminate between cross-reactive ZIKV antibodies and boosted DENV antibodies from a previous infection, comparative VNT's should ideally be performed against ZIKV and DENV type 1–4 (or the specific flaviviruses endemic to the region of exposure [12]), as recently suggested in CDC Morbidity and Mortality Weekly report [21]. Unfortunately, even in VNT's cross-reactivity has been observed and most routine diagnostic laboratories will not have the capacity to perform VNT and thus are dependent on available commercial assays.

In conclusion, we have demonstrated that the recent ZIKV outbreak in Latin America substantially affects the DENV serology in routine diagnostic laboratories. We confirmed a high specificity of DENV NS1 antigen but showed that in our cohort of 93 travelers with confirmed ZIKV infection, about one third of ZIKV IgM and more than half of ZIKV IgG antibodies cross-reacted in the Euroimmun DENV ELISA. Diagnostics are complicated by the observed high rates of DENV IgG background in this group of returning travelers of which clinical laboratories should be aware. It is therefore recommended to: i) promote early sampling enabling DENV NS1 antigen and ZIKV and DENV qRT-PCR testing, ii) always perform ZIKV and DENV serology in parallel enabling more informative comparative serology iii) include, when available, virus neutralization in paired samples in high risk groups for adverse infection, like pregnant women, and iv) be aware of high rates of cross-reactivity in routine DENV IgM and IgG serology in an increasing number of returning travelers who can present with an acute or past ZIKV infection.

Conflicts of interest

None.

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Ethical approval

Ethical approval was obtained from the Erasmus MC Medical Ethical Committee (MEC-2015-306) to anonymously analyze the used samples of all patients.

Author contributions

MvM, CR and CGvK designed the study and analyzed the data. RM, JK and FC performed the serology and virus neutralization experiments and acquired the corresponding data. SP acquired the results of the molecular diagnostics on ZIKV and DENV. MvM and CGvK drafted the manuscript. CR, AvdE, SP and MK critically revised the manuscript. All authors approved the final version of the manuscript.

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