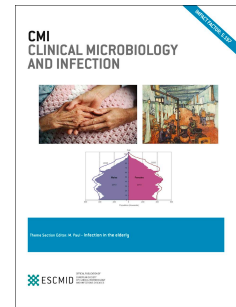# Accepted Manuscript

Sexually acquired Zika virus: a systematic review

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CMI
CLINICAL MICROBIOLOGY AND INFECTION

ESCMID

Please cite this article as: Moreira J, Peixoto TM, Machado de Siqueira A, Lamas CC, Sexually acquired Zika virus: a systematic review, *Clinical Microbiology and Infection* (2017), doi: 10.1016/j.cmi.2016.12.027.

Title: Sexually acquired Zika virus: a systematic review

Short title: Zika & Sex

Category: Systematic review

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1   Title: Sexually acquired Zika virus: a systematic review

2   Length of abstract: 232 words

3   Length of the paper (excluding references): 3052 words

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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.

The 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 intercourse (7. 4%).The median time between onset of symptoms in the index partner and presumed sexual transmission was 13 (range:4-44) days. ZIKV RNA was detected in semen as late as 188 (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.

PROSPERO systematic review registration number: CRD42016041475**.**

Keywords: Zika; Sex; Semen

1

2 **Introduction**

3 Zika (ZIKV) is an emerging flavivirus belonging to the family Flaviviridae, which is

4 currently responsible for a major outbreak in the Americas (1). As of 7th December

5 2016, a total of 69 countries and territories have reported evidence of vector-

6 borne ZIKV transmission since the start of the outbreak in 2015 (2).

7 The disease usually presents as a self-limited febrile illness, but mounting data

8 have established an association between ZIKV infection and adverse pregnancy

9 and fetal outcomes, with microcephaly being the most prominent, as well as

10 other neurologic syndromes, especially Guillain-Barre Syndrome (3, 4). By these

11 close associations, in February 2016 the World Health Organization (WHO)

12 declared that the situation represented a Public Health Emergency of

13 International Concern (PHEIC) (5). Recently, during the fifth meeting of the

14 Emergency Committee on ZIKV convened by WHO, the team felt that ZIKV no

15 longer represented a PHEIC, but emphasis was made that it remains a significant

16 enduring public health challenge that requires a long-term response

17 mechanism(6).

18 The main mode of transmission of Zika virus disease (ZVD) in urban and suburban

19 environments is by mosquito bite – *Aedes aegypti* and, to a lesser extent, *A.*

1 *albopictus* (1). However, non-mosquito transmission does occur but the

2 magnitude of the contribution of different ZIKV transmission routes to initiate or

3 maintain the epidemics in different regions remains unclear(7). Here we

4 summarize the current evidence about sexually acquired ZIKV infection.

5 Secondarily, we assess the shedding of ZIKV in genital fluids. We hope that by

6 assembling the available data we will be able to contribute to the much-needed

7 evidence to support the WHO's interim guidance regarding the prevention of the

8 sexual transmission of ZIKV. Furthermore, we highlight the gaps in the current

9 knowledge that should be addressed to improve our understanding of the

10 transmission of ZIKV through sex.

11 **Methods**

12 **Search strategy**

13 This study is a systematic review, and the PRISMA guidelines were followed(8).

14 The PubMed and Scopus databases from inception to December 8, 2016, were

15 searched to identify published clinical reports describing ZIKV infection acquired

16 through sex, and studies reporting the presence of ZIKV in genital fluids. The

17 following keywords were used in search engines: "Zika", "Zika infection", "Zika

18 fever", "Zika virus disease", "Sex", and "Semen". Additionally, we searched for

19 unpublished sexually-acquired ZIKV cases which were notified to the WHO by

1 National International Health Regulations Focal Points (NFP) and the PROMED-

2 mail database. Neither time nor language restrictions were imposed. Manual

3 searches were also performed from the reference lists of the included articles.

4 The study is registered at PROSPERO (CRD42016041475).

5 **Study criteria**

6 Published observational studies (i.e., case series, and case reports) were

7 considered for inclusion. Studies lacking primary data were excluded. Sexually

8 acquired infections were considered for individuals without a history of residing

9 or traveling to areas of active ZIKV transmission who acquired ZIKV infection from

10 a sex partner with ZIKV infection, as defined by WHO ZIKV disease interim case

11 definitions(9). For the sake of clarity, index subjects were those who were the

12 likely source of infection with suspected, probable, or confirmed ZIKV infection

13 residing in or with a history of travel to areas of active ZIKV transmission or a

14 recent ZIKV outbreak. Non-human cases were excluded. Moreover, eligible

15 studies could include individuals in whom the presence or persistence of ZIKV was

16 evaluated in the genital fluids throughout the disease process. Two review

17 authors (JM, TP) independently screened the titles and abstracts of studies based

18 on the inclusion criteria. If there was a disagreement, a consensus was arrived at

19 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 (STI), 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 the 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

1   Two independent review authors (JM, TP) assessed the quality of the individual

2   case reports or case series using a modified National Institute of Health (NIH)

3   Tool(10).

4   **Results**

5   Our initial search result yielded 88 records, but only 33 articles were considered

6   for the qualitative analysis (Figure 1). A total of 18 reports described sexually-

7   acquired ZIKV cases, whereas 21 described the kinetics of ZIKV in genital fluids. Six

8   articles described both outcomes.

9   **Risk of bias assessment**

10   Four studies were of low quality, 27 of medium quality and 2 of high quality,

11   indicating a moderate risk of bias for the total analysis (Supplementary Tables 1 &

12   2).

13   **Transmission of ZIKV through sexual intercourse**

14   We found 18 studies reporting person-to-person transmission of ZIKV through

15   sexual intercourse, corresponding to 27 episodes of probable or confirmed sexual

16   transmission of ZIKV(11-28). Table 1 describes the probable or confirmed cases of

17   sexually acquired ZIKV. Figure 2 shows the countries reporting sexual transmission

18   of ZIKV. The median index case age was 41 (range = 20-61) years. Fifteen studies

19   reported male to female transmission in 25 couples; one reported male to male

1 transmission(19), and another reported female to male transmission(24). Modes

2 of sexual transmission were unprotected vaginal intercourse in 96.2% (26/27),

3 oral intercourse in 18.5% (5/27), anal intercourse in 7.4%(2/27). Time of sexual

4 intercourse concerning index case symptom onset was reported in 13/27 (48%)

5 couples. Sexual intercourse occurred before, during, and after the index's

6 symptom onset in five (38.4%), seven (53.8%), and one (7.6%), respectively.

7 The most commonly reported signs and symptoms in the index partner were,

8 fever (83.3%), rash (79.1%), arthralgia (58.3%), conjunctivitis hyperemia (33.3%),

9 and headache (25%). Fever was absent in four patients, and three were entirely

10 asymptomatic(14, 26) (20). Laboratory evidence of ZIKV infection in the index

11 patient included positive serologic test results in 20 (74%) cases and positive

12 reverse transcriptase polymerase chain reaction (RT-PCR) in nine (33.3%) cases.

13 ZIKV RT-PCR was detected in seminal plasma (1/27,3.7%), serum (2/27, 7.4%),

14 urine (5/27,18.5%), and semen (6/27,22.2%). In six (22.2%) cases ZIKV was

15 confirmed through both serology and RT-PCR. In 4 cases (reported in three

16 studies), ZIKV was suspected in the index patient, and probable sexual

17 transmission was defined based on the epidemiological and clinical history of the

18 subjects(13, 14, 18).

1 Among the 15 symptomatic index cases with known travel dates, patients

2 reported becoming ill a median of one day after returning home (range= 3 days

3 before return to 6 days after return). The most frequently reported regions with

4 active ZIKV transmission visited by index cases were the Caribbean (n=5), Central

5 America (n=5), and one each for the Maldives, Senegal, Thailand, and Pacific

6 islands.

7 A detailed medical history, as well as epidemiological and environmental

8 investigations, was obtained to exclude other transmission routes in all cases (i.e.,

9 exclusion of vector and blood-borne transmission). Sexual transmission was

10 further confirmed by phylogenetic analysis in two cases (22, 27). Investigations for

11 other sexually transmitted infections (STI) were conducted in two cases, and were

12 negative in both (17, 22). The median time between onset of symptoms in the

13 index partner and presumed sexual transmission was 13 (range: 4-44) days.

14 **Shedding of ZIKV in genital fluids**

15 The description of the 21 studies that reported shedding of ZIKV in genital fluids

16 (15, 22, 23, 25, 27-43) is shown in Table 2. An in-house ZIKV RT-PCR assay was

17 used in five studies, and a commercial ZIKV RT-PCR assay was employed in the

18 remaining studies (i.e.; RealStar Zika Virus RT-PCR Kit 1.0; Altona Diagnostics

1   GmbH; Hamburg, Germany). All but three reported on male genital secretions

2   (i.e., semen, seminal plasma)(29, 31, 39).

3   **ZIKV shedding in male genital tract**

4   ZIKV RNA was detected in semen as late as 188 days following symptom onset

5   (range: 3-188 days after symptom onset) The longest recorded time for ZIKV

6   viability (i.e., an infectious virus in cell culture) in semen was 69 days after

7   symptom onset(15). In five instances, cultures of ZIKV particles from semen in

8   Vero cells were also obtained(15, 35, 36, 44, 45).

9   **ZIKV shedding in female genital tract**

10   No study reported long-term viral persistence in female genital secretions, but

11   shedding occurred up to 13 days after onset of symptoms, and an attempt to

12   culture ZIKV at the same time failed(31).

13   **Timing of ZIKV shedding in different body compartments**

14   ZIKV RNA detection occurred in genital fluids, and in other body compartments

15   such as serum, urine, saliva, and cerebrospinal fluid samples. The detection times

16   for ZIKV in urine (2 days before to 91 days after symptom onset), serum (2 days

17   before to 34 days after symptom onset), cerebrospinal fluids (7 days after

18   symptom onset), and saliva (2 days before to 91 days after symptom onset) were

19   much shorter than genital secretion samples (3-188 days after symptom onset).

1 **Hematospermia as a surrogate clinical marker of positive ZIKV-RNA in men**

2 Hematospermia was reported in three studies, corresponding to four patients (16,

3 33, 41). It was the clinical sign that prompted seeking medical treatment in three

4 patients. One of them also reported symptoms of prostatitis(16). Hematospermia

5 was macroscopic in three, and microscopic in one. All of them had ZIKV-RNA

6 detected in the semen, and viable ZIKV particles were cultured in one case(41).

7 Other features concerning semen characteristics (i.e. sperm count, the presence

8 of leukocytes) were altered in two patients but reverted to normal after viral

9 clearance.

10 **Discussion**

11 The results of this review confirm (i) that ZIKV is potentially sexually transmitted

12 and (ii) that ZIKV may persist for a longer period after symptom onset in male

13 genital secretions. The confirmation that ZIKV may be a sexually transmitted

14 pathogen has wide implications for clinical care and public health policy(46).

15 The finding that asymptomatic and female patients transmit the disease sexually

16 is alarming. First, asymptomatic infection represents up to 80% of those

17 individuals with ZVD, and thus, would enhance the risk of sexual transmission(1,

18 47). Second, the identification of male to female sexual transmission raises the

19 issue of the vertical transmission of ZIKV and its deleterious consequences

1 especially in women of child-bearing age. The link of pregnancy-related ZVD with

2 microcephaly and severe fetal outcomes is undisputed(3).

3 Furthermore, we found that ZIKV could be sexually transmitted very late after

4 symptom onset (i.e., 44 days after symptom onset) due to its persistence in

5 semen. To date, the longest that ZIKV RNA was detected in semen was 188 days

6 after the onset of illness(43), with viable ZIKV RNA isolation in cell cultures

7 achieved as late as 69 days after symptom onset. Here we recognize the fact that

8 long-term persistence of ZIKV in male genital tract does not necessarily reflect

9 infectivity, and more research should be done to confirm ZIKV isolation and the

10 mechanisms of ZIKV adaptation and persistence in the genital tract. The patient in

11 whom ZIKV was detected in semen up to 188 days after symptom onset did not

12 infect his regular female partner, and that he had used condoms following

13 diagnosis(43); for the other patient, with 181 days detection in semen, no onward

14 transmission through sex was described (42). Therefore, despite the detection of

15 ZIKV in urine and saliva, condom use seemed effective in preventing ZIKV

16 transmission.

17 The current guidance about the prevention of sexual acquisition of ZIKV in regions

18 with no active ZIKV circulation states that both men and women returning from

19 areas where ZIKV occurs should adopt safer sex practices, or consider abstinence

1   for at least six months upon return to prevent ZIKV infection through sexual

2   transmission(48).

3   We acknowledge that the magnitude of person-to-person ZIKV transmission is

4   substantially underestimated, and the role of sexual-borne transmission is

5   potentially much larger than previously appreciated. A major limitation for better

6   estimation of the magnitude of sexual transmission of ZIKV is the lack of methods

7   that could ascertain this route in areas of active mosquito transmission. In an

8   elegant approach, Coelho et al., could partially overcome this limitation using

9   data on the incidence of ZIKV in 2015 to 2016, and dengue in 2015 to 2016, and

10 2013 for the city of Rio de Janeiro, Brazil, and suggested that women of

11 reproductive age were 90% more likely to acquire ZIKV than their male

12 counterparts(49). This is an important epidemiological study conducted in an area

13 of active ZIKV transmission, which controlled for gender-related health seeking

14 behavior or the systematic testing of pregnant women was the cause of higher

15 reporting of ZVD in females. Its implications are that controlling the vector alone

16 may not be sufficient to control ZVD and that safe sex is highly advisable (as it is

17 widely known but not widely practiced).

18 Our study has limitations. Firstly, we did not include studies that were reported in

19 local government sites or published in news agency, as we could not confirm their

1 authenticity and clinical and laboratory data are almost always lacking. Secondly,

2 in some articles, ZIKV was suspected, and the confirmation of sexual transmission

3 was missing. This is critical as other specimens could also be implicated in ZIKV

4 transmission, and represent a potential interpretation bias (i.e. saliva instead of

5 genital fluids may be involved in ZIKV transmission through deep kissing or in oral

6 sex)(50). However, the fact that ZIKV sustains high titers and for prolonged

7 periods of time after symptom onset in genital fluids is a compelling argument for

8 this as the source of the infection. Thirdly, we were unable to compare the ZIKV

9 RNA titers between the studies, as different methodologies were used and no

10 international standardized curve exists to convert CT values into viral loads.

11 Finally, we could not provide a true estimate of the incidence of sexual-acquired

12 ZIKV cases, due to imprecision regarding the total number of reported cases of

13 ZIKV. The strength of the present review is the evaluation of the currently

14 available evidence using a focused systematic approach.

15 **Future outlook**

16 Our findings can be used to help guide future research and identify gaps in

17 knowledge regarding the transmission of ZIKV through sex. Based on

18 experimental animal models and the available, but limited human observations,

19 we hypothesize that ZIKV maintains an active replication process in immune-

1 privileged sites (i.e., such as the testis) after the symptoms and viremia has

2 abated(51, 52). A dissociation between RNA results in blood and genital samples

3 does occur, and might represent a unique opportunity to extend the time for viral

4 recovery in the convalescent period. Whether testing of semen should be

5 routinely implemented or tested in certain situations (i.e., after the initial viremia

6 phase faded) is a question that needs further investigation.

7 More research is also required on the role of STI in increasing shedding of ZIKV in

8 genital fluids, especially in situations where the serum viral load is undetectable.

9 Studies from other non-arboviral infections suggest that the risk of sexual

10 transmission is incremented in the presence of other STI(53). Similarly, it will be

11 important to estimate the average risk of ZIKV transmission from specific modes

12 of unprotected sex. Considering that not all kinds of exposure are equal, and the

13 existing data from the non-arboviral literature (i.e., HIV and HCV), it is plausible

14 that even in the ZIKV context, receptive anal sex may carry a much higher risk of

15 ZIKV transmission and acquisition than receptive vaginal and oral sex(54, 55).

16 Furthermore, it is crucial to understand the lasting effects of ZIKV on testicular

17 and reproductive function in infected males. In one report, signs of semen

18 inflammation were reported in subjects who had a positive ZIKV seminal load(33).

19 Similarly, others have described symptoms of prostatitis in men with ZIKV-

1 infected semen(16). Furthermore, the experimental evidence of persistent

2 damage of testes in mice raises the need to investigate whether ZIKV can result in

3 male infertility(56). Future studies should address the question of male fertility as

4 well as the long-term effects of ZIKV on sperm production.

5 Although hematospermia remains a rare manifestation of ZVD in men, those who

6 presented with it had ZIKV detected in seminal fluid, perhaps suggesting a tropism

7 to genital tissues such as the prostate and testis. Individuals with hematospermia

8 may present with active ZIKV replication in the testes, and therefore should be

9 closely monitored and messages about safe sex should be reinforced in those

10 individuals.

11 Here, we underscore that the ZIKV transmission chain is complex, and prevention

12 efforts toward ZIKV control should focus on (i) vector control and insect bite

13 precautions (i.e.; reduction of standing water, provision of repellants, application

14 of insecticides and larvicides); (ii) avoidance of unprotected sex contact from

15 symptomatic ZIKV-infected patients and individuals residing or with travel history

16 from areas of active ZIKV transmission; and (iii) routine screening of ZIKV in

17 blood—banks.

18 In summary, our review shows that ZIKV is potentially sexually transmitted either

19 from symptomatic or asymptomatic infected individuals, that both genders can

1 potentially transmit the virus, and that ZIKV shedding in male genital tract can

2 occur for a prolonged period after symptom onset. To address some of the issues

3 highlighted here, there must be a robust and organized response from health

4 authorities and scientific community, focusing on the need to design prospective

5 cohort studies (i) to estimate the exact risk of ZIKV transmission and acquisition

6 from different modes of sexual exposures; (ii) to estimate the true prevalence and

7 kinetics ZIKV infection in the genital tract;(iii) to define the role of genital fluids

8 testing during ZVD; and (iv) to determine the viability of viral shedding in genital

9 secretions.

10 Such knowledge gaps may be addressed in ongoing cohort studies such as the

11 ZIKERNCOL cohort study on Sincelejo, Colombia(57), the ZIKA cohort study at

12 Fundação Oswaldo Cruz (FIOCRUZ) in Rio de Janeiro, Brazil(58), and the Zika

13 cohort study in men at the Institute of Tropical Medicine in Antwerp, Belgium

14 (NCT 02733796).

15 **Acknowledgments**

1 **Transparency declaration**

2 The authors declare that they have no conflicts of interest.

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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 2nd 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 hemagglutination inhibition antibody titer 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

Fréour 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
D´Ortenzi o E, *et al.* 2016, [22] Mathero n 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,	Positive serum ZIKV RNA (rRT-PCR); Positive urine ZIKV RNA (rRT-PCR)	6

						arthralgia, swelling (extremities), numbness and tingling (hands and feet)		
Harrower J, et al. 2016 [25]	CR	New Zealand	M-F	vaginal intercourse	51	Fever, rash, arthralgia, ankle edema	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, arthalgias, 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	9 couples M-F	9 had vaginal intercourse, 4 had oral sex,	20-55	8 with ash 8 with fever 5 with arthralgia 3 with conjunctivitis	1 had positive semen RT-PCR 8 had positive ZIKV serology	10-19

1 had anal sex

CR stands for case reports; CS case-series; F female; M male; NA not applicable; ND not described; NFP National International Health Regulations Focal Point, RT-PCR reverse transcriptase polymerase chain reaction; ZIKV Zika virus

Table 2. Timeline and results of ZIKV RNA detection on specimens from patients with active shedding of ZIKV 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[§] (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	ND

							log/mL)	
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[§] (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[§] (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	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-	ND	ND	ND	ND	positive	Negativ

		house rRT-PCR					(45.63)	e
	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-	ND	ND	positive	ND	ND	

		PCR						
	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	
Hee-Chang J. [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	positive (3.1	ND	ND	positive (8.6	positive

			log/mL)	log/mL)			log/mL)	
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	ND	ND	ND	ND	negative	

		rRT-PCR						
D'Ortenzio & Matheron S.[22,23]	16	rRT-PCR	negative	positive (4 x 10^3 copies/mL)	negative	ND	ND	
	18	rRT-PCR	ND	ND	ND	ND	positive (2.9 x 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 x 10^4 copies/mL)	ND	ND	positive (3.5 x 10^7 copies/mL)	positive
	80	rRT-PCR	ND	positive (2.4 x 10^2 copies/mL)	ND	ND	positive (1.2 x 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§	ND

	11	rRT-PCR	negative	negative	ND	ND	positive[§]	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 x 10^7 copies/mL)	Positive
	ND	In-house rRT-PCR	negative	positive (3.8 x 10^3 copies/mL)	ND	ND	positive (1.1 x 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 x 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
	47	In-	negative	negative	positive	ND	positive	

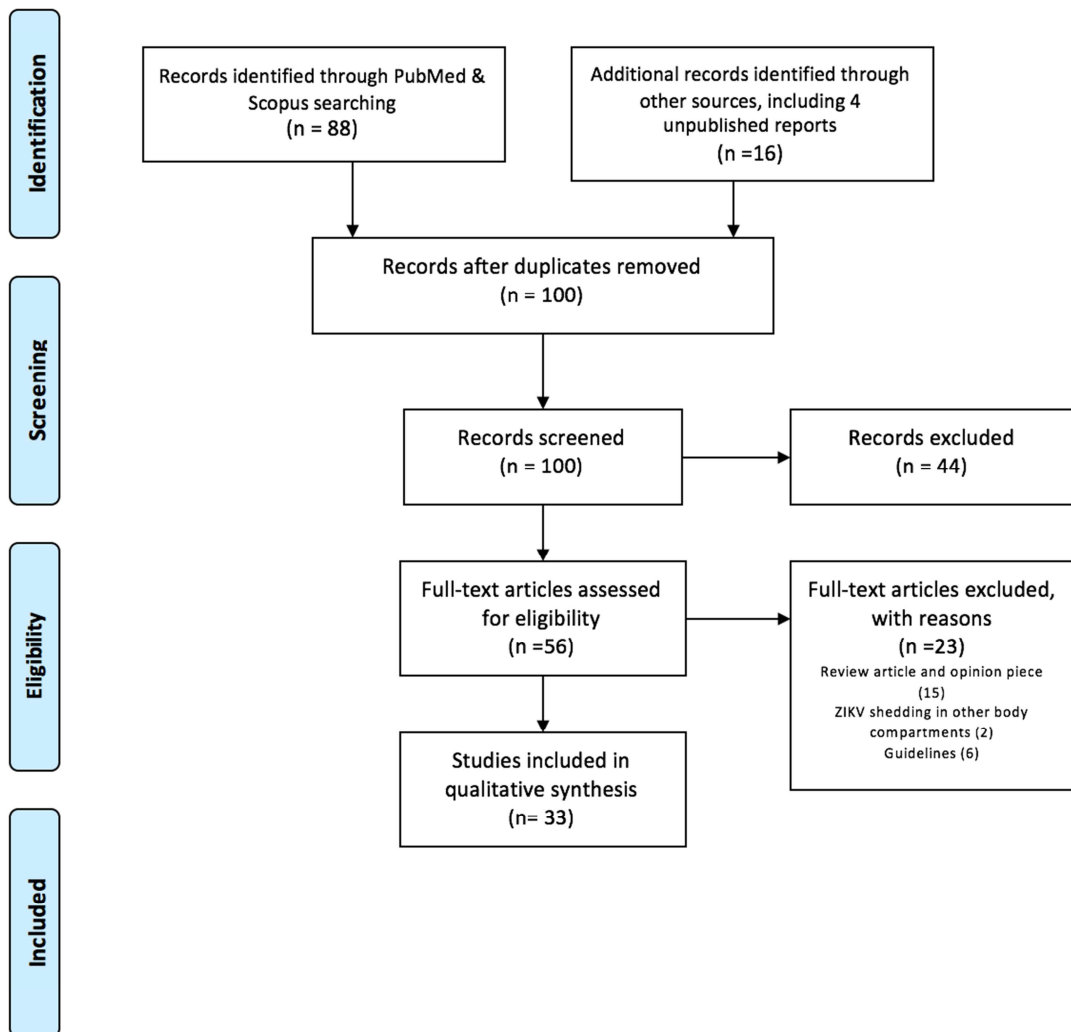|								
		house rRT-PCR						
	181	In-house rRT-PCR	negative	negative	negative	ND	positive	
Nicastri E [43]	17	rRT-PCR	negative	negative	positive (36.4)	ND	ND	
	91	rRT-PCR	negative	positive (36.1)	positive (35.4)	ND	positive (29.6)	Negative
	134	rRT-PCR	negative	negative	negative	ND	positive (32.5)	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	equivoca¶	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	equivoca¶	
	60	rRT-PCR	ND	ND	ND	ND	negative	

Dpi stands for days' post onset of illness; ND not determined; RT-PCR reverse transcription polymerase chain reaction; 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.

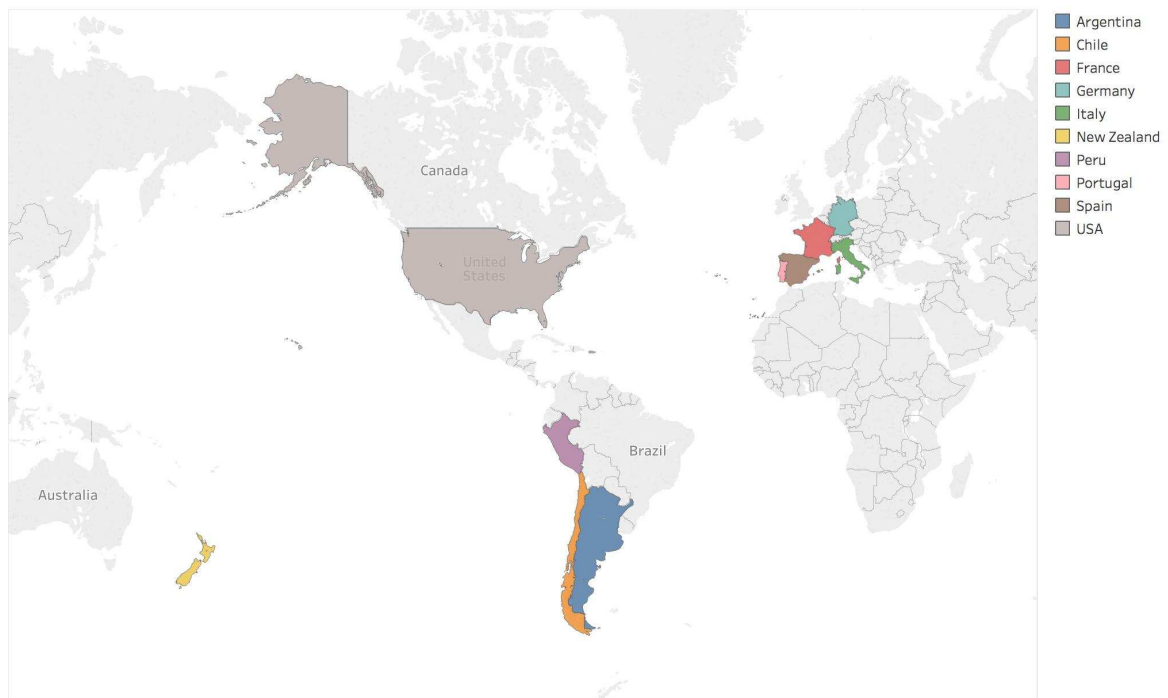§means that female genital tract samples were used for Zika virus detection

¶means that the results of the first PCR assay for ZIKV RNA was discordant with the second assay (i.e. one was positive and another negative).

PRISMA Flow Diagram

Identification

Records identified through PubMed &
Scopus searching
(n = 88)

Additional records identified through
other sources, including 4
unpublished reports
(n =16)

Screening

Records after duplicates removed
(n = 100)

Records screened
(n = 100)

Records excluded
(n = 44)

Eligibility

Full-text articles assessed
for eligibility
(n =56)

Full-text articles excluded,
with reasons
(n =23)
Review article and opinion piece
(15)
ZIKV shedding in other body
compartments (2)
Guidelines (6)

Included

Studies included in
qualitative synthesis
(n= 33)

Countries reporting sexual transmission of ZIKV, 2011-2016

■	Argentina
■	Chile
■	France
■	Germany
■	Italy
■	New Zealand
■	Peru
■	Portugal
■	Spain
■	USA

Map based on Longitude (generated) and Latitude (generated). Color shows details about Country. Details are shown for Country.