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Systematic Review

Sexually acquired Zika virus: a systematic review

J. Moreira [1, *], T.M. Peixoto [2], A.M. Siqueira [1, 4], C.C. Lamas [1, 2, 3, 4]

[1] Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil
[2] Universidade do Grande Rio (Unigranrio), Rio de Janeiro, Brazil
[3] Unidade de pesquisa cardiovascular, Instituto Nacional de Cardiologia, Rio de Janeiro, Brazil

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ABSTRACT

Background: Zika virus (ZIKV) is transmitted to humans primarily by *Aedes* mosquito bites. However, circumstantial evidence points to a sexual transmission route.
Objectives: To assess the sexually acquired ZIKV cases and to investigate the shedding of ZIKV in genital fluids.
Data sources: PubMed, Scopus, Pro-MED-mail and WHO ZIKV notification databases from inception to December 2016.
Selection criteria: Reports describing ZIKV acquisition through sex and studies reporting the detection or isolation of ZIKV in the genital fluids were included.
Risk-of-bias assessment: The risk of bias was assessed using the National Institute of Health Tool.
Results: Eighteen studies reporting on sex-acquired ZIKV and 21 describing the presence of ZIKV in genital fluids were included. The overall risk of bias was moderate. Sexual transmission was male–female (92.5%), female–male (3.7%) and male–male (3.7%). Modes of sexual transmission were unprotected vaginal (96.2%), oral (18.5%) and anal (7.4%) intercourse. The median time between onset of symptoms in the index partner and presumed sexual transmission was 13 days (range 4–44 days). ZIKV RNA was detected in semen as late as 188 days (range 3–188 days) following symptom onset, and infectious virus was isolated in semen up to 69 days after symptom onset. No study reported ZIKV isolation from female genital samples, but detection did occur up to 13 days after symptom onset.
Conclusions: ZIKV is potentially sexually transmitted and persists in male genital secretions for a prolonged period after symptom onset.
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Introduction

Zika virus (ZIKV) is an emerging flavivirus belonging to the family *Flaviviridae*, which is currently responsible for a major outbreak in the Americas [1]. As of 7 December 2016, a total of 69 countries and territories have reported evidence of vector-borne ZIKV transmission since the start of the outbreak in 2015 [2].

The disease usually presents as a self-limited febrile illness, but mounting data have established an association between ZIKV infection and adverse pregnancy and fetal outcomes, with microcephaly being the most prominent, as well as other neurological syndromes, especially Guillain–Barré syndrome [3,4]. By these close associations, in February 2016 WHO declared that the situation represented a Public Health Emergency of International Concern [5]. Recently, during the fifth meeting of the Emergency Committee on ZIKV convened by WHO, the team felt that ZIKV no longer represented a Public Health Emergency of International Concern, but emphasis was made that it remains a significant enduring public health challenge that requires a long-term response mechanism [6].

The main mode of transmission of Zika virus disease (ZVD) in urban and suburban environments is by mosquito bite—*Aedes aegypti* and, to a lesser extent, *Aedes albopictus* [1]. Non-mosquito transmission does occur but the magnitude of the contributions

* Corresponding author. J. Moreira, Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz (FIOCRUZ), Avenida Brasil, 4365—Manguinhos, Rio de Janeiro, CEP: 21040-360, Brazil.
E-mail address: jose.moreira@ini.fiocruz.br (J. Moreira).
[4] André Machado de Siqueira and Cristiane C Lamas contributed equally to this article.

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of different ZIKV transmission routes to initiate or maintain the epidemics in different regions remains unclear [7]. Here we summarize the current evidence about sexually acquired ZIKV infection. Second, we assess the shedding of ZIKV in genital fluids. We hope that by assembling the available data we will be able to contribute much-needed evidence to support WHO's interim guidance regarding the prevention of the sexual transmission of ZIKV. Furthermore, we highlight the gaps in the current knowledge that should be addressed to improve our understanding of the transmission of ZIKV through sex.

Materials and methods

Search strategy

This study is a systematic review, and the PRISMA guidelines were followed [8] The PubMed and Scopus databases from inception to 8 December 2016, were searched to identify published clinical reports describing ZIKV infection acquired through sex, and studies reporting the presence of ZIKV in genital fluids. The following keywords were used in search engine: 'Zika', 'Zika infection', 'Zika fever', 'Zika virus disease', 'Sex' and 'Semen'. Additionally, we searched for unpublished sexually acquired ZIKV cases that were notified to WHO by National International Health Regulations Focal Points and the PROMED-mail database. Neither time nor language restrictions were imposed. Manual searches were also performed from the reference lists of the included articles. The study is registered at PROSPERO (CRD42016041475).

Study criteria

Published observational studies (i.e. case series and case reports) were considered for inclusion. Studies lacking primary data were excluded. Sexually acquired infections were considered for individuals without a history of residing in or travelling to areas of active ZIKV transmission who acquired ZIKV infection from a sex partner with ZIKV infection, as defined by WHO ZIKV disease interim case definitions [9]. For the sake of clarity, index subjects were those who were the likely source of infection with suspected, probable or confirmed ZIKV infection residing in or with a history of travel to areas of active ZIKV transmission or a recent ZIKV outbreak. Non-human cases were excluded. Moreover, eligible studies could include individuals in whom the presence or persistence of ZIKV was evaluated in the genital fluids throughout the disease process. Two review authors (JM, TP) independently screened the titles and abstracts of studies based on the inclusion criteria. If there was a disagreement, a consensus was arrived at through discussion with a third reviewer.

Data extraction

We extracted the following key information from the included studies: study design, country and date of publication, modes of sexual transmission, age and clinical manifestations relative to the index patients, diagnostic workout of ZVD, secondary incubation period relative to the other sex partner, presence of other sexually transmitted infections, country of ZIKV acquisition, timing of sexual intercourse in relation to disease onset in the index case (i.e. before, during or after the symptom onset), and the investigations used to exclude other non-sexual transmission routes.

For the studies that evaluated ZIKV shedding in genital fluids, we extracted the timing and the viral load of ZIKV in genital fluids in relation to other specimens tested (i.e. serum, saliva, urine, cerebrospinal fluid).

Aims

The primary aim was to assess the number of suspected, probable or confirmed sexually acquired ZIKV cases. The secondary aim was to describe the number of ZIKV-infected cases in which ZIKV shedding in genital fluids was documented.

Risk-of-bias assessment

Two independent review authors (JM, TP) assessed the quality of the individual case reports or case series using a modified National Institute of Health Tool [10].

Results

Our initial search result yielded 88 records, but only 33 articles were considered for the qualitative analysis (Fig. 1). A total of 18 reports described sexually acquired ZIKV cases, whereas 21 described the kinetics of ZIKV in genital fluids. Six articles described both outcomes.

Risk-of-bias assessment

Four studies were of low quality, 27 of medium quality and two of high quality, indicating a moderate risk of bias for the total analysis (see Supplementary material, Tables S1 and S2).

Transmission of ZIKV through sexual intercourse

We found 18 studies reporting person-to-person transmission of ZIKV through sexual intercourse, corresponding to 27 episodes of probable or confirmed sexual transmission of ZIKV [11–28]. Table 1 describes the probable or confirmed cases of sexually acquired ZIKV. Fig. 2 shows the countries reporting sexual transmission of ZIKV. The median index case age was 41 years (range 20–61 years). Fifteen studies reported male to female transmission in 25 couples; one reported male to male transmission [19], and another reported female to male transmission [24]. Modes of sexual transmission were unprotected vaginal intercourse in 96.2% (26/27), oral intercourse in 18.5% (5/27) and anal intercourse in 7.4% (2/27). Time of sexual intercourse concerning index case symptom onset was reported in 13/27 (48%) couples. Sexual intercourse occurred before, during and after the index's symptom onset in five (38.4%), seven (53.8%) and one (7.6%), respectively.

The most commonly reported signs and symptoms in the index partner were, fever (83.3%), rash (79.1%), arthralgia (58.3%), conjunctivitis hyperaemia (33.3%) and headache (25%). Fever was absent in four patients, and three were entirely asymptomatic [14,20,26]. Laboratory evidence of ZIKV infection in the index patient included positive serological test results in 20 (74%) cases and positive RT-PCR in nine (33.3%) cases. ZIKV RT-PCR was detected in seminal plasma (1/27, 3.7%), serum (2/27, 7.4%), urine (5/27, 18.5%) and semen (6/27, 22.2%). In six (22.2%) cases ZIKV was confirmed through both serology and RT-PCR. In four cases (reported in three studies), ZIKV was suspected in the index patient, and probable sexual transmission was defined based on the epidemiological and clinical history of the participants [13,14,18].

Among the 15 symptomatic index cases with known travel dates, patients reported becoming ill a median of 1 day after returning home (range 3 days before return to 6 days after return). The most frequently reported regions with active ZIKV transmission visited by index cases were the Caribbean ($n = 5$), Central America ($n = 5$) and one each for the Maldives, Senegal, Thailand and Pacific islands.

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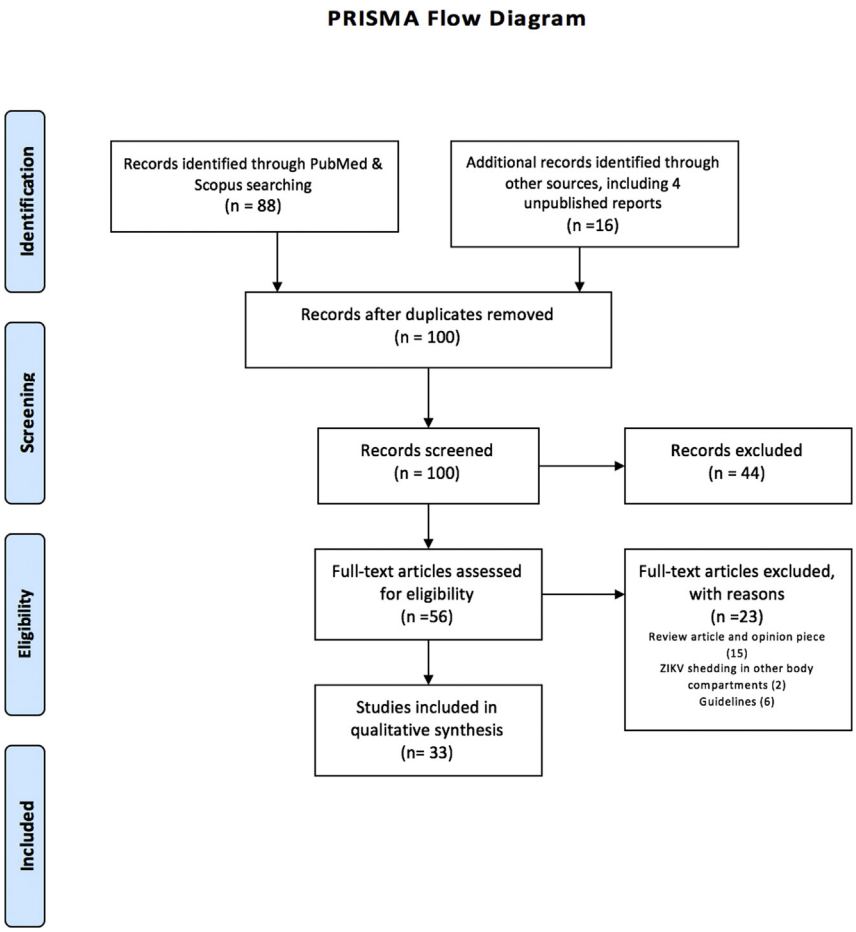

Fig. 1. PRISMA flow diagram.

A detailed medical history, as well as epidemiological and environmental investigations, was obtained to exclude other transmission routes in all cases (i.e. exclusion of vector and blood-borne transmission). Sexual transmission was further confirmed by phylogenetic analysis in two cases [22,27]. Investigations for other sexually transmitted infections were conducted in two cases, and were negative in both [17,22]. The median time between onset of symptoms in the index partner and presumed sexual transmission was 13 days (range 4–44 days).

Shedding of ZIKV in genital fluids

The description of the 21 studies that reported shedding of ZIKV in genital fluids [15,22,23,25,27–43] is shown in Table 2. An in-house ZIKV RT-PCR assay was used in five studies, and a commercial ZIKV RT-PCR assay was employed in the remaining studies (i.e. RealStar Zika Virus RT-PCR Kit 1.0; Altona Diagnostics GmbH; Hamburg, Germany). All but three reported on male genital secretions (i.e. semen, seminal plasma) [29,31,39].

ZIKV shedding in male genital tract

ZIKV RNA was detected in semen as late as 188 days following symptom onset (range 3–188 days after symptom onset). The longest recorded time for ZIKV viability (i.e. an infectious virus in cell culture) in semen was 69 days after symptom onset [15]. In five instances, cultures of ZIKV particles from semen in Vero cells were also obtained [15,35,36,41,44].

ZIKV shedding in female genital tract

No study reported long-term viral persistence in female genital secretions, but shedding occurred up to 13 days after onset of symptoms, and an attempt to culture ZIKV at the same time failed [31].

Timing of ZIKV shedding in different body compartments

ZIKV RNA detection occurred in genital fluids, and in other body compartments such as serum, urine, saliva and cerebrospinal fluid samples. The detection times for ZIKV in urine (2 days before to 91 days after symptom onset), serum (2 days before to 34 days after symptom onset), cerebrospinal fluids (7 days after symptom onset) and saliva (2 days before to 91 days after symptom onset) were much shorter than genital secretion samples (3–188 days after symptom onset).

Haematospermia as a surrogate clinical marker of positive ZIKV RNA in men

Haematospermia was reported in three studies, corresponding to four patients [16,33,41]. It was the clinical sign that prompted the seeking of medical treatment in three patients. One of them also reported symptoms of prostatitis [16]. Haematospermia was macroscopic in three, and microscopic in one. All of them had ZIKV RNA detected in the semen, and viable ZIKV particles were cultured in one case [41]. Other features concerning semen characteristics

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Table 1
Probable or confirmed cases of sexually acquired Zika virus (2011–2016)

First author, year, [reference]	Study design	Country	Category of sexual transmission	Pattern of sexual transmission	Index patient age (years)	Clinical manifestation in the index patient	Diagnostic framework in the index patient	Period of second transmission after illness onset in index patient (days)
Peru NFP [11]	CR	Peru	M–F	Vaginal intercourse	ND	ND	Positive ZIKV RT-PCR in serum, urine and semen	12
Chile NFP [12]	CR	Chile	M–F	Vaginal intercourse	ND	ND	Positive IgM and IgG for ZIKV	ND
Argentina NFP [13]	CR	Argentina	M–F	Vaginal intercourse	ND	ND	ND	ND
Portugal NFP [14]	CR	Portugal	M–F	Vaginal intercourse	ND	Asymptomatic	ND	ND
Arsuaga M, 2016 [15]	CR	Spain	M–F	Vaginal intercourse	53	Fever, rash, headache, weakness, myalgias, arthritis	Positive ZIKV RT-PCR seminal plasma Positive serum IgG and IgM for ZIKV	14
Foy BD, *et al*., 2011, [16]	CR	USA	M–F	Vaginal intercourse	36	Arthralgia, rash, fatigue, headache, *no fever*, lip ulcers, prostatitis, haematospermia	Positive serum plaque-neutralization test for ZIKV Positive haemagglutination inhibition antibody titre for ZIKV Negative serum ZIKV PCR	4
Venturi G, *et al*., 2016, [17]	CR	Italy	M–F	Vaginal intercourse	30	Rash, fever, conjunctivitis, headache	Positive serum plaque-neutralization for ZIKV Negative serum ZIKV PCR	19
Hills SL, *et al*., 2016, [18]	CS	USA	M–F	Vaginal intercourse	ND	Fever, arthralgia, conjunctivitis, rash, pruritus	ND	13-to-14
Hills SL, *et al*., 2016, [18]	CS	USA	M–F	Vaginal intercourse	ND	Fever, arthralgia, pruritus, myalgia, eye discomfort	Positive serum ZIKV IgM	10
Hills SL, *et al*., 2016, [18]	CS	USA	M–F	Vaginal intercourse	ND	Fever, arthralgia, myalgia, rash, conjunctivitis, headache	ND	13
Deckard DT, *et al*., 2016, [19]	CR	USA	M–M	Insertive anal sex	ND	Fever, rash, conjunctivitis	Positive serum ZIKV IgM Positive plaque-reduction-neutralization for ZIKV Equivocal semen ZIKV RNA (RT-PCR)	7
Freour T, *et al*., 2016, [20]	CR	France	M–F	Vaginal intercourse	ND	Asymptomatic	Positive urine and semen ZIKV RNA Positive serum ZIKV IgM. Negative serum ZIKV PCR	15
Turmel JM, *et al*., 2016, [21]	CR	France	M–F	Vaginal sexual intercourse	61	Rash, conjunctivitis, arthralgia; *no fever*	Positive serum ZIKV IgM Positive anti-ZIKV neutralizing antibodies	44
DÓrtenzio E, *et al*., 2016, [22] Matheron S. *et al*., 2016 [23]	CR	France	M–F	Vaginal intercourse, without ejaculation; oral sex with ejaculation	46	Fever, asthenia, myalgia, chills, rash,	Positive urine ZIKV RNA (RT-PCR) Positive semen ZIKV RNA (RT-PCR) Positive ZIKV IgM	13
Davidson A, *et al*., 2016 [24]	CR	USA	F–M	Vaginal intercourse	20s	Headache, abdominal cramping, fever, fatigue, rash, myalgia, arthralgia, swelling (extremities), numbness and tingling (hands and feet)	Positive serum ZIKV RNA (rRT-PCR) Positive urine ZIKV RNA (rRT-PCR)	6
Harrower J, *et al*. 2016 [25]	CR	New Zealand	M–F	Vaginal intercourse	51	Fever, rash, arthralgia, ankle oedema	Positive ZIKV IgM and IgG antibodies Positive semen RT-PCR	10
Brooks RB, *et al*. 2016 [26]	CR	USA	M–F	Vaginal intercourse; oral sex	ND	Asymptomatic	Positive ZIKV IgM antibody	10-14
Frank C, *et al*., 2016, [27]	CR	Germany	M–F	Vaginal intercourse	35	Fatigue, lymphadenopathy, arthralgias, rash; headache *no fever*	Positive serum ZIKV IgM and IgG Positive urine ZIKV RNA Positive semen ZIKV RNA	12
Russell K, *et al*. 2016, [28]	CS	USA	Nine couples M–F	Nine had vaginal intercourse, four had oral sex, one had anal sex	20–55	Eight with rash Eight with fever Five with arthralgia Three with conjunctivitis	One had positive semen RT-PCR Eight had positive ZIKV serology	10-19

Abbreviations: CR, case reports; CS, case-series; F, female; M, male; NA, not applicable; ND, not described; NFP, National International Health Regulations Focal Point, ZIKV, Zika virus.

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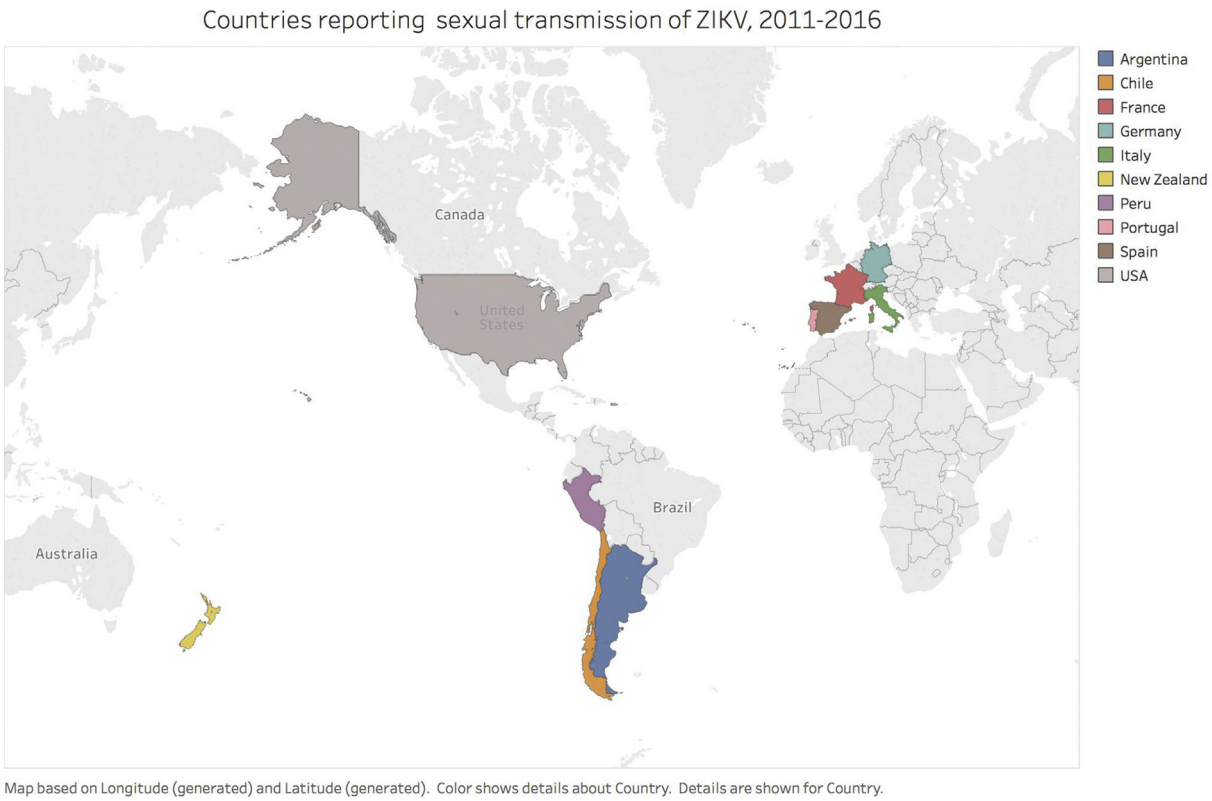

Fig. 2. Countries reporting sexual transmission of Zika virus, 2011–2016.

(i.e. sperm count, the presence of leucocytes) were altered in two patients but reverted to normal after viral clearance.

Discussion

The results of this review confirm (a) that ZIKV is potentially sexually transmitted and (b) that ZIKV may persist for a longer period after symptom onset in male genital secretions. The confirmation that ZIKV may be a sexually transmitted pathogen has wide implications for clinical care and public health policy [45].

The finding that asymptomatic and female patients transmit the disease sexually is alarming. First, asymptomatic infection represents up to 80% of those individuals with ZVD, and so would increase the risk of sexual transmission [1,46]. Second, the identification of male-to-female sexual transmission raises the issue of the vertical transmission of ZIKV and its deleterious consequences, especially in women of child-bearing age. The link of pregnancy-related ZVD with microcephaly and severe fetal outcomes is undisputed [3].

Furthermore, we found that ZIKV could be sexually transmitted very late after symptom onset (i.e. 44 days after symptom onset) due to its persistence in semen. To date, the longest that ZIKV RNA was detected in semen was 188 days after the onset of illness [43], with viable ZIKV RNA isolation in cell cultures achieved as late as 69 days after symptom onset. Here we recognize that long-term persistence of ZIKV in the male genital tract does not necessarily reflect infectivity, and more research should be done to confirm ZIKV isolation and the mechanisms of ZIKV adaptation and persistence in the genital tract. The patient in whom ZIKV was detected in semen up to 188 days after symptom onset did not infect his regular female partner, and had used condoms following diagnosis [43]; for the other patient, with detection in semen of 181 days, no onward transmission through sex was described [42]. Therefore, despite the detection of ZIKV in urine and saliva, condom use seemed effective in preventing ZIKV transmission.

The current guidance about the prevention of sexual acquisition of ZIKV in regions with no active ZIKV circulation states that both men and women returning from areas where ZIKV occurs should adopt safer sex practices, or consider abstinence for at least 6 months upon return to prevent ZIKV infection through sexual transmission [47].

We acknowledge that the magnitude of person-to-person ZIKV transmission is substantially underestimated, and the role of sexual transmission is potentially much larger than previously appreciated. A major limitation for better estimation of the magnitude of sexual transmission of ZIKV is the lack of methods that could ascertain this route in areas of active mosquito transmission. In an elegant approach, Coelho *et al.* could partially overcome this limitation using data on the incidence of ZIKV in 2015–2016 and dengue virus in 2015–2016 and 2013 for the city of Rio de Janeiro, Brazil, and suggested that women of reproductive age were 90% more likely to acquire ZIKV than their male counterparts [48]. This is an important epidemiological study conducted in an area of active ZIKV transmission, which controlled for gender-related health-seeking behaviour or the systematic testing of pregnant women being the cause of higher reporting of ZVD in females. Its implications are that controlling the vector alone may not be sufficient to control ZVD and that safe sex is highly advisable (as is widely known but not widely practiced).

Our study has limitations. First, we did not include studies that were reported in local government sites or published in news agencies, as we could not confirm their authenticity and clinical and laboratory data are almost always lacking. Second, in some articles, ZIKV was suspected, and the confirmation of sexual transmission was missing. This is critical as other specimens could also be implicated in ZIKV transmission, and represent a potential

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Table 2
Timeline and results of Zika virus RNA detection on specimens from patients with active shedding of virus in genital fluids, 2015–2016

Author	Dpi	ZIKV RT-PCR assay	Serum RT-PCR	Urine RT-PCR	Saliva RT-PCR	CSF RT-PCR	Genital RT-PCR	Genital ZIKV culture
Visseaux B [29]	2	rRT-PCR	Positive (38.1)	Positive (27.2)	ND	ND	ND	
	11	rRT-PCR	ND	Positive (30.8)	ND	ND	Positive[a] (36.7)	ND
	17	rRT-PCR	Negative	Positive (35.3)	ND	ND	Negative	
	37	rRT-PCR	ND	Negative	ND	ND	Negative	
Mansuy JM [30]	1	rRT-PCR	Positive	ND	ND	ND	ND	
	4	rRT-PCR	Positive	Positive	ND	ND	ND	
	10	rRT-PCR	Positive	Positive	ND	ND	Positive (8.6 log/mL)	ND
	18	rRT-PCR	Positive	Positive	ND	ND	Positive	ND
	25	rRT-PCR	Positive	Positive	ND	ND	Positive	ND
	34	rRT-PCR	Positive	Positive	ND	ND	Positive	ND
	46	rRT-PCR	Negative	Negative	ND	ND	Positive	ND
	53	rRT-PCR	Negative	Negative	ND	ND	Positive	ND
	67	rRT-PCR	Negative	Negative	ND	ND	Positive	ND
	98	rRT-PCR	Negative	Negative	ND	ND	Positive	ND
	118	rRT-PCR	Negative	Negative	ND	ND	Positive	ND
	139	rRT-PCR	Negative	Negative	ND	ND	Positive	ND
	141	rRT-PCR	Negative	Negative	ND	ND	Positive (3.5 log/mL)	ND
Arsuaga M [15]	47	rRT-PCR	Negative	Negative	ND	ND	Positive (27.3)	ND
	69	rRT-PCR	ND	ND	ND	ND	Positive (30.1)	positive
	96	rRT-PCR	ND	ND	ND	ND	Positive (34.07)	Negative
	124	rRT-PCR	ND	ND	ND	ND	Negative	ND
Nicastri E [31]	6	rRT-PCR	Positive (32.9)	Positive (34.2)	ND	ND	ND	
	7	rRT-PCR	Negative	Positive (31.8)	Positive (29.9)	Positive (37.0)	Positive[a] (31.1)	ND
	10	rRT-PCR	Negative	Positive (32.4)	Positive (33.5)	ND	Negative	ND
	13	rRT-PCR	Negative	Positive (29.8)	Positive (34.1)	ND	Positive[a] (34.3)	ND
	17	rRT-PCR	Negative	Positive (32.1)	Negative	Negative	Negative	
	28	rRT-PCR	Negative	Positive (32.2)	Negative	ND	Negative	
Gaskell KM [32]	3	In-house rRT-PCR	Negative	Positive	ND	ND	ND	
	22	In-house rRT-PCR	ND	ND	ND	ND	Positive (21.3)	Negative
	55	In-house rRT-PCR	ND	ND	ND	ND	Positive (30.1)	Negative
	92	In-house rRT-PCR	ND	ND	ND	ND	Positive (37.2)	Negative
	132	In-house rRT-PCR	ND	ND	ND	ND	Negative	Negative
	174	In-house rRT-PCR	ND	ND	ND	ND	Negative	Negative
RMHG H. (case 1) [33]	3	In-house rRT-PCR	Negative	Positive (34.22)	ND	ND	ND	
	4	In-house rRT-PCR	Negative	Positive (34.06)	ND	ND	ND	
	10	In-house rRT-PCR	ND	ND	ND	ND	Positive (18.22)	Negative
	11	In-house rRT-PCR	ND	Positive (40.82)	ND	ND	ND	
	20	In-house rRT-PCR	ND	ND	ND	ND	Positive (23.56)	Negative
	31	In-house rRT-PCR	ND	ND	ND	ND	Positive (28.16)	Negative
	41	In-house rRT-PCR	ND	ND	ND	ND	Positive (35.7)	Negative
	58	In-house rRT-PCR	ND	ND	ND	ND	Positive (45.63)	Negative
	68	In-house rRT-PCR	ND	ND	ND	ND	Negative	
RMHG H. (case 2) [33]	16	In-house rRT-PCR	ND	Positive (26.96)	Negative	ND	Positive (23.85)	ND
	31	In-house rRT-PCR	ND	Negative	Negative	ND	ND	
	44	In-house rRT-PCR	ND	ND	ND	ND	Positive (35.15)	ND
	50	In-house rRT-PCR	ND	ND	ND	ND	Positive (40.95)	ND
	56	In-house rRT-PCR	ND	ND	ND	ND	Negative	
	64	In-house rRT-PCR	ND	ND	ND	ND	Negative	
Wu D. [34]	-2	rRT-PCR	Positive	Positive	Positive	ND	ND	
	0	rRT-PCR	Positive	Positive	Positive	ND	ND	
	2	rRT-PCR	ND	ND	Positive	ND	ND	
	3	rRT-PCR	ND	Positive	Positive	ND	Positive	ND
	4	rRT-PCR	ND	ND	Positive	ND	ND	
	5	rRT-PCR	ND	Positive	Positive	ND	ND	
	6	rRT-PCR	ND	ND	Positive	ND	ND	

	7	rRT-PCR	ND	Positive	Positive	ND	ND	
	8	rRT-PCR	ND	Positive	ND	ND	ND	
	9	rRT-PCR	ND	Positive	ND	ND	ND	
	10	rRT-PCR	ND	Positive	ND	ND	ND	
	12	rRT-PCR	ND	Positive	ND	ND	ND	
Harrower J. [25]	19	rRT-PCR	Negative	ND	ND	ND	ND	
	21	rRT-PCR	Negative	Negative	ND	ND	ND	
	23	rRT-PCR	ND	ND	ND	ND	Positive (25)	Negative
	35	rRT-PCR	ND	ND	ND	ND	Positive (29)	
	76	rRT-PCR	ND	ND	ND	ND	Positive (35)	
	99	rRT-PCR	ND	ND	ND	ND	Negative	
	117	rRT-PCR	ND	ND	ND	ND	Negative	
Jang H-C. [35]	6	rRT-PCR	Positive	ND	ND	ND	ND	
	7	rRT-PCR	Positive	Positive	Positive	ND	Positive	Positive
	14	rRT-PCR	Negative	Positive	Positive	ND	ND	
	21	rRT-PCR	ND	Negative	Negative	ND	ND	
Mansuy JM [36]	14	rRT-PCR	Positive (2.8 log/mL)	Positive (3.1 log/mL)	ND	ND	Positive (8.6 log/mL)	Positive
Reusken C [37]	6	In-house rRT-PCR	Positive	Positive	Positive	ND	ND	
	10	In-house rRT-PCR	Negative	Positive	Negative	ND	Positive	Negative
	13	In-house rRT-PCR	ND	POSITIVE	ND	ND	Positive	Negative
	19	In-house rRT-PCR	ND	Positive	ND	ND	Positive	Negative
	20	In-house rRT-PCR	ND	Positive	ND	ND	Positive	Negative
	32	In-house rRT-PCR	ND	ND	ND	ND	Positive	Negative
	34	In-house rRT-PCR	ND	ND	ND	ND	Positive	Negative
	47	In-house rRT-PCR	ND	ND	ND	ND	Positive	Negative
	62	In-house rRT-PCR	ND	ND	ND	ND	Negative	
D'Ortenzio & Matheron S. [22,23]	16	rRT-PCR	Negative	Positive (4×10^3 copies/mL)	Negative	ND	ND	
	18	rRT-PCR	ND	ND	ND	ND	Positive (2.9×10^8 copies/mL)	Positive
	22	rRT-PCR	ND	Negative	Negative	ND	ND	
	23	rRT-PCR	ND	Negative	ND	ND	ND	
	24	rRT-PCR	ND	Positive (2.1×10^4 copies/mL)	ND	ND	Positive (3.5×10^7 copies/mL)	Positive
	80	rRT-PCR	ND	Positive (2.4×10^2 copies/mL)	ND	ND	Positive (1.2×10^3 copies/mL)	
	93	rRT-PCR	ND	Negative	ND	ND	Negative	
Atkinson B [38]	3	In-house rRT-PCR	Positive (35)	ND	ND	ND	ND	
	27	In-house rRT-PCR	Negative	Negative	ND	ND	Positive (29)	ND
	62	In-house rRT-PCR	Negative	Negative	ND	ND	Positive (33)	ND
Prisant N [39]	0	rRT-PCR	Positive	Negative	ND	ND	ND	ND
	3	rRT-PCR	ND	ND	ND	ND	Positive[a]	ND
	11	rRT-PCR	Negative	Negative	ND	ND	Positive[a]	ND
Mansuy JM [40]	93	rRT-PCR	Negative	Negative	ND	ND	Positive (3.7 log/mL)	ND
Musso D [41]	ND	In-house rRT-PCR	Negative	ND	ND	ND	Positive (2.9×10^7 copies/mL)	Positive
	ND	In-house rRT-PCR	Negative	Positive (3.8×10^3 copies/mL)	ND	ND	Positive (1.1×10^7 copies/mL)	Positive
Frank C [27]	5	rRT-PCR	Negative	ND	ND	ND	ND	
	13	rRT-PCR	Negative	Positive	Negative	ND	ND	
	45	rRT-PCR	Negative	Negative	ND	ND	Positive (6×10^4 copies/mL)	Negative
Barzon L [42]	3	In-house rRT-PCR	Positive (175 copies/mL)	Positive (25.600 copies/mL)	ND	ND	ND	
	5	In-house rRT-PCR	Positive	Positive	Positive (58.700 copies/mL)	ND	Positive (175 copies/mL)	Negative
	9	In-house rRT-PCR	Positive	Positive	Positive	ND	Positive	Negative
	15	In-house rRT-PCR	Negative	Positive	Positive	ND	Positive	Negative

(*continued on next page*)

Table 2 (*continued*)

Author	Dpi	ZIKV RT-PCR assay	Serum RT-PCR	Urine RT-PCR	Saliva RT-PCR	CSF RT-PCR	Genital RT-PCR	Genital ZIKV culture
	47	In-house rRT-PCR	Negative	Negative	Positive	ND	Positive	
	181	In-house rRT-PCR	Negative	Negative	Negative	ND	Positive	
Nicastri E [43]	17	rRT-PCR	Negative	Negative	Positive (36.4)	ND	ND	
	91	rRT-PCR	Negative	Positive (36.1)	Positive (35.4)	ND	Positive (29.6)	Negative
	134	rRT-PCR	Negative	Negative	Negative	ND	Positive (32.5)	Negative
	188	rRT-PCR	ND	ND	ND	ND	Positive (30.2)	Negative
Russell K (case1) [28]	41	rRT-PCR	ND	Negative	ND	ND	ND	
	42	rRT-PCR	ND	ND	ND	ND	Negative	ND
Russell K (case2) [28]	27	rRT-PCR	ND	Equivocal[b]	ND	ND	ND	
	28	rRT-PCR	ND	ND	ND	ND	Positive	ND
	39	rRT-PCR	ND	ND	ND	ND	Positive	
	46	rRT-PCR	ND	ND	ND	ND	Equivocal[b]	
	60	rRT-PCR	ND	ND	ND	ND	Negative	

Abbreviations: Dpi, days post onset of illness; ND, not determined; rRT-PCR, real-time reverse transcription PCR; ZIKV, Zika virus.
Zika virus-specific rRT-PCR is a commercial PCR assay; i.e. RealStar Zika Virus RT-PCR Kit 1.0; Altona Diagnostics GmbH; Hamburg, Germany.
When reported, numbers in parentheses indicate ZIKV RNA levels expressed as threshold cycle values; in log/mL; or in copies per mL.
[a] Female genital tract samples were used for Zika virus detection.
[b] Results of the first PCR assay for ZIKV RNA were discordant with the second assay (i.e. one was positive and the other was negative).

interpretation bias (i.e. saliva instead of genital fluids may be involved in ZIKV transmission through deep kissing or in oral sex) [49]. However, the fact that ZIKV sustains high titres and for prolonged periods of time after symptom onset in genital fluids is a compelling argument for this as the source of the infection. Third, we were unable to compare the ZIKV RNA titres between the studies, as different methodologies were used and no international standardized curve exists to convert CT values into viral loads. Finally, we could not provide a true estimate of the incidence of sexually acquired ZIKV cases, due to imprecision regarding the total number of reported cases of ZIKV. The strength of the present review is the evaluation of the currently available evidence using a focused systematic approach.

Future outlook

Our findings can be used to help guide future research and identify gaps in knowledge regarding the transmission of ZIKV through sex. Based on experimental animal models and the available, but limited, observations in humans, we hypothesize that ZIKV maintains an active replication process in immune-privileged sites (i.e. such as the testis) after the symptoms and viraemia have abated [50,51]. A dissociation between RNA results in blood and genital samples does occur, and might represent a unique opportunity to extend the time for viral recovery in the convalescent period. Whether testing of semen should be routinely implemented or tested in certain situations (i.e. after the initial viraemia phase faded) is a question that needs further investigation.

More research is also required on the role of sexually transmitted infections in increasing shedding of ZIKV in genital fluids, especially in situations where the serum viral load is undetectable. Studies from other non-arboviral infections suggest that the risk of sexual transmission is incremented in the presence of other sexually transmitted infections [52]. Similarly, it will be important to estimate the average risk of ZIKV transmission from specific modes of unprotected sex. Considering that not all kinds of exposure are equal, and the existing data from the non-arboviral literature (i.e. human immunodeficiency virus and hepatitis C virus), it is plausible that even in the ZIKV context, receptive anal sex may carry a much higher risk of ZIKV transmission and acquisition than receptive vaginal and oral sex [53,54].

Furthermore, it is crucial to understand the lasting effects of ZIKV on testicular and reproductive function in infected males. In one report, signs of semen inflammation were reported in participants who had a positive ZIKV seminal load [33]. Similarly, others have described symptoms of prostatitis in men with ZIKV-infected semen [16]. Furthermore, the experimental evidence of persistent damage of testes in mice raises the need to investigate whether ZIKV can result in male infertility [55]. Future studies should address the question of male fertility as well as the long-term effects of ZIKV on sperm production.

Although haematospermia remains a rare manifestation of ZVD in men, those who presented with it had ZIKV detected in seminal fluid, perhaps suggesting a tropism to genital tissues such as the prostate and testis. Individuals with haematospermia may present with active ZIKV replication in the testes, and therefore should be closely monitored and messages about safe sex should be reinforced in those individuals.

Here, we underscore that the ZIKV transmission chain is complex, and prevention efforts toward ZIKV control should focus on (a) vector control and insect bite precautions (i.e. reduction of standing water, provision of repellants, application of insecticides and larvicides); (b) avoidance of unprotected sex contact from symptomatic ZIKV-infected patients and individuals residing or

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with travel history from areas of active ZIKV transmission; and (c) routine screening of ZIKV in blood banks.

In summary, our review shows that ZIKV is potentially sexually transmitted either from symptomatic or asymptomatic infected individuals, that both genders can potentially transmit the virus, and that ZIKV shedding in the male genital tract can occur for a prolonged period after symptom onset. To address some of the issues highlighted here, there must be a robust and organized response from health authorities and the scientific community, focusing on the need to design prospective cohort studies (a) to estimate the exact risk of ZIKV transmission and acquisition from different modes of sexual exposures; (b) to estimate the true prevalence and kinetics of ZIKV infection in the genital tract; (c) to define the role of genital fluids testing during ZVD; and (d) to determine the viability of viral shedding in genital secretions.

Such knowledge gaps may be addressed in ongoing cohort studies such as the ZIKERNCOL cohort study on Sincelejo, Colombia [56], the ZIKA cohort study at Fundação Oswaldo Cruz (FIOCRUZ) in Rio de Janeiro, Brazil [57] and the Zika cohort study in men at the Institute of Tropical Medicine in Antwerp, Belgium (NCT 02733796).

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Transparency declaration

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.12.027.

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