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Transmission of Zika virus through breast milk and other breastfeeding-related bodily-fluids: a systematic review

Susannah Colt,^a Maria N Garcia-Casal,^b Juan Pablo Peña-Rosas,^b Julia L. Finkelstein,^a Pura Rayco-Solon,^b Zita Weise Prinzo,^b & Saurabh Mehta^a

^aDivision of Nutritional Sciences, Cornell University, 314 Savage Hall, Ithaca, New York 14853, USA

^bDepartment of Nutrition for Health and Development, World Health Organization, Geneva, Switzerland

Correspondence to Saurabh Mehta (email: smehta@cornell.edu)

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Abstract

Objective

Zika virus (ZIKV) infection is an emerging mosquito-borne disease, which is associated with an increase in central nervous system malformations and newborn microcephaly cases. This review investigated evidence of breastfeeding transmission from ZIKV-infected mothers to their children and the presence of ZIKV infection in breastfeeding-related fluids.

Methods

We conducted a systematic review of observational studies, case studies, and surveillance reports involving breastfeeding women with ZIKV infection in several international databases. Data extraction and analysis were conducted following a PROSPERO-registered protocol.

Findings

From 471 non-duplicate records, one case report met criteria for inclusion. A second case report was included after the search was conducted. We reviewed three cases of ZIKV infection among lactating mothers near the time of delivery. Two of the three (2/3) associated newborns had evidence of ZIKV infection. ZIKV was detected in breast milk of all three mothers. Breast milk detection results were positive in all mothers (3/3) by RT-PCR, one was positive by culture (1/3), and none were tested for ZIKV-specific antibodies. Serum samples were ZIKV positive in all mothers (3/3), and sweat was not tested for ZIKV.

Conclusion

We describe three cases of ZIKV-infected breastfeeding mothers who were symptomatic within three days of delivery, and two cases with ZIKV-infected newborns. While ZIKV was detected in the breast milk of all three mothers, the data are not sufficient to conclude ZIKV transmission via breastfeeding. More evidence is needed to distinguish breastfeeding transmission from other perinatal transmission routes.

Key words

Zika, flavivirus, breastfeeding, breast milk, mother-to-child transmission

Introduction

Zika virus (ZIKV) infection is an emerging vector-borne disease of the *Flaviviridae* family, which includes dengue, yellow fever, Japanese encephalitis, and West Nile viruses (1). ZIKV infection causes a mild, self-limiting influenza-like illness with a 10-day incubation for most cases and shares similarities with other circulating arthropod-borne viral infections like the alphavirus chikungunya (1, 2). Many cases of ZIKV infection are asymptomatic and therefore unreported.

The World Health Organization has developed an interim case definition to classify and report cases of ZIKV infection (Figure 1). A **suspect case** is a person presenting with rash and/or fever and at least one of the following: arthralgia, arthritis or conjunctivitis. A **probable case** is a suspected case with presence of IgM antibody against ZIKV and an epidemiological link; and a **confirmed case** is a person with laboratory confirmation of recent ZIKV infection: by presence of ZIKV RNA or antigen in serum or other samples **or** IgM antibody against ZIKV positive and plaque reduction neutralization test $\geq 90\%$ (PRNT₉₀) for ZIKV with titre ≥ 20 and ZIKV PRNT₉₀ titre ratio ≥ 4 compared to other flaviviruses (3). Due to the possible cross reactivity with other members of the *Flaviviridae* family, the presence of IgM is not enough to rule out ZIKV infection, and the PRNT₉₀ will determine if the *in vitro* inhibition of cell growth is produced by antibodies against ZIKV (4, 5). An enzyme linked immunoassay (ELISA) for ZIKV has been developed by the Centers for Disease Control and Prevention, but is only available upon request for emergency use (6).

The timing and the test performed could be crucial for detecting the ZIKV infection. During the first 7 days, viral RNA can often be identified by RT-PCR, but as viremia decreases, a negative RT-PCR does not exclude flavivirus infection, and serologic testing should be performed. On the other hand virus-specific IgM antibodies may be detectable >4 days after onset of illness, however a sample taken within 7 days of illness onset may not have detectable virus-specific IgM antibodies (7).

ZIKV transmission occurs primarily via the bite of *Aedes aegypti* mosquitoes, in addition to *Aedes* spp. *Ae. africanus*, *Ae. albopictus*, *Ae. hensilli*, and *Ae. luteocephalus* (2, 8-12). However, perinatal, transfusion, and sexual transmission have also been reported (13-17). Among infected individuals, evidence of ZIKV has been detected in serum, saliva, urine, semen, and breast milk (13, 18-22). Generally, transmission of antibodies through breast milk has been described, particularly for IgA, conferring passive immunity (23). The presence of IgA, IgG, or IgM antibodies against similar flaviviruses such as West Nile Virus has been reported in breast milk (24). Given recent increases of ZIKV cases in Central and South America and suggested associations with congenital microcephaly and other non-congenital neurological or autoimmune disorders, an investigation of transmission via breast milk is needed (25).

Until recently, outbreaks of ZIKV were sporadic. During the last 50 years, widespread infection throughout Africa and Southeast Asia is suspected, but the asymptomatic nature and limited diagnostics have likely hampered disease surveillance (12, 26-28). In 2007, the disease migrated to Oceania where an outbreak in Yap State in the Federated States of Micronesia infected roughly 5,000 individuals, nearly 75% of the island population (2). The next outbreaks occurred in French Polynesia (396 confirmed), New Caledonia (1,400 confirmed), and the Cook Islands

(50 confirmed) in 2013-2014 (29-31). The first official outbreak in the Americas arrived to Easter Island, Chile in early 2014 with 51 confirmed cases (32). In April, 2015, Brazil reported the first confirmed autochthonous case of ZIKV infection (33). Since then, an epidemic has rapidly expanded affecting 35 countries and territories in South and Central America (34). The Brazilian Ministry of Health estimates the number of ZIKV cases in 2015 between 0.4-1.3 million (8).

During the Brazilian ZIKV epidemic, clinicians have observed a 20-fold increase in suspected cases of microcephaly in newborns (35). Reported microcephaly and/or central nervous system malformations have affected 7,150 individuals in Brazil between 22 October 2015 and 16 April 2016 (34). Flaviviruses have not been known to cause microcephaly, however ZIKV has been confirmed in recent microcephaly cases, which has prompted global concern for pregnant women and a large-scale investigation (36). A recent report from the World Health Organization indicated that there is scientific consensus that Zika virus is a cause of microcephaly and Guillain-Barré syndrome (34), based on new evidence from studies (37, 38). Many mothers of infants with microcephaly reported no illness or symptoms associated with Zika infection (39). Regardless of symptoms, pregnant women are at risk for infection and potential complication in any trimester (13, 40). At this time, the World Health Organization recommends standard breastfeeding practices for all mothers, regardless of ZIKV infection (41), unless there is an acceptable medical reason for permanent or temporary avoidance of breastfeeding (42).

The primary objective of this systematic review was to determine the transmission of ZIKV through breastfeeding. For the purposes of this review, ZIKV infection included suspected, probable, or confirmed cases as described by the WHO interim case definition. A secondary objective assessed the presence of ZIKV or ZIKV-specific antibodies in breast milk and breastfeeding-related bodily fluids (i.e. blood or sweat) of lactating women. We sought to address the following questions:

Primary Outcome: In ZIKV-free infants or children, does breastfeeding (any or exclusive) from a ZIKV-infected lactating mother, compared to not breastfeeding, result in evidence of ZIKV infection in the infant?

Secondary Outcome: Are there ZIKV specific antibodies present in breast milk?

Methodology

Study criteria

We included observational studies, case studies, and surveillance reports. Study participants were limited to adolescents or adult women who were lactating, or expressing milk, with ZIKV infection. This includes lactating participants who were currently breastfeeding or not, as well as those who were breastfeeding prior to a ZIKV presumptive diagnosis.

Exposure criteria were described as any mothers with ZIKV infection who were breastfeeding or expressing breast milk. Primary outcomes included any ZIKV infection in infants or children, within 30 days of breastfeeding or receiving expressed breast milk from a mother with ZIKV infection or detection of ZIKV in breast milk, maternal blood, or maternal sweat. Detection methods include:

- ZIKV RNA by reverse transcriptase polymerase chain reaction (RT-PCR)
- ZIKV-specific IgM antibody by ELISA
- PRNT₉₀ for ZIKV with titre > 20 and ZIKV PRNT₉₀ titre ratio > 4 compared to other flaviviruses
- ZIKV isolation in culture

Search strategy

A search overview is provided in Appendix 1.

Electronic databases: Search terms included variations and permutations of United States National Library of Medicine Medical Subject Headings (MeSH) terms and text words relating to flaviviruses (Zika, West Nile, and yellow fever), breastfeeding, transmission fluids (breast milk, blood, and sweat), and participants (mother or child) (See appendix for full search strategy). No language or time restrictions were applied. The following electronic databases were searched:

- MEDLINE & MEDLINE in Process (OVID) (1946 to 9/3/16)
- PubMed
- CINAHL (Ebsco) (1982 to March 2016)
- Web of Science (ISI) SCI, SSCI, CPCI & CPCI-SSH to 2/3/16
- Popline to March 2016
- LILACS (Birme) 1982 to March 2016
- PAHO (Birme) to March 2016
- WHOLIS (Birme) to March 2016
- WPRIM to March 2016
- IMSEAR to March 2016

Additional search strategy: To identify ongoing and unpublished studies or case reports, we searched the WHO International Clinical Trials Registry Platform (ICTRP - <http://apps.who.int/trialsearch/Default.aspx>), and the PAHO Zika Research Portal (<http://www.paho.org/zika-research/index.php>) separately on March 11, 2016. We also contacted CDC and the WHO and PAHO Zika outbreak teams for recent or unpublished findings.

Study Selection

Screening of search results was performed using Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. Two authors independently screened the titles and abstracts of studies based on the inclusion criteria. A third author assessed and resolved disagreements on study selection. All irrelevant titles were excluded. For studies that met eligibility criteria, full text articles were obtained and managed using EndNote, version X7.5 © 2016 Thomson Reuters, reference management software.

Data extraction and management

A data extraction form was tailored for this review. One author extracted study characteristics and two authors extracted study outcome data according to the pre-designed data extraction form. For each study, information pertaining to the source, eligibility, methods, participants, exposures, outcomes, and results was entered into the data extraction form. When relevant, effect estimates including odds ratios, relative risks, mean differences, or summary effects were extracted for each outcome. All potential modifiers or confounders of study outcomes were included in the extraction form.

Our study design followed a pre-established protocol based on methods for systematic reviews described in the Cochrane Handbook for Systematic Reviews (43). The review was registered in PROSPERO, the international prospective register of systematic reviews of the University of York and the National Institute for Health Research, under the number CRD42016036667.

Results

The databases described were searched on March 11, 2016 and yielded 472 records after duplicates were removed (Figure 2). Initial screening retained 42 records. At the time of screening, inclusion criteria included terms for West Nile and yellow fever viruses. For the purposes of this review, only ZIKV was considered for quantitative analyses, which yielded 1 record for review (Figure 2). A total of 2 studies (that included 3 mother child pairs) were included for analysis. The main reasons for exclusion were non-ZIKV infections or ineligible populations.

A case report from the ZIKV outbreak in French Polynesia described two mothers, who had recently given birth, with ZIKV infection (Table 1) (13). Mother 1 initiated breastfeeding to Newborn 1 on the day of delivery. On day 2 following delivery, mother 1 had a confirmed case of ZIKV detected by serum RT-PCR and saliva RT-PCR. On day 3, the breast milk from mother 1 was found to contain ZIKV by RT-PCR, however ZIKV breast milk culture was negative. Also on day 3, Newborn 1 had confirmed ZIKV infection by serum RT-PCR and saliva RT-PCR.

Mother 2 was confirmed with ZIKV infection on days 1 and 5 post delivery by serum RT-PCR and initiated breastfeeding on day 3. On day 8, the ZIKV RT-PCR results from Mother 2 were serum negative, urine positive, and breast milk positive, however ZIKV breast milk culture was negative. Newborn 2 tested negative for ZIKV on day 0 and day 3 by serum RT-PCR, but had confirmed ZIKV infection on days 4 and 7 by serum RT-PCR and on day 8 by urine RT-PCR. On day 9, Newborn 2 urine was ZIKV negative by RT-PCR. These case reports confirmed ZIKV infection in 2 breastfeeding mothers and their newborns as well as detected ZIKV in serum and

breast milk of both mothers. Both mothers had clinical signs of rash within days of delivery, and the authors conclude that vertical ZIKV transmission likely occurred during vaginal delivery.

A second case report describes a mother (referred to as Case 3 in Table 1) from New Caledonia who initiated breastfeeding on the day of delivery and developed fever and maculopapular rash in the following days (44). On day 3 post delivery, the mother tested positive for ZIKV infection by serum RT-PCR and the newborn's test results were reported as ambiguous. Breast milk was ZIKV positive by RT-PCR on day 4 and ZIKV breast milk culture was also positive. While vertical transmission was not described in this case, the presence of ZIKV in breast milk was confirmed. As shown in Table 2, the quality of the evidence is very low for all the proposed outcomes.

Discussion

The cases presented in these two reports confirm the presence of ZIKV RNA in breast milk from three ZIKV-infected mothers. The presence of Zika-specific antibodies was not reported in these cases. Of the three newborns delivered to ZIKV-infected mothers who were receiving breast milk with confirmed presence of ZIKV, only two were confirmed to be infected with ZIKV with no reported adverse outcomes. With regard to the presence of ZIKV in breastfeeding-related fluids, ZIKV was detected by RT-PCR in breast milk and blood of the three mothers; sweat was not measured.

Like other viral infections, mother-to-child transmission of ZIKV infection can potentially occur during antepartum, intrapartum, or postnatal periods (45). Given the variable incubation period for ZIKV, it can be difficult to distinguish breastfeeding transmission from other perinatal routes. For the two newborns who contracted ZIKV from ZIKV-infected mothers expressing ZIKV-infected breast milk, antepartum or intrapartum transmission is suspected. Even if a newborn is ZIKV negative following delivery from a ZIKV-infected mother and contracts ZIKV infection while consuming breast milk with ZIKV, there remains a possibility for separate mosquito transmission. Identifying the time of infection and duration of an incubation period is further complicated by the asymptomatic nature of acute ZIKV infection.

There is limited evidence describing breastfeeding transmission for other flavivirus infections. West Nile virus (WNV), dengue virus, and yellow fever virus have been detected in breast milk (46, 47). Of these infections, WNV has been associated with breastfeeding transmission in a small number of cases (24). Like ZIKV, breastfeeding transmission for other flavivirus infections is likely underreported due to asymptomatic illness and limited access to diagnostics. We intend to review breastfeeding transmission for related flavivirus infections in the near future.

Our systematic review for ZIKV breastfeeding transmission resulted in two studies and three cases of lactating women with confirmed ZIKV infection. As new data emerges from these current outbreaks, further investigation is needed to explore ZIKV breastfeeding transmission dynamics. This includes understanding the mechanics of transmission with regards to timing of infection for mother and infant, breast milk viral load, and exposure duration as well as assessing the frequency and distribution of breastfeeding transmission among affected populations. In addition to determining viral transmission risk, research should also explore the protective properties of ZIKV-specific immunoglobulin in breast milk transferred from mothers who have

experienced ZIKV infection. At this time, there is no evidence of ZIKV transmission via breastfeeding, and the authors support the WHO breastfeeding guidelines currently in place recommending initiating breastfeeding within one hour of delivery, exclusively for 6 months and extended until 2 years or beyond (48).

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Figures and Tables

Figure 1. Case definitions and main diagnostic tests interpretations for zika virus

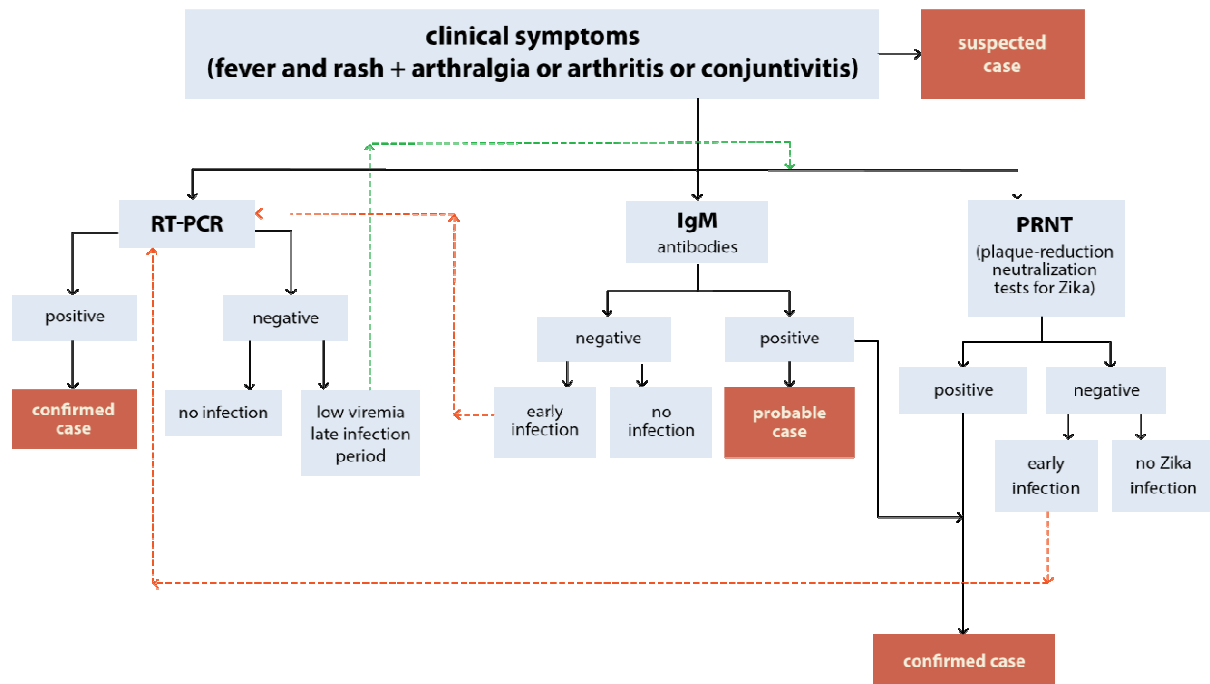


Figure 2. Study flow diagram

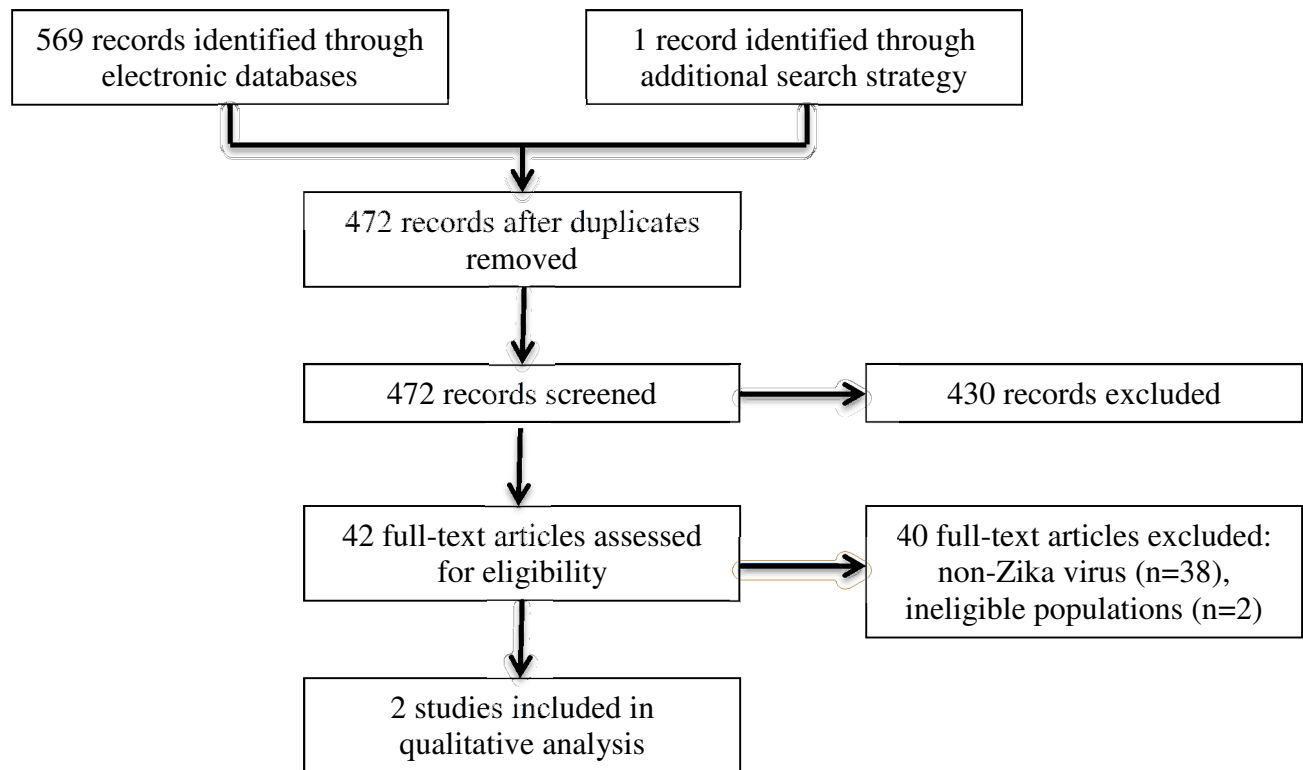


Table 1. Detection of ZIKV in breastfeeding mother-infant pairs

Case	Days After Delivery	Maternal					Newborn		
		Clinical Sign	Serum RT-PCR ¹	Saliva RT-PCR	Breast RT-PCR	Milk Culture	Clinical Sign	Serum RT-PCR	Saliva RT-PCR
1	-2	Rash	-	-	-	-	-	-	-
	0	-	-	-	-	-	-	-	-
	1	Rash	-	-	-	-	-	-	-
	2	Rash	Positive	Positive	-	-	-	-	-
	3	-	-	-	Positive	Negative	-	Positive	Positive
2	0	-	-	-	-	-	-	Negative	-
	1	-	Positive	-	-	-	-	-	-
	3	Rash, Mild fever	-	-	-	-	-	Negative	-
	4	-	-	-	-	-	Rash	Positive	-
	5	-	Positive	-	-	-	-	-	-
	7	-	-	-	-	-	-	Positive	-
	8	-	Negative	-	Positive	Negative	-	-	-
	11	-	Negative	-	-	-	-	-	-
13	-	Negative	-	-	-	-	-	-	
3	0	Fever	-	-	-	-	-	-	-
	1	Fever	-	-	-	-	-	-	-
	3	-	Positive	-	-	-	-	Ambiguous	-
	4	-	-	-	Positive	Positive	-	-	-

¹. RT-PCR = Reverse transcription polymerase chain reaction

Table 2. GRADE evidence profile table for the primary outcome

Quality assessment							№ of patients		Effect		Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Breastfeeding from mothers infected with Zika virus	Not breastfeeding from mothers with Zika virus infection	Relative (95% CI)	Absolute (95% CI)		
Confirmed Zika virus infection among infants or young children breastfeeding or consuming breast milk from mothers with Zika virus infection												
2	observational studies	very serious ¹	not serious	not serious	not serious		2/3 (66.7%)	-	-	-	⊕○○○ VERY LOW	Critical

CI: Confidence interval

GRADE Working Group grades of evidence

High quality: We are very confident that the true effect lies close to that of the estimate of the effect

Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

^{1.} The evidence is based on three mother-infant pairs from two case reports.

Appendix – Search Overview

Zika search results March 2016

Database searched	Date searched	Number of results
MEDLINE & MEDLINE in Process (OVID) 1946 to 9/3/16	10/03/16	308
PubMed	10/03/16	48
CINAHL (Ebsco) 1982 to March 2016	10/03/16	82
Web of Science (ISI) SCI, SSCI, CPCI & CPCI-SSH to 2/3/16	10/03/16	94
Popline to March 2016	10/03/16	20
LILACS (Birme) 1982 to March 2016	10/03/16	14
PAHO (Birme) to March 2016	10/03/16	0
WHOLIS (Birme) to March 2016	10/03/16	0
WPRIM to March 2016	10/03/16	0
IMSEAR to March 2016	10/03/16	2
Total		569
After de-duplication		471

MEDLINE and Medline in Progress(OVID)

- 1 Flavivirus/ (1367)
- 2 Flavivirus Infections/ (506)
- 3 zika.tw. (333)
- 4 (Flaviviridae or Flavivirus).tw. (4045)
- 5 ZIKV.tw. (53)
- 6 West Nile Fever/ (3381)
- 7 West Nile virus/ (3775)
- 8 Yellow Fever/ (2564)
- 9 Yellow fever virus/ (1148)
- 10 (yellow fever* or west nile* or Kunjin).tw. (10075)
- 11 exp Breast Feeding/ (31065)
- 12 Milk, Human/ (16293)
- 13 (((breastfeed* or breast) adj2 feed*) or lactat*).tw. (138436)
- 14 ((breast or human or mother*) adj2 milk).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (24003)

- 15 or/11-14 (172163)
- 16 or/1-10 (14313)
- 17 Sweat/ (3545)
- 18 exp Blood/ (990614)
- 19 Mucus/ (8604)
- 20 Saliva/ (34692)
- 21 (sweat* or blood* or mucus or serum* or sera or fluid* or saliva).tw. (2582998)
- 22 Infectious Disease Transmission, Vertical/ (12798)
- 23 Antibodies, Viral/ (66607)
- 24 or/17-23 (3289755)
- 25 mothers/ (31502)
- 26 exp Infant/ (996971)
- 27 (mother* or infant* or baby or babies or newborn*).tw. (557321)
- 28 or/25-27 (1234144)
- 29 24 and 28 (198442)
- 30 15 and 16 (55)
- 31 16 and 29 (267)
- 32 30 or 31 (308)

CINAHL (EBSCO)

- S30 S28 OR S29
- S29 S13 AND S27
- S28 S13 AND S14
- S27 S22 AND S26
- S26 S23 OR S24 OR S25
- S25 (mother* or infant* or baby or babies or newborn*)
- S24 (MH "Infant+")
- S23 (MH "Mothers")
- S22 S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21
- S21 (MH "Antibodies, Viral")
- S20 (MH "Disease Transmission, Vertical")
- S19 (sweat* or blood* or mucus or serum* or sera or fluid* or saliva)
- S18 (MH "Saliva")
- S17 (MH "Mucus")
- S16 (MH "Blood+")
- S15 (MH "Sweat")
- S14 S9 OR S10 OR S11 OR S12
- S13 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8
- S12 ((breast or human or mother*) N2 milk)
- S11 (((breastfeed* or breast) N2 feed*) or lactat*)
- S10 (MH "Milk, Human")
- S9 (MH "Breast Feeding+")
- S8 (yellow fever* or west nile* or Kunjin)
- S7 (MH "Yellow Fever")

S6 (MH "West Nile Virus")
S5 (MH "West Nile Fever")
S4 ZIKV
S3 Flaviviridae or Flavivirus
S2 zika
S1 (MH "Flavivirus") OR (MH "Flavivirus Infections+")

Web of Science (SCI, SSCI, CPCI & CPCI-SSH) (ISI)

14 #13 OR #12

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

13 #11 AND #4

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

12 #8 AND #4

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

11 #10 AND #9

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

10 TOPIC: (((mother* or infant* or baby or babies or newborn*)))

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

9 TOPIC: (((sweat* or blood* or mucus or serum* or sera or fluid* or saliva)))

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

8 #7 OR #6 OR #5

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

7 TOPIC: (((breast or human or mother*) near/2 milk)))

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

6 TOPIC: ((lact*))

Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

5 TOPIC: (((breastfeed* or breast) near/2 (feed*)))
Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

4 #3 OR #2 OR #1
Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

3 TOPIC: (("yellow fever*" or "west nile*" or Kunjin))
Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

2 TOPIC: ((Flaviviridae or Flavivirus))
Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

1 TOPIC: ((zika))
Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

Popline

(Zika OR Flaviviridae OR Flavivirus OR yellow fever OR west nile OR Kunjin) AND (mother OR women)

PAHO, WHOLIS and LILACS (BIRME)

zika or yellow fever\$ or west nile\$ or Kunjin or Flaviviridae or Flavivirus [Words] and sweat\$ or blood\$ or mucus or serum\$ or sera or fluid\$ or saliva or breastfeed\$ or breast feed\$ or lactat\$ or breast milk\$ or mother\$ milk or human milk [Words] and mother\$ or infant\$ or baby or babies or newborn\$ [Words]

IMSEAR & WPRIM

zika or yellow fever or west nile or Kunjin or Flaviviridae or Flavivirus