International Journal of Infectious Diseases 78 (2019) 39–43

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Incidence of Zika virus infection in a prospective cohort of Belgian travellers to the Americas in 2016

Ralph Huits[a,*], Dorien Van Den Bossche[a], Kaat Eggermont[a], Erica Lotgering[a], Anne-marie Feyens[a], Idzi Potters[a], Jan Jacobs[a,b], Marjan Van Esbroeck[a], Lieselotte Cnops[a,1], Emmanuel Bottieau[a,1]

[a] Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[b] Department of Microbiology and Immunology, University of Leuven, Leuven, Belgium

ARTICLE INFO

Article history:
Received 8 September 2018
Received in revised form 15 October 2018
Accepted 19 October 2018
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Zika virus
Americas
Incidence rate
Prospective cohort study
Enzyme-linked immunosorbent assay
Virus neutralization testing
Real-time reverse transcription polymerase chain reaction
Filter paper

ABSTRACT

Background: The incidence rate of Zika virus (ZIKV) infection in travellers from non-endemic areas to the Americas during the ZIKV outbreak in 2016 is unknown.
Methods: Belgian adults who planned to travel to South America, Central America, and the Caribbean were recruited prospectively to study the incidence and characteristics of ZIKV. Demographic data and sera were collected at baseline. Participants were trained to collect capillary blood on filter paper (BFP). When ill during travel, the participants completed a questionnaire and they sampled BFP for post-hoc analysis. All symptomatic participants were screened for ZIKV using ZIKV-specific RT-PCR on serum or urine, or BFP, and antibody detection assays (ELISA). Follow-up sera of asymptomatic travellers, obtained at least 20 days post travel, were tested by ZIKV ELISA only. All positive ELISA results were subject to confirmation by virus neutralization testing (VNT).
Results: Forty-nine participants completed follow-up: 38 women and 11 men, with a median age of 32 years (range 19–64 years). Travel destinations were countries in South America ($n = 20$), Central America ($n = 24$), and the Caribbean ($n = 5$). The total travel duration was 67.8 person-months. Illness was reported by 24 participants (49.0%). ZIKV infection was confirmed in nine cases, by RT-PCR ($n = 5$) and by VNT ($n = 4$). Only one of nine ZIKV cases (11.1%) was asymptomatic. The ZIKV incidence rate was 17.0% (95% confidence interval 7.8–32.2%) per month of travel.
Conclusions: The ZIKV incidence rate in adult travellers from non-endemic countries to the epidemic territories during the 2016 outbreak was high. Asymptomatic ZIKV infection was rare in this population.

Introduction

Following the epidemic spread of an Asian strain of Zika virus (ZIKV) in the New World, a causal association was recognized between ZIKV infection during pregnancy and adverse neonatal outcomes (Petersen et al., 2016). Although primarily transmitted by *Aedes spp* mosquitoes, evidence has emerged that ZIKV could also be transmitted through sexual intercourse (D'Ortenzio et al., 2016; Moreira et al., 2017). In addition, prolonged virus shedding has often been observed in the semen of infected men (Huits et al., 2017). Forty-eight countries in the Americas reported autochthonous vector-borne transmission of ZIKV in 2016–2017 (Pan American Health Organization, 2017). As many of these countries are popular travel destinations, travellers from non-endemic countries were particularly concerned about the risk of infection and of secondary sexual transmission upon return (Hamer et al., 2017). Data collection by surveillance networks like GeoSentinel has further indicated that travel-associated infection with ZIKV may drive its global spread (Hamer et al., 2017). However, incidence rates of ZIKV infection in travellers remain unknown because denominator data are unavailable (Leder et al., 2015). This prospective observational study was performed to assess the incidence of ZIKV infection in adults travelling to the Americas in 2016.

Methods

In February 2016, a prospective cohort study was started to determine the incidence and aetiology of febrile illness during

* Corresponding author at: Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.
E-mail address: rhuits@itg.be (R. Huits).
[1] These authors contributed equally to this manuscript.

https://doi.org/10.1016/j.ijid.2018.10.010

travel in (sub-)tropical areas (protocol registered at ClinicalTrials.gov, NCT02900066). As the start of recruitment coincided with the ZIKV outbreak in the Americas (PAHO, 2016), it was aimed to determine the incidence and clinical characteristics of ZIKV infection in travellers to destinations in South America, Central America, and the Caribbean, from February to December 2016.

Belgian participants aged 18 years or older were actively recruited through travel organizations and educational institutions. Before travel, baseline demographic and clinical data were obtained during a structured interview by a physician (RH) at the Institute of Tropical Medicine in Antwerp, Belgium. A baseline serum sample was stored. Participants were trained to draw blood from a finger-prick using an auto-retractable safety lancet, and to collect the capillary blood on filter paper (BFP) (Whatman 903 Filter Paper).

All participants were provided with a kit that contained the necessary study materials (see **Supplementary material**). In the event of illness during travel, participants were requested to record data on symptoms, protective measures and exposure to health hazards, diagnostics, and case management abroad in a structured questionnaire. When symptoms matched the clinical criteria of the World Health Organization case definition for ZIKV infection, i.e. fever or rash (WHO, 2016), participants were instructed to collect BFP for laboratory diagnosis upon return from travel. Participants with disease were further advised to seek medical attention during travel and they were offered consultations at the Institute of Tropical Medicine upon their return. All symptomatic and asymptomatic participants were also contacted by telephone for completion of data missing from the questionnaires and to request a follow-up serum sample to determine seroconversion rates.

For participants who were symptomatic during travel or at presentation to the Institute of Tropical Medicine, ZIKV-specific real-time reverse transcription PCR (RT-PCR) (RealStar Zika Virus RT-PCR Kit; Altona Diagnostics GmbH, Hamburg, Germany) was performed on BFP obtained during the acute phase (details provided in the **Supplementary material**), or on serum or urine samples obtained within 7 or 14 days post symptom onset. Post-travel serum samples were screened for ZIKV-specific immunoglobulins (Ig) by ZIKV IgG/IgM ELISA (Euroimmun, Lübeck, Germany). Serum samples from asymptomatic participants were obtained at least 20 days post travel. The baseline sera of all participants were assessed for the presence of antibodies against dengue virus (DENV) using the Dengue Virus IgM Capture DxSelect and Dengue Virus IgG DxSelect assays (Focus Diagnostics, Cypress, CA, USA), as indicators of previous DENV or related flavivirus infection. Samples for which positive or equivocal ELISA results were obtained were further tested in a virus neutralization testing assay (VNT) using the 90% neutralizing antibody titre. In internal validation procedures, it was demonstrated that cross-reactivity with DENV antibodies for this VNT was limited to ZIKV VNT titres between 1:10 and 1:100. Symptomatic and asymptomatic participants with a positive RT-PCR or VNT result were defined as ZIKV cases, and participants with negative results were defined as non-ZIKV cases.

Primary ZIKV infection is believed to induce protective immunity (Dudley et al., 2016; Aliota et al., 2016). The time at risk was defined by subtraction of the travel time after symptom onset for cases from the total travel duration in endemic areas. As the time to symptom onset could not be ascertained for asymptomatic travellers, the median time to symptom onset was used for symptomatic participants in this study to estimate the time at risk for asymptomatic cases with VNT-confirmed ZIKV infection. The incidence rate of ZIKV infection in this cohort was then calculated using the following formula: (number of cases)/((travel duration of cases) – (travel duration of cases – time to symptom onset)) (see **Supplementary material**). The 95% confidence interval (CI) for the incidence rate estimate was calculated using the Poisson distribution.

Results

A total of 55 participants were recruited, of whom 49 (89.1%) had a complete follow-up (38 women and 11 men). The median age was 32 years (range 19–64 years). Travel destinations were countries in South America ($n = 20$), Central America ($n = 24$), and the Caribbean ($n = 5$). The purpose of travel was tourism for 40 participants (81.6%), student internship for six participants (12.2%), and relief work for three participants (6.1%). The median duration of travel was 22 days (range 16–197 days), with a total duration of 2068 person-days (67.8 person-months). Twenty-four participants (49.0%) reported an illness and nine cases of ZIKV infection were identified, including one asymptomatic case (Table 1). The predominant symptoms in the ZIKV cases were rash, headache, and diarrhoea. A skin rash was the only symptom that distinguished ZIKV infection from other illnesses (odds ratio 45, 95% CI 3.4–594). No neurological complications after ZIKV infection were observed.

Five ZIKV cases were diagnosed by RT-PCR: three on BFP collected during travel (cycle threshold (Ct) values 37.2, 37.3, and 34.9) and two on urine collected post travel (Ct values 28.9 and 37.3). The remaining four cases were diagnosed by seroconversion of ZIKV-specific IgG antibodies, confirmed by VNT (Table 2). ZIKV-infected participants had returned from South America (Colombia, $n = 1$), Central America ($n = 6$), and the Caribbean ($n = 2$). The median time from arrival in an endemic area to symptomatic ZIKV infection was 13 days (range 4–20 days).

The time at risk was calculated to be 53.0 person-months. The ZIKV incidence rate was 17.0% (95% CI 7.8–32.2; Poisson distribution) per month of travel.

Self-reported exposure and protective measures to prevent mosquito bites and the use of chloroquine as malaria chemoprophylaxis did not differ between ZIKV cases and non-cases (Table 1). All ZIKV cases and 70% of non-ZIKV cases had been vaccinated against yellow fever virus. Forty-six baseline serum samples were available for DENV-IgG antibody testing. Anti-DENV antibodies were detected in the pre-travel samples of three participants, and none of these acquired ZIKV infection during the study period.

Discussion

This prospective cohort study indicated that adult participants who travelled to areas with epidemic vector-borne transmission were at high risk of acquiring ZIKV infection. The calculated incidence rate of 17.0% per month of travel during the 2016 outbreak in the Americas exceeded surveillance-based estimates of all travel-associated illness frequencies, except for travellers' diarrhoea (Leder et al., 2013). ZIKV cases in this study cohort had visited Colombia, Honduras, Mexico, and Nicaragua, at a time when the respective national health authorities reported more than 200 (suspected and confirmed) ZIKV cases per epidemiological week (EW) to the Pan American Health Organization (PAHO) (Figure 1) (Pan American Health Organization, 2017). The detection of ZIKV-RNA on BFP collected by a symptomatic traveller to Haiti in EW 39 (Figure 1E), confirmed ongoing transmission of ZIKV beyond EW 33, when Haiti sent its last update to PAHO (Pan American Health Organization, 2017; Journel et al., 2017). This finding highlights the role of travellers as sentinels for ZIKV circulation (Leder et al., 2017). It also illustrates the utility of BFP, which allowed us to obtain a molecular diagnosis of ZIKV in three cases. In addition, it permitted the time from arrival to symptom onset to be established in symptomatic ZIKV infections, even when the duration of travel was much longer (Table 2 and Figure 1F, cases 2 and 8).

Only one of nine ZIKV infections was asymptomatic. The sensitivity of the NS1-based ELISA has been debated, but seroconversion for ZIKV-specific IgG is very likely at 20 days post

Table 1
Demographic and travel data, risk factors, and symptoms for 49 travellers to the Americas, 24 of whom reported symptoms and nine of whom had confirmed Zika virus infections (including one asymptomatic case).

	Total	ZIKV cases ($n=9$), n	(%)	Non-ZIKV cases ($n=40$), n	(%)	OR (95% CI)	p-Value
Age (years), median (range)	32 (19–64)	29 (22–41)		37 (19–64)			0.11[a]
Sex ratio, F:M	38:11	7:2		31:9		1.0 (0.2–5.6)	NS
Travel destination							
South America	20	1	(11.1)	19	(47.5)		0.06[b]
Central America	24	6	(66.7)	18	(45.0)		0.29[b]
Caribbean	5	2	(22.2)	3	(7.5)		0.22[b]
Travel type							
Tourist	40	6	(66.7)	34	(85.0)		0.34[b]
Student	3	0	(0.0)	3	(7.5)		1[b]
Relief worker	6	3	(33.3)	3	(7.5)		0.07[b]
Travel duration							
Days, median (IQR)	21 (16–197)	22 (21–197)		22 (16–118)			NS
Person-months	67.8	18.8		49.0			
Exposure/protection		9	(100)	40	(100)		
Insect bites	42	8	(88.9)	34	(85.0)	1.4 (0.15–13.4)	NS
Insect repellent[c]	46	8	(88.9)	38	(95.0)	0.42 (0.03–5.2)	NS
Protective clothing	29	4	(44.4)	25	(62.5)	0.48 (0.11–2.1)	NS
Camping in open air	19	3	(33.3)	16	(40.0)	0.75 (0.16–3.4)	NS
Bed net	25	7	(77.8)	18	(45.0)	4.3 (0.79–23.2)	NS
Chloroquine use[d]	13	3	(33.3)	10	(25.0)	1.5 (0.3–7.1)	NS
Yellow fever vaccination	37	9	(100)	28	(70.0)	–	
Previous dengue infection[e]	3	0/7	(0.0)	3/39	(7.7)	–	
Symptoms	24	8	(100)	16	(100)		
Time to onset (days), median (range)		13 (4–20)		12 (4–86)			NS
Rash	7	6	(75.0)	1	(6.3)	45 (3.4–594)	
Conjunctivitis	0	0	(0.0)	0	(0.0)	–	
Fever	9	2	(25.0)	7	(43.8)		NS
Arthralgia	3	2	(25.0)	1	(6.3)		NS
Myalgia	6	2	(25.0)	4	(25.0)		NS
Headache	6	4	(50.0)	2	(12.5)		NS
Fatigue	6	3	(37.5)	3	(18.8)		NS
Cough	2	0	(0.0)	2	(12.5)		NS
Throat ache	2	0	(0.0)	2	(12.5)		NS
Abdominal pain	6	2	(25.0)	4	(25.0)		NS
Diarrhoea	17	5	(62.5)	12	(75.0)		NS
Constipation	1	0	(0.0)	1	(2.5)		NS
Nausea	8	2	(25.0)	6	(37.5)		NS
Vomiting	7	1	(12.5)	6	(37.5)		NS

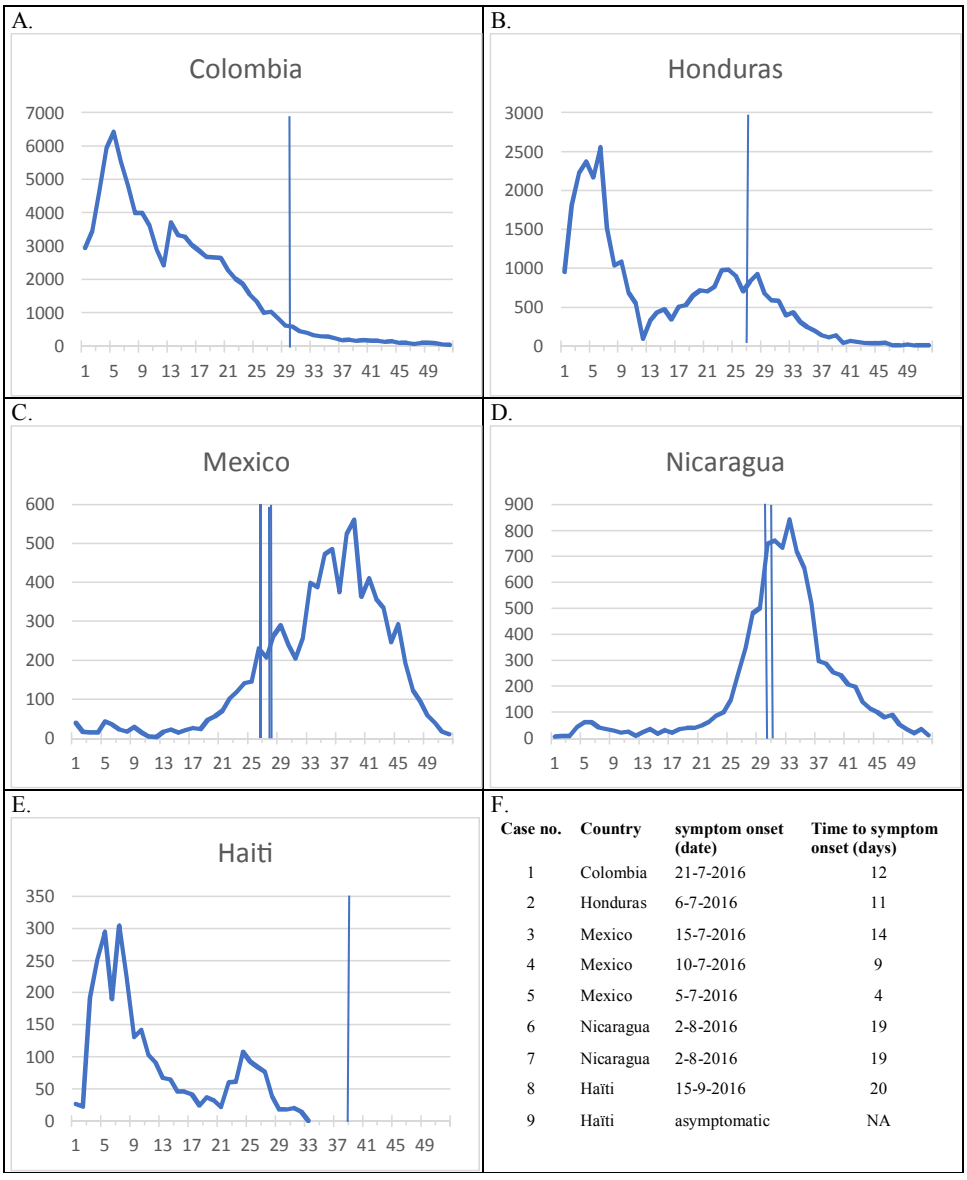
OR, odds ratio; CI, confidence interval; F, female; M, male; IQR, interquartile range; NS, not significant.
[a] Mann–Whitney *U*-test.
[b] Each row represents cases vs. non-cases per travel destination, using the other destinations as a comparator (Fisher's exact test, two-tailed probability).
[c] Insect repellent: *N,N*-diethyl-meta-toluamide (DEET).
[d] Malaria chemoprophylaxis: chloroquine, 300 mg base (500 mg salt) orally, once/week.
[e] Presence of dengue IgG in baseline serum is an indicator of previous dengue virus or related flavivirus infection; note: baseline serum missing for two Zika virus cases (Table 2, cases 7 and 8).

travel-associated exposure (Lessler et al., 2016; Lustig et al., 2018; Huits et al., 2018). Given the estimates of symptomatic-to-asymptomatic ZIKV infection ratios in non-traveller populations in Micronesia and French Polynesia (1:4.4 and 1:1, respectively) (Duffy et al., 2009; Aubry et al., 2017 Aubry et al., 2017), a higher frequency of asymptomatic infection had been anticipated. However, several studies have already suggested that asymptomatic ZIKV infections occur in only 0–27% of non-pregnant returning

Table 2
Demographic and travel data, characteristics, and diagnosis of nine Zika cases in the case-cohort study of travellers to the Americas, 2016.

Case	Age (years)	Sex	Destination	Travel type[a]	Travel duration (days)	Symptoms	Time to symptom onset (days)	ZIKV RT-PCR[b]	Ct value	ZIKV ELISA IgG[c]	ZIKV ELISA IgM[c]	ZIKV NT[d]
1	30	F	Colombia	T	22	Yes	12	BFP	37.2	Positive	Positive	243
2	41	F	Honduras	R	76	Yes	11	BFP	34.9	Positive	Positive	640
3	31	M	Haiti	R	169	Yes	20	BFP	37.3	Positive	Negative	640
4	29	M	Nicaragua	T	21	Yes	19	Urine	37.3	Positive	Negative	416
5	28	F	Nicaragua	T	21	Yes	19	Urine	28.9	Positive	Negative	152
6	30	F	Mexico	T	22	Yes	14	.		Positive	Negative	640
7	22	F	Mexico	T	22	Yes	9	.		Positive	Negative	416
8	24	F	Mexico	T	22	Yes	4	.		Positive	Negative	416
9	29	F	Haiti	R	197	No	NA	.		Positive	Negative	640

F, female; M, male; ZIKV, Zika virus; NA, not applicable; Ct, cycle threshold.
[a] Travel type: T denotes tourism, R denotes relief work.
[b] Real-time reverse transcription PCR (RealStar Zika Virus RT-PCR Kit, Altona Diagnostics GmbH, Hamburg, Germany). The matrix for RT-PCR is reported in the column (BFP = capillary blood on filter paper, collected during illness abroad).
[c] ZIKV IgG/IgM enzyme linked immunosorbent assay (ELISA) (Euroimmun, Lübeck, Germany).
[d] In-house Zika virus neutralization testing assay, 90% neutralizing antibody titre.



F.

Case no.	Country	symptom onset (date)	Time to symptom onset (days)
1	Colombia	21-7-2016	12
2	Honduras	6-7-2016	11
3	Mexico	15-7-2016	14
4	Mexico	10-7-2016	9
5	Mexico	5-7-2016	4
6	Nicaragua	2-8-2016	19
7	Nicaragua	2-8-2016	19
8	Haïti	15-9-2016	20
9	Haïti	asymptomatic	NA

Figure 1. Countries and timelines of Zika virus (ZIKV) infection among participating travellers in relation to the number of autochthonous cases reported to the Pan American Health Organization (PAHO) by the national health authorities (2016). Panels A–E show the epidemic curves of ZIKV infection per country, based on the counts of suspected and confirmed cases (vertical axes) per epidemiological week (EW) in 2016 (horizontal axes), as reported to the PAHO. Note: The scales of the vertical axes are different in the different panels. The vertical lines in the graphs indicate the timing of ZIKV infection in symptomatic travellers who participated in the study. Panel F presents the country, date of symptom onset, time from arrival in the country to symptom onset, and mode of diagnosis for ZIKV cases in the study cohort.
Source: PAHO—Countries and territories with autochthonous transmission in the Americas reported in 2015–2017 (http://ais.paho.org/phip/viz/ed_zika_countrymap.asp).
Note: The latest update on the Zika epidemic received by PAHO from the Haiti national authorities was for EW 33 of 2016. However, vector-borne transmission of ZIKV was ongoing beyond EW 33, as reported byJournel *et al.* (2017).

travellers (Dasgupta et al., 2016; de Laval et al., 2016; Díaz-Menéndez et al., 2018).

In line with other series, it was confirmed that skin rash is a predominant feature and this was the only clinical predictor of ZIKV infection in travellers (Hamer et al., 2017; Meltzer et al., 2016).

Persistent adherence to mosquito bite prevention measures could not be ascertained, but the data suggest that these did not protect against ZIKV infection. However, the study lacked power to draw conclusions in this regard. Chloroquine has shown antiviral activity to ZIKV in various cell models (Delvecchio et al., 2016). When taken as malaria chemoprophylaxis, chloroquine (dosage of 300 mg base orally, once a week) did not seem to protect against ZIKV infection in three participants.

The strengths of this study are the prospective design, the collection of data and BFP during travel, the availability of follow-up sera for asymptomatic patients, and the use of RT-PCR and VNT assays to confirm the ZIKV diagnosis. The study also has several limitations. Most importantly, the sample size was small because of the sharp decline in cases towards the end of 2016. Secondly, the risk of acquiring a vector-borne disease is governed by many factors. The analysis was restricted to travellers to countries that reported epidemiological updates on the ZIKV outbreak to PAHO. It is important to be aware that this is an oversimplification of the dynamic and variable risk factors (geographic, climate, population density, activities, etc.) for acquiring vector-borne disease during travel.

In conclusion, the timing of recruitment into an observational cohort study presented the unique opportunity to study the incidence and characteristics of ZIKV infection in adult travellers prospectively. The incidence rate of ZIKV in the study participants who travelled to the epidemic territories during the 2016 outbreak

was estimated at 17% per month of travel. It exceeded most other known travel hazards. In retrospect, this finding justifies the precautionary measures and travel restrictions recommended for persons at risk of complications of ZIKV infection, including pregnant women (World Health Organization, 2016; Centers for Disease Control and Prevention (CDC), 2016). Most cases of ZIKV infection were symptomatic, with skin rash as the only predicting symptom. Asymptomatic ZIKV in adult travellers from non-endemic areas may occur less frequently than previously thought.

Acknowledgements

This work was supported by the ZikaPLAN Consortium, which received funding from the European Union's Horizon 2020 research and innovation programme under ZikaPLAN grant agreement number 734584. This work was also supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System to The National Reference Centre of Arboviruses (Institute of Tropical Medicine). The funding organizations had no role in the study design, in the collection, analysis and interpretation of the data, in the writing of the manuscript, or in the decision to submit the manuscript for publication. We thank all travellers who volunteered to participate in this study. We thank Achilleas Tsoumanis for his comments on the statistical analysis.

Ethical approval

Ethical approval was obtained from the Institutional Review Board at the Institute of Tropical Medicine and the Ethics Committee of Antwerp University Hospital, Antwerp, Belgium. All participants gave written informed consent.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceived and designed the study: RH, JJ, EB. Recruitment and training of travellers: DVDB, EL, AF, IP. Data collection and follow-up: RH, EL. Validation of RT-PCR on filter paper: RH, KE, LC. Laboratory work: DVDB, KE, LC, MVE. Analyzed the data: RH. Wrote the paper: RH, JJ, MvE, LC, EB. All authors read and approved the final version of the manuscript.

Data availability statement

The data supporting the findings of this study/publication are retained at the Institute of Tropical Medicine, Antwerp and will not be made openly accessible due to ethical and privacy concerns. Data can however be made available after approval of a motivated and written request to the Institute of Tropical Medicine at ITMresearchdataaccess@itg.be/.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2018.10.010.

References

Aliota MT, Dudley DM, Newman CM, Mohr EL, Gellerup DD, Breitbach ME, et al. Heterologous protection against Asian Zika virus challenge in rhesus macaques. PLoS Negl Trop Dis 2016;10(12):1–22.

Aubry M, Teissier A, Huart M, Merceron S, Vanhomwegen J, Roche C, et al. Zika virus seroprevalence, French Polynesia, 2014–2015. Emerg Infect Dis 2017;23(4) 2014–5 Available from: http://wwwnc.cdc.gov/eid/article/23/4/16-1549_article.htm.

Centers for Disease Control and Prevention (CDC). Zika travel information — updated March 9, 2018. 2016 Available from: https://wwwnc.cdc.gov/travel/page/zika-information.

Díaz-Menéndez M, de la Calle-Prieto F, Montero D, Antolín E, Vazquez A, Arsuaga M, et al. Experiencia inicial en infección importada por virus Zika en España. Enferm Infecc Microbiol Clin 2018;36(1):4–8.

\D'Ortenzio E, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, et al. Evidence of sexual transmission of Zika virus. N Engl J Med 2016;374 (June (22))2195–8 Available from: http://www.ncbi.nlm.nih.gov/pubmed/27074370.

Dasgupta S, Reagan-Steiner S, Goodenough D, Russell K, Tanner M, Lewis L, et al. Patterns in Zika virus testing and infection, by report of symptoms and pregnancy status — United States, January 3–March 5, 2016. MMWR Morb Mortal Wkly Rep 2016;65(April (15))395–9 Available from: http://www.cdc.gov/mmwr/volumes/65/wr/mm6515e1.htm.

Delvecchio R, Higa LM, Pezzuto P, Valadão AL, Garcez PP, Monteiro FL, et al. Chloroquine, an endocytosis blocking agent, inhibits Zika virus infection in different cell models. Viruses 2016;8(12):1–15.

de Laval F, Matheus S, Maquart M, Yvrard E, Barthes N, Combes C, et al. Prospective Zika virus disease cohort: systematic screening. Lancet 2016;388(10047):868.

Dudley DM, Aliota MT, Mohr EL, Weiler AM, Lehrer-Brey G, Weisgrau KL, et al. A rhesus macaque model of Asian-lineage Zika virus infection. Nat Commun 2016;7(June (3))12204 Available from: http://biorxiv.org/lookup/doi/10.1101/046334.

Duffy MR, Chen T-H, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360 (24):2536–43.

Hamer DH, Barbre KA, Chen LH, Grobusch MP, Schlagenhauf P, Goorhuis A, et al. Travel-associated Zika virus disease acquired in the Americas through February 2016: a GeoSentinel analysis. Ann Intern Med 2017;166(January (2))99–108 Available from: http://www.ncbi.nlm.nih.gov/pubmed/27893080.

Huits R, De Smet B, Ariën KK, Van Esbroeck M, Bottieau E, Cnops L. Zika virus in semen: a prospective cohort study of symptomatic travellers returning to Belgium. Bull World Health Organ 2017;95(December (12))802–9 Available from: http://www.ncbi.nlm.nih.gov/pubmed/29200521.

Huits R, Van Den Bossche D, Bottieau EVEM. Re lack of Zika virus antibody response in confirmed patients in non-endemic countries. J Clin Virol 2018;99–100 (February)31–4 Elsevier B.V. Available from: https://doi.org/10.1016/j.jcv.2018.07.005.

Journel I, Andrécy LL, Metellus D, Pierre JS, Faublas RM, Juin S, et al. Transmission of Zika virus — Haiti, October 12, 2015–September 10, 2016. MMWR Morb Mortal Wkly Rep 2017;66(6)172–6 Available from: http://www.cdc.gov/mmwr/volumes/66/wr/mm6606a4.htm.

Leder K, Torresi J, Libman MD, Cramer JP, Castelli F, Schlagenhauf P, et al. GeoSentinel surveillance of illness in returned travelers, 2007–2011. Ann Intern Med 2013;158(6):456–68.

Leder K, Steffen R, Cramer JP, Greenaway C. Risk assessment in travel medicine: How to obtain, interpret, and use risk data for informing pre-travel advice. J Travel Med 2015;22(1):13–20.

Leder K, Grobusch MP, Gautret P, Chen LH, Kuhn S, Lim PL, et al. Zika beyond the Americas: travelers as sentinels of Zika virus transmission. A GeoSentinel analysis, 2012 to 2016. PLoS One 2017;12(10) Available from: https://doi.org/10.1371/journal.pone.0185689.

Lessler J, Ott CT, Carcelen AC, Konikoff JM, Williamson J, Bi Q, et al. Times to key events in Zika virus infection and implications for blood donation: a systematic review (and source data from https://github. com/HopkinsIDD/ZikaLitReview). Bull World Health Organ 2016;94(November (11))841–9 Available from: http://biorxiv.org/lookup/doi/10.1101/041913.

Lustig Y, Cotar AI, Ceianu CS, Castilletti C, Zelena H, Burdino E, et al. Lack of Zika virus antibody response in confirmed patients in non-endemic countries. J Clin Virol 2018;99–100(October):31–4.

Meltzer E, Leshem E, Lustig Y, Gottesman G, Schwartz E. The clinical spectrum of Zika virus in returning travelers. Am J Med 2016;129(10)1126–30 Elsevier Inc. Available from: https://doi.org/10.1016/j.amjmed.2016.04.034.

Moreira J, Peixoto TM, Siqueira AM, Lamas CC. Sexually acquired Zika virus: a systematic review. Clin Microbiol Infect 2017;23(May (5))296–305 Elsevier Ltd. Available from: https://doi.org/10.1016/j.cmi.2016.12.027.

PAHO. Regional Zika epidemiological update. 2016 2016. Available from: https://www.paho.org/hq/index.php?option=com_content&view=article&id=11599&Itemid=41691.

Pan American Health Organization. Countries and territories with autochthonous transmission of Zika virus in the Americas reported in 2015–2017. 2017 Available from: http://www.paho.org/hq/index.php?option=com_content&view=article&id=11603&Itemid.

Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. N Engl J Med 2016;374 (April (16))1552–63 Available from: http://www.nejm.org/doi/10.1056/NEJMra1602113.

WHO. Zika virus disease — interim case definitions. WHO/ZIKV/SUR/16.1. WHO Website; 2016 2016. (February) Available from: http://www.who.int/csr/disease/zika/case-definition/en/.

World Health Organization. Information for travellers visiting Zika affected countries — updated April 19, 2017. 2016 Available from: www.who.int/csr/disease/zika/information-for-travelers/en/%0A.