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Letter to the editor

Lack of Zika virus antibody response in confirmed patients in non-endemic countries

Zika virus (ZIKV) has spread in the last 2 years throughout America and South-Eastern Asia causing a widespread epidemic [1]. Detection of ZIKV RNA in body fluids confirms ZIKV infection, however ZIKV antibody testing is much more complex due to possible cross-reactivity with closely related flaviviruses [2].

From December 2015 to February 2017, 401 patients from eight reference laboratories in the Czech Republic, Israel, Italy, the Netherlands, Romania, Slovenia, and the United Kingdom had been confirmed for ZIKV infection by detection of ZIKV RNA in body fluids [2–4] (Table 1). Of these 401 patients, 148 were negative for ZIKV directed against IgM and IgG in serum collected at the time of PCR-positivity as tested by ELISA (7 laboratories, Euroimmun, Lübeck, Germany) or IFA and ELISA (2 laboratories, Euroimmun, Lübeck, Germany). For 80 of these 148 seronegative confirmed patients a second, follow-up serum sample was available. Altogether, 5 of these 80 patients remained without seroconversion in consecutive samples (Table 2) for ZIKV antibodies tested by ELISA and virus neutralization (VNT) (Table 2). The acute samples of these 5 patients were re-extracted and retested from original material which confirmed the presence of ZIKV RNA. Material from patients 1 and 2 were sequenced [5]. Ideally, each of the samples from the 5 patients would also have been tested in at least one of the other laboratories, but because of insufficient clinical material, this wasn't possible. Most importantly, none of the sero-negative patients had any indication of immune-deficiency. Two patients were pregnant.

One explanation for the lack of detection of ZIKV IgM or IgG antibodies in 5 of our patients is low sensitivity of the assays. Indeed, a few studies have previously demonstrated low sensitivity of the Euroimmun NS1 ELISA [6–9]. However, since neutralization is widely accepted as the gold standard test for arboviral infections and unlike the NS1 ELISA, neutralization primarily recognizes antibodies against surface proteins, the probability that both tests failed to detect ZIKV antibodies is low. Another explanation is that production of ZIKV antibodies was suppressed in these cases maybe due to a previous flavivirus infection which might suppress ZIKV immune response including the production of neutralizing antibodies (original antigenic sin [10,11]).

In conclusion, our results show absence of ZIKV specific antibodies using routine serological assays in 5 of 80 of convalescent sera from PCR confirmed ZIKV cases in returning travelers. This may suggest significant under-diagnosis of ZIKV infections when diagnosis relies on serology alone. This is especially of importance in cases where congenital Zika syndrome might be involved such as diagnosis of pregnant women or males with pregnant partners. As serum of pregnant women, whole blood and semen provide a longer window of detection for PCR [12–15], these samples should be tested by RT-PCR alongside serology. Relating the absence of detectable ZIKV immune responses to the absence/severity of clinical symptoms and previous flavivirus antigen exposure in larger cohort studies might provide insight into the groups at risk for such under-diagnosis.

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Table 1
Criteria and numbers of ZIKV testing in travelers.

Laboratory name/Country	Rare and Imported Pathogens Laboratory, PHE Porton, UK	Central Virology Laboratory, Ministry of Health, Israel	Cantacuzino National Institute for Research, Bucharest, Romania	National Institute for infectious diseases Lazzaro Spallanzani, Rome, Italy	Institute of Public Health, Ostrava, Czech Republic	Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, Torino, Italy	Institute of Microbiology and Immunology, Ljubljana, Slovenia	WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever, Rotterdam, The Netherlands
ZIKV testing criteria	Only patients with symptoms suggestive of ZIKV infection tested	Patients with symptoms or pregnant women with or without symptoms tested	Patients with symptoms or pregnant women with or without symptoms tested	Patients with symptoms or pregnant women with or without symptoms tested	Patients with symptoms or pregnant women with or without symptoms tested	Patients with symptoms or pregnant women with or without symptoms tested	Patients with symptoms, pregnant women with or without symptoms tested	Patients with symptoms, pregnant women and their partners with or without symptoms tested
Total number of ZIKV PCR positive cases between December 2015 and February 2017	148	14	3	35	18	10	9	164
Number of ZIKV PCR positive cases with no antibody detected in serum at the time of the PCR positive result	87	4	3	6	6	3	1	38
Number of initially ZIKV PCR positive but seronegative cases with follow-up serum samples submitted	53	4	3	5	5	2	1	7
Number of these cases with ZIKV antibodies detected in follow-up serum	53	3	2	5	5	2	1	4

Table 2
Results of Zika serology assays from RT–PCR positive Zika virus infection patients.

No.	Country of origin	Country of Acquisition	Gender/ age	Symptoms (description)	RT PCR Result (Ct), sample type	Median (range) Ct values of all PCR positive patients	Dengue Virus IgM/ IgG result for first serum sample	1st serum sample				2nd serum sample				3rd serum sample			
								Time from onset (days)	Neutralization result	IgM	IgG	Time from onset (days)	Neutralization result	IgM	IgG	Time from onset (days)	Neutralization result	IgM	IgG
1	Israel	Vietnam	M/61	Yes (Fever, malaise, headache)	Pos (34), WB*	35.2 (29.4–39.5)	Neg/Neg	10	Neg	Pos	Neg,	79	Neg	Neg,	Neg,	No results	No results	No results	No results
2	Israel	Guatemala, Mexico	F/30	Yes (Fever)	Pos (31), WB	35.2 (29.4–39.5)	Neg/Neg	26	Neg	Neg,	Neg,	48	Neg	Neg,	Neg	No results	No results	No results	No results
3	NL	unknown	M/41	no	Pos (34.8) WB	Too few data	Not performed	unknown	Not performed	Neg	Neg	Unknown (38 days after sample with pos PCR result)	Neg	Neg	Neg	No results	No results	No results	No results
4	NL	Curacao	F/29 (pregnant)	no	Pos (36.5) urine	33.6 (24.2–38.5)	Neg/Pos	18 days after returning to NL	Neg	Neg	Neg	32 days after returning to NL	Neg	Neg	Neg	No results	No results	No results	No results
5	NL	British Virgin Islands	F/30 (pregnant)	no	Pos (37.7) serum	35.5 (27.7–38)	Neg/Neg	10 days after returning to NL	Not performed	Neg	Neg	34 days after returning to NL	Neg	Neg	Neg	41 days after returning to NL	Neg	Neg	Neg

All laboratories used the ZIKV Euroimmun ELISA Zika RT-PCR was performed in all laboratories as descibed in Lanciotti et. al. [2].
NL = Netherlands; WB = whole blood; Neg = Negative, Pos = Positive.
* Positive for ZIKV RNA in WB on day 58 after symptom's onset.

Conflict of interest

All authors declare that they have no competing or conflict of interest.

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Ethical approval

Ethical approval was given by Sheba Medical Center, Ref. no. 4420-17-SMC.

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