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Characteristics of Zika virus infection among international travelers: A prospective study from a Spanish referral unit

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ABSTRACT

Background: From the first Zika virus (ZIKV) description, it has progressively widespread worldwide. We analyzed demographic, clinical, microbiologic and travel-related characteristic from returned patients from a ZIKV endemic country in a referral Tropical Medicine Unit.

Method: A prospective cohort study performed in a Spanish referral center with the aim of determining the significant factors associated with confirmed Zika virus (ZIKV) infection.

Results: 817 patients, (56% women, median age 36 [IQR, *Interquartile Range*: 32–42]) were enrolled. Most had returned from Latin America (n = 486; 59.4%), travelled for tourism (n = 404; 49.4%) and stayed a median of 18 days (IQR: 10–30). 602 (73.6%) presented symptoms, but only 25 (4%) were finally diagnosed with confirmed ZIKV infection (including two pregnant women, without adverse fetal outcomes), 88% (n:22) presented with fever and 92% (n:23) with rash. 56% (n:14) arthralgia and/or myalgia and 28% (n:7) conjunctivitis. The presence of conjunctivitis, fever and rash were associated with an 8.9 (95% CI: 2.2–34.9), 6.4 (95% CI: 1.2–33.3) and 72.3 (95% CI: 9.2–563.5) times greater probability of confirmed ZIKV infection, respectively.

Conclusion: Travel characteristics and clinical presentation may help clinicians to optimize requests for microbiological testing. Diagnosis of arboviriasis in travellers arriving form endemic areas remains a challenge for clinicians, but must be detected for the possible transmission outside endemic areas, where the vector is present.

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1. Introduction

Zika virus (ZIKV) disease has emerged as one of the greatest public health threats worldwide, attracting media attention for its significance and repercussions. The shortage of reported cases in Africa and Asia since its first isolation in Uganda in 1947 [1] led to an initial lack of information about the disease. Characterization of patients during the outbreaks on Yap Island (2007) [2]and French Polynesia (2013) [3] enabled description of the primary characteristics of the disease. A more complete spectrum of ZIKV disease, however, including new routes of infection and associated complications, has been outlined while the infection has explosively spread across Americas since 2015 [4–6].

The growing numbers of investigators, publications and funded projects related to ZIKV infection in recent years reflect the importance of the disease for the scientific community. The identification of novel pathways in fetal microcephaly [7] and the development of a preventive ZIKV vaccine [8] are some examples of the exponential increase in ZIKV research. However, and despite the evident advances achieved, there are still areas of ignorance.

Diagnosis of ZIKV remains a challenge due to serologic cross-reactions and clinical similarity of ZIKV infection with other flaviviruses, e.g. dengue.This fact, together with limited access to diagnostic tests in endemic countries, has led to underdiagnosis in many cases, making it difficult to estimate the real risk of infection for both the endemic population and travelers [5].

The role of tourists and others returning from ZIKV risk areas is crucial for international authorities. ZIKV-infected travelers are particularly important in geographical locations where *Aedes albopictus* and/or *Aedes aegypti,* the primary vectors, are present [9]. It becomes critical, therefore, to identify those travelers at risk of potential introduction of the disease to new areas, to prevent outbreaks or endemic circulation of the virus, as occurred previously with dengue virus (DENV) and chikungunya virus (CHIKV) in Europe [10,11]. The lack of accurate tools, however, hinders the management of travelers, which often leads to inadequate use of serologic testing. Therefore, discovering relevant clinical factors to identify at-risk cases would be an effective way to focus the diagnostic approach and reduce costs from unnecessary testing.

This study aims to describe the characteristics of ZIKV screening and to identify the risk factors associated with a confirmed diagnosis in a large cohort of travelers who returned from ZIKV-risk countries.

2. Methods

2.1. Study design

We performed a descriptive study among all patients evaluated in the Tropical Medicine Unit of Hospital Universitario La Paz- Carlos III in Madrid, Spain and tested for ZIKV infection from January 2016 to January 2017. The hospital Ethics Committee reviewed and approved the study protocol. Symptomatic patients (suggestive symptoms of an arboviral infection) and asymptomatic patients who were planning to conceive, pregnant women and their sexual partners returning from a ZIKV endemic area were included. Demographic, clinical, laboratory and travel-related data were collected in a database that met the requirements of the hospital's ethics committee.

2.2. Analyzed data

- Demographic data: Age, sex and nationality were collected for all patients. Patients were categorized into four risk groups: pregnant women, patients planning to conceive (women and men), partners of pregnant women and other.
- Clinical data: Patients were classified according to the presence of symptoms. Among symptomatic travelers, symptoms were categorized into two groups: general symptoms (e.g., fever, rash, arthralgia, conjunctivitis) and neurological symptoms (e.g., headache, paresthesia, loss of strength). The final diagnosis was also recorded.
- Characteristics of travel: Five travel purposes were designated: tourism, visiting friends and relatives (VFR), business, humanitarian service and other. Geographical destination was categorized into five areas: Latin America (including Mexico and Central America), Caribbean, Asia, Africa and USA. Date of departure and arrival were also collected.
- Microbiological data: All blood samples were screened for ZIKV infection by serological testing (indirect immunofluorescent or ELISA technique for IgM ad IgG, in both cases using commercially-available reagents from Euroinmun®, Madrid, Spain). If indicated according to international recommendations [12,13], reverse transcription polymerase chain reaction (RT-PCR) (RealStar Zika Virus RT-PCR Kit 1.0; Altona Diagnostics®, Hamburg, Germany) was performed on various biological fluids (urine, blood, semen, umbilical cord blood or amniotic fluid). A plaque reduction neutralization test (PRNT) for ZIKV was performed at the National Microbiology Center in Madrid, the reference laboratory in Spain, when needed for confirmation. ZIKV neutralization was tested by an in-house test using Vero E6 cells and 100 TCID50 of ZIKV (strain MR-766) also at the National Microbiology Center in Madrid, Spain. For neutralizing antibodies, samples were tested in two-fold dilutions from 1/32. Samples were considered positive if neutralization of viral growth at a dilution > 1/512 was observed; samples with titers between 1/32 and 1/512 were considered indeterminate; and samples < 1/32 were considered negative. DENV serology (ELISA IgM capture Vircell SA, ELISA IgG Vircell SA) and a DENV rapid test NS1 (Biosynex, Strasbourg, France) were also performed.

2.3. Diagnostic criteria and management

We followed national and international protocols for case definitions and surveillance of ZIKV infection [12,14]. A positive RT-PCR on any fluid and/or PRNT against ZIKV in the presence of IgM antibodies against ZIKV was considered a confirmed positive case. A probable acute case was defined as a positive IgM ZIKV serology without PRNT confirmation. A positive IgG with a negative IgM was considered a probable past infection and cases with negative IgG and IgM were considered a non-ZIKV-infected patient. Pregnant women with all negative markers (including neutralizing antibodies) but positive IgG were also considered a non-ZIKV-infected patient.

Fetal scan/ultrasound, including neurosonography was performed on all pregnant women who were screened for ZIKV regardless of whether they were ultimately a positive or negative case. Fetal ultrasound was repeated every 3 weeks in positive-confirmed and probable-infected women until delivery, and RT-PCR of amniotic fluid was offered in those cases from 20 to 21 weeks of gestation.

2.4. Statistical analysis

The general characteristics of the ZIKV-tested patients were analyzed according to the presence of symptoms. Continuous variables were expressed as median and interquartile range (IQR) and compared using the Mann-Whitney *U* test or a *t*-test. Frequency distributions and the chi-squared or Fisher's exact test were used for categorical variables. Multivariate logistic regression was performed including confirmed ZIKV infection as dependent variable. A predictive model was built with those variables with a significance of $p \leq 0.1$.

3. Results

During the study period, a total of 817 travelers were tested for ZIKV infection. The median age was 36 years (IQR: 32–42), and 459

(56%) were women. The majority were Spanish (626; 76%) and had travelled for tourism (404; 49.4%) to Latin America (484; 59.3%) and Asian countries (116; 14.2%). More than half (602; 73.6%) presented with symptoms suggestive of ZIKV infection. In those who were asymptomatic, the main reasons for requesting the test were the following: pregnant women who had travelled to a ZIKV-risk area during gestation; sexual partners of pregnant women; and couples planning to conceive after travelling to a ZIKV-risk area. Given the baseline differences between the two groups, symptomatic and asymptomatic travelers were analyzed separately. General characteristics of both groups are described in Table 1.

3.1. Symptomatic patients

There were 602 symptomatic patients with a median age of 35 years (IQR: 32–38), and a proportion of 54% women. The travel destinations were Ecuador (50 cases; 8.3%), Brazil (48; 8%), Mexico and the Dominican Republic (both 45; 7.5%). Tourism was the principal reason for travel (49.2%). The median stay at the destination was 18 days. The date of symptom onset was available for 567 patients. Symptoms started a median of 8 days (IQR: 3–23.5) before returning to Spain in 262 (46.2%) patients; on the day of arrival in 80 (14.1%); and a median of 5 days (IQR: 2–16) after arrival in 225 (39.7%) patients. More than half of the patients (390; 64.8%) presented with fever, accompanied by other symptoms in 219 (56%) cases. The most frequently associated symptoms were fever and arthralgia in 48 patients (7.9%); fever, rash and arthralgia in 38 (6.3%); and fever and rash in 36 (6%). Other symptoms were present in 171 (28.4%) patients and corresponded to general symptoms such as asthenia, odynophagia, rhinorrhea, general discomfort and digestive symptoms (vomiting or diarrhea). Headache was the most frequent neurological symptom (127; 21%). A significant association was found between the presence of rash, arthralgia, headache and conjunctivitis and the confirmation of ZIKV infection ($p < 0.001$). The presence of other symptoms was significantly associated with not having a ZIKV diagnosis ($p = 0.04$).

3.2. Zika-confirmed patients

ZIKV serology was suggestive of acute infection in 47 cases (7.8%), but infection could only be confirmed in 25 of them (22 were probable, with only serological diagnosis). The remaining patients were classified as indeterminate in 10 cases (1.6%), past infection in 59 (9.8%) and negative in 486 (80.7%). Table 2 summarizes the general patient characteristics according to the ZIKV microbiological test results. Confirmation was made by a positive RT-PCR in any body fluid (most frequently urine) in 21 cases, by PRNT in 3 cases and by both methods

Table 1
General characteristics of ZIKV-tested patients.

	Symptomatic n = 602	Asymptomatic n = 215	*p*
Median age (IQR)	35 (32–38)	37 (31–44)	0.018
Number of women (%)	326 (54)	133 (62)	0.038
Nationality region (N, %)	Europe: 483 (80.3)	Europe: 156 (72.5)	0.015
	Latin America: 90 (14.9)	Latin America: 51 (23.7)	
	Caribbean: 6 (0.8)	Caribbean: 4 (1.8)	
	Africa: 14 (2.3)	Africa: 1 (0.5)	
	Asia: 6 (1.15)	Asia: 1 (0.5)	
	ND: 3 (0.5)	ND: 2 (1)	
Risk group (N, %)	Pregnant: 34 (5.6)	Pregnant: 102 (47.4)	< 0.001
	PTC: 53 (8.8)	PTC: 71 (33)	
	PSP: 19 (3.1)	PSP: 42 (19.5)	
	Other: 496 (82.4)		
Travel area (N, %)	LA: 334 (55.4)	LA: 152 (70.6)	< 0.001
	Caribbean: 76 (12.6)	Caribbean: 24 (11)	
	Asia: 98 (16.2)	Asia: 18 (8.3)	
	Africa: 86 (14.2)	Africa: 3 (1.4)	
	USA: 6 (1)	USA: 16 (7.5)	
	ND: 2 (0.3)	ND 2 (1)	
Reason for travel (N, %)	Tourism: 296 (49.2)	Tourism: 108 (50.2)	0.130
	VFR: 106 (17.6)	VFR: 39 (18)	
	Business: 129 (21.4)	Business: 48 (22.3)	
	Humanitarian: 39 (6.5)	Humanitarian: 4 (2)	
	Other: 14 (2.3)	Other: 9 (4.2)	
	ND: 18 (3)	ND: 7 (3.2)	
Median time of stay (d, IQR)	18 (10–30)	15 (8–25)	0.018
Median time from arrival to visit (d, IQR)	14 (5–38)	37.5 (16–76.5)	< 0.001
General symptoms (N, %)	Fever 390 (64.8)	None	
	Rash: 137 (22.7)		
	Arthralgia: 151 (25)		
	Conjunctivitis: 24 (4)		
	Other: 171 (28)		
Neurological symptoms (N, %)	Headache 127 (21)	None	
	Paresthesia 8 (1.3)		
	Strength loss 4 (0.6)		
ZIKV microbiological results (N, %)	Probable AI: 22 (3.6)	Probable AI: 3 (1.4)	0.006
	Confirmed AI: 25 (4)	Confirmed AI: 1 (0.4)	
	Indeterminate: 10 (1.6)	Indeterminate: 3 (1.40)	
	Negative or PI: 545 (90.5)	Negative or PI: 208 (96.7)	
DENV microbiological results (N, %)	Probable AI: 45 (7.3)	Probable AI: 3 (1.40)	< 0.001
	Confirmed AI: 14 (2.3)	Confirmed AI: 0	
	Indeterminate: 10 (1.7)	Indeterminate: 7 (3.26)	
	Negative or PI: 492 (81.7)	Negative or PI: 165 (76.74)	

AI: acute infection; d: days; DENV: dengue virus; IQR: interquartile range; LA: Latin America; ND: no data; PI: past infection; PSP: Pregnant sexual partner; PTC: Planning to conceive; USA: United States of America; VFR: visiting friends and relatives; ZIKV: Zika virus.

in one case. Confirmed cases were distributed almost equally between both sexes (13 women, 12 men), median age 38 years (IQR: 32.5–43). More than half had travelled for tourism (52%; n:13), 32% (n:8) were VFR, 12% (n:3) travelled for business and 4% (n:1) for humanitarian proposes. The majority (92%) had returned from Latin America, and only 2 travelers had arrived from the Maldives. The Dominican Republic was the most frequent destination (28%), followed by Colombia (20%), Nicaragua and Cuba (12% and 8% respectively). Median time of stay at the ZIKV-risk area and median time between arrival to Spain and a Zika test was 20 days (IQR:13.8–24.8) and 10 days (IQR: 5–21) respectively. 96% of confirmed patients presented symptoms: 88% (n:22) presented with fever and rash. 56% (n:14) of patients presented with arthralgia and/or myalgia and 28% (n:7) with conjunctivitis. Regarding diagnostic tools, 56% of patients had positive IgM for ZKV (24% of these patients also had positive serology for DNV), 16% had a positive PRNT, and 88% had a positive RT-PCR. Median time between symptom onset and first ZKV RT-PCR was 7.5 days (IQR: 3.5–15.7). There were two confirmed pregnant women who had been infected during the first trimester in Colombia and Mexico. They were tested by RT-PCR for ZIKV in amniotic fluid during pregnancy and in umbilical

Table 2
General characteristics according to microbiological ZIKV results in all symptomatic tested patients.

	Confirmed AI n = 25 (4%)	Probable AI n = 22 (4%)	Indeterminate n = 10	Negative or PI n = 545	Total N = 602 (100%)	*p*
Number of women (%)	13 (4)	15 (5)	4 (1)	294 (91)	326	0.4
Nationality						0.07
Europe	14 (3)	15 (3)	7 (1)	447 (93)	483	
Latin America	11 (12.2)	6 (6.6)	2 (2.2)	71 (79)	90	
Africa	0	1 (7)	0	13 (93)	14	
Asia	0	0	0	7 (100)	7	
Caribbean	0	0	1 (20)	4 (80)	5	
Not identified	0	0	0	3 (100)	3	
Risk group						0.5
Gestational desire	1 (2)	2 (3.8)	1 (2)	48 (92.3)	52	
Pregnant	2 (6)	2 (6)	2 (6)	28 (82)	34	
Pregnant sexual partner	2 (11)	0	1 (5)	16 (84)	19	
Other	20 (4)	18 (4)	6 (1)	452 (91)	496	
Travel area						0.8
Latin America	16 (4.8)	13 (3.9)	6 (1.8)	298 (89.5)	333	
Caribbean	7 (9)	4 (5)	3 (4)	64 (82)	78	
Asia	2 (2.2)	2 (2.2)	1 (1.1)	84 (94.4)	89	
Africa	0	3 (4)	0	82 (96)	85	
USA	0	0	0	6 (100)	6	
Reason for travel						0.5
Tourism	13 (4)	8 (3)	5 (2)	270 (91)	296	
VFR	6 (6)	9 (8)	2 (2)	89 (84)	106	
Business	3 (2)	4 (3)	3 (2)	119 (92)	129	
Humanitarian	1 (1)	1 (3)	0	37 (95)	39	
Other	1 (7)	0	0	13 (93)	14	
General symptoms						0.063
Fever						
Yes	22 (6)	14 (4)	7 (2)	343 (89)	386	
No	3 (1)	8 (4)	3 (1)	202 (94)	216	
Rash						0.001
Yes	23 (17)	12 (9)	4 (3)	98 (72)	137	
No	2 (0,5)	10 (2)	6 (1)	446 (96)	453	
Arthralgia						< 0.001
Yes	14 (9)	14 (9)	3 (2)	118 (79)	149	
No	11(2)	8 (2)	7 (2)	427 (94)	453	
Conjunctivitis:						< 0.001
Yes	8 (35)	1 (4)	0	14 (61)	23	
No	17 (3)	21 (4)	10 (2)	531 (92)	579	
Other						0.04
Yes	1 (1)	3 (2)	1 (1)	165 (97)	170	
No	**24 (6)**	**19 (4)**	9 (2)	380 (88)	432	
Neurological symptoms						
Headache						
Yes	8 (6)	7 (6)	2(2)	107 (86)	124	< 0.001
No	17 (45)	15 (3)	8(2)	438 (92)	478	
Paresthesia						
Yes	1 (13)	0	1 (13)	6 (75)	8	0.1
No	24 (4)	22 (4)	9(2)	539 (91)	594	
Strength loss						
Yes	0	0	0	4 (100)	4	0.7
No	25 (4)	22 (4)	10 (2)	541 (90)	598	

AI: acute infection; d: days; DENV: dengue virus; IQR: interquartile range; ND: no data; PI: past infection; USA: United States of America; VFR: visiting friends and relatives; ZIKV: Zika virus.

cord blood, vaginal fluid and breast milk after delivery. All of the samples analyzed were negative for ZIKV. They remained in follow-up until childbirth and normal neonates were born in both cases. Among the 12 positive men (11 symptomatic and 1 asymptomatic), RT-PCR on semen was performed in 10 cases, with a positive result found in half of them. Median of time between symptom onset and time to first semen sample collection, in this group of patients, was 32.5 days (IQR: 16.7–47.5). A more detailed description of the characteristics of all confirmed patients is summarized in Table 3 (supplemental material).

Multivariate logistic regression was performed from the total of symptomatic patients. The presence of conjunctivitis, fever and rash were associated with an 8.9 (95% CI: 2.2–34.9), 6.4 (95% CI: 1.2–33.3), and 72.3 (95% CI: 9.2–563.5) times greater risk of having a confirmed Zika virus infection, respectively. A more detailed description of the characteristics of all confirmed patients is summarized in Table 3 (supplemental material).

3.3. Dengue-confirmed patients

DENV serology was also performed in 567 symptomatic patients. Of these, 59 (7.6%) patients were classified with acute DENV infection (including probable and confirmed infections). A DENV NS1 rapid test was performed in 150 cases and was positive in 15 (10%). 74 patients had simultaneously positive serology (IgG or IgM) for ZIKV and DENV (probably cross-reaction, although the possibility of co-infections cannot be excluded). PRNT was performed in 25 of these (33.8%), being positive in 7 (28%) and indeterminate in 5 (20%). None of the confirmed cases were coinfected with the two viruses.

3.4. Other diagnoses

The remaining 433 (69.6%) symptomatic patients who were not diagnosed with ZIKV or DENV were classified as unspecified febrile illness (207; 48%), travelers' febrile diarrhea (68; 15.7%), upper respiratory infections (43; 10%), probable CHIKV infection (34; 7.8%), urinary tract infection (12; 2.7%) and other diagnoses, including generalized reaction to an insect bite (e.g., prurigo) (9; 2%), schistosomiasis (9; 2%) and malaria (6; 1.4%), among others.

3.5. Asymptomatic travelers

The 215 asymptomatic travelers were mainly women (132; 62%) and were a median of 2 years older than symptomatic patients. 132 (47.4%) were pregnant women, and Latin American origin was more frequent among this group. The stay in the endemic ZIKV-risk area was 3 days shorter, and they took an average of 23.5 days more to arrive at the consultation after returning from the trip. All baseline characteristics but one (the reason for travel) were significantly different between both groups. There was only one confirmed case of ZIKV infection among asymptomatic travelers (a male with positive RT-PCR in semen). A past infection was identified in 19 patients (8.8%) and ZIKV serology was negative in the majority (189; 88%) of cases. There were no confirmed cases of DENV in this group. All serological results of asymptomatic patients are summarized in Table 1.

4. Discussion

We found that the probability of a confirmed ZIKV infection was significantly higher in travelers presenting with fever, rash or conjunctivitis. Importantly, only half of those who had initial clinical and serological data concordant with an acute ZIKV infection were finally confirmed. The average time between symptom onset and medical consultation (and sample collection) in our study was 14 days. This delay considerably reduces the possibility of obtaining a positive RT-PCR in serum, whose narrow timeframe for detection is up to 14 days after symptom onset [15]. Moreover, due to the initial ignorance about the presence of ZIKV in different body fluids, RT-PCR in urine was not requested for all patients. In our study, we found a urine sample to be positive up to 50 days from arrival [16]; thus, persistence of RT-PCR in samples other than blood may help clinicians make a late diagnosis after leaving the ZIKV-risk endemic area or after symptom onset. Of note, half of the men with a confirmed infection had a positive RT-PCR for ZIKV in semen for a median period of 32.5 days until 96 days (in one isolated symptomatic case) from symptom onset [17]. In addition, one of them was asymptomatic. These findings, together with the others previously reported [18–20] suggest that sexual transmission (unprotected oral, vaginal and anal-receptive sex) [21] may be possible regardless of the presence of symptoms, and could be associated with independent risk factors [22]. A recent systematic review of ZIKV infection in travelers revealed 73 cases of ZIKV sexual transmission in patients without a history of travel between 2016 and 2017 [23]. This information reinforces insistence on recommending protected sex for at least 3 months after a male has travelled to an ZIKV-risk area [24]. However, in our cohort differing from the findings in endemic areas, and similarly to other travellers cohorts [25] the majority of ZIKV infections in travellers were symptomatic.

Confirmed ZKV cases in our study represented 4% of all travelers screened. Other series of returning travelers shows similar proportion of confirmed cases (6%) [26]. Suggesting as in our cohort, over-testing. The absence of clinical data and a diagnostic consensus at the beginning of the epidemic, along with the clear increase of imported ZIKV infection in travelers, which was later identified by some authors (799 cases reported in 2016 compared to 131 in 2015, in a systematic review of imported ZIKV in travelers) [23] led us to test anyone returning from a ZIKV-risk zone regardless the presence of symptoms or their risk group. Current guidelines only recommend testing symptomatic individuals and pregnant women, even if they are asymptomatic [27]. Routine testing in non-pregnant asymptomatic individuals and preconception screening is no longer recommended [28].

Clinical manifestations in our cohort of confirmed patients was similar to those described in other non-endemic countries (with rash [88%], fever [76%] and arthralgia [72%] being the most common symptoms) [29], but there were some differences with other cohorts in ZIKV-risk areas (rash [97%], arthralgia [63%], conjunctivitis [56%] and fever [36%]) [30]. This may be explained by the difference in the time lapse from symptom onset to seeking medical care. Regarding the predictive factors for ZIKV infection, our confirmed patients were more likely to have rash (odds ratio [OR] 72.3), conjunctivitis (OR: 8.9) and fever (OR: 6.4) compared with unconfirmed ZIKV cases. Other studies have compared symptoms in patients diagnosed with ZIKV or DENV, showing a strong association between the presence of conjunctivitis and ZIKV diagnosis (odds ratio [OR] 30.1, 95% CI 9.57–94.44; $p < 0.001$) [31].

False positive results due to serological cross-reactivity between ZIKV and DENV have been observed in early studies [32]. We identified this potential problem in 74 travelers with simultaneous positive serology for ZIKV and DENV. This result hinders the diagnosis and forces the clinician to request a confirmatory PRNT, which is sometimes not readily available [15,32]. In these circumstances, comprehensive and updated knowledge of local epidemiology plays a crucial role in the interpretation of the serologic results. In our center, a corroborative PRNT is exclusively requested for pregnant women and is performed at the National Microbiology Center (Majadahonda, Spain). However, extending the differential diagnosis to include arboviruses other than ZIKV is important given the possibility of cotransmission (when being bitten by multiple mosquitoes carrying various viruses), and coinfection (when being infected simultaneously by diverse arboviruses) [33]. Moreover, travelers play a key role as gateways in the global spread of arboviruses, increasing the risk of dissemination to areas where there are suitable and efficient vectors and opportune climate conditions [34]. Several cases of imported ZIKV have been reported in ZIKV-free countries and appropriate vector control in these areas should be

performed to minimize the risk of establishment and spread of this disease. However, despite the presence of viremic patients in areas of Spain where the presence of *A. albopictus* has been identified so far [35–37] there have been no autochthonous cases in Spain up to date. However, we must be aware to this possibility, as the first cases of indigenous Zika in nearby France have recently been described [38].

Social alarm may have played an important role in trends in testing. On the one hand, we detected an increasing number of ZIKV requests near August and September, coinciding with the Olympic Games in Rio de Janeiro. At that time, there were international warnings that even recommended relocating or delaying the Olympics, though ultimately no cases of ZIKV infection among spectators, athletes or anyone associated with the Olympics were identified [39,40]. On the other hand, asymptomatic pregnant women constitute the largest group of tested travelers in our series. Confirmation of the scientific link between infection during pregnancy and congenital brain abnormalities [41,42] could have encouraged pregnant women to seek medical advice after travelling, regardless of the presence of symptoms. Only two pregnant women were confirmed to have ZIKV infection in our series and no fetal outcomes were registered. However, a large series of pregnant women in Catalonia (Spain) founded an incidence of infection of 1.3% (95%CI: 0.7–2.2%) during the study period with three adverse fetal outcomes: two miscarriages and one newborn with microcephaly [43].

This study had several limitations. Our cohort represents only those returned travelers who presented to our unit; therefore, our conclusions may not be representative of all global travelers. Also, social and medical alarm could have led to over-screening of ZIKV, especially in sexual partners of pregnant women and couples trying to conceive. In addition, the habit of applying the ZIKV test has changed over time, as knowledge and research on the virus and its epidemiology has progressed, and therefore the criteria for requesting testing may not have been homogeneous throughout the study. This limitation could lead to a lack of uniformity among our cohort of patients during the study period. Nonetheless, this is a limitation of all emerging infections.

Although better characterized than at the beginning of the epidemic, ZIKV infection remains a challenge for clinicians. Our data may help raise awareness of clinical manifestations suggestive of ZIKV infection. However, given the substantial overlap with DENV and CHIKV infection, testing for all three arboviruses if the epidemiology is in accordance may be necessary.

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Declaration of competing interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2019.101543.

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