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Breast Milk Transmission of Flaviviruses in the Context of Zika Virus: A Systematic Review

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Abstract

Background—Since the Zika virus epidemic in the Americas began in 2015, Zika virus transmission has occurred throughout the Americas. However, limited information exists regarding possible risks of transmission of Zika virus and other flaviviruses through breast feeding and human milk. We conducted a systematic review of the evidence regarding flaviviruses detection in and transmission through milk, specifically regarding Zika virus, Japanese encephalitis virus, tick-borne encephalitis virus, Powassan virus, West Nile virus, dengue virus, and yellow fever virus.

Methods—Medline, Embase, Global Health, CINAHL, Cochrane Library, Scopus, Popline, Virtual Health Library, and WorldCat were searched through June 12, 2017. Two authors independently screened potential studies for inclusion and extracted data. Human and nonhuman (animal) studies describing: 1) confirmed or suspected cases of mother-to-child transmission through milk; or 2) the presence of flavivirus genomic material in milk.

Results—Seventeen studies were included, four animal models and thirteen observational studies. Dengue virus, West Nile virus, and Zika virus viral ribonucleic acid was detected in human milk, including infectious Zika virus and dengue virus viral particles. Human breast-feeding transmission was confirmed for only yellow fever virus. There was evidence of milk-related transmission of dengue virus, Powassan virus, and West Nile virus in animal studies

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Conclusions—Because the health advantages of breast feeding are considered greater than the potential risk of transmission, the World Health Organization recommends that mothers with possible or confirmed Zika virus infection or exposure continue to breast feed. This review did not identify any data that might alter this recommendation.

Keywords

Zika virus; flavivirus; breast milk; breast feeding transmission

Introduction

Zika virus (ZIKV) was first discovered in 1947 in the Zika Forest of Uganda, with transmission reported over subsequent decades from other countries in Africa, Asia, and the Pacific.[1] In February 2014, health officials reported the first autochthonous ZIKV infection in the Americas on Easter Island (Chile) in the South Pacific,[2] and in May 2015 local transmission was confirmed in northeastern Brazil.[2–5] During 2015–2017, the outbreak expanded throughout much of the Americas. Because of concern about a possible link between ZIKV infection and birth defects, including brain abnormalities, microcephaly, and other neurologic disorders, the World Health Organization (WHO) declared the ZIKV epidemic to be a Public Health Emergency of International Concern in February 2016.[6,7]

ZIKV is related to other flaviviruses including dengue virus (DENV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), Powassan virus (POWV), West Nile virus (WNV), and yellow fever virus (YFV). ZIKV is transmitted by mosquito vectors (e.g. *Aedes aegypti* and *Aedes albopictus*), in addition to other routes of transmission: sexual transmission, transfusion of blood products, and mother-to-child transmission (MTCT) in utero and perinatally.[8–10] However, limited information exists regarding the potential transmission risk associated with breast feeding and human milk.

The health benefits of breast feeding are well known and widely recognized.[11–13] In the United States, both the American Academy of Pediatrics and the American Academy of Family Physicians recommend that infants exclusively breast feed for the first six months of life and continue breast feeding until at least one year of age, or as long thereafter as mutually desired by mother and child.[11,12] Among infants born in the United States, 81% initiate breast feeding, 52% continue some breast feeding until at least six months of age (22% exclusively breast feed), and 31% breast feed until at least one year of age.[13] Given the maternal and child health advantages associated with breast feeding, most women are encouraged to breast feed. However, in North America and other regions where safe and affordable replacement feeding is available, mothers with infections such as human immunodeficiency virus type 1 or *Mycobacterium tuberculosis* are discouraged from breast feeding.[11] Based on the limited data available regarding the potential infectivity of human milk, the WHO determined the benefits of breast feeding for the infant and mother outweigh any potential risk of ZIKV transmission through breast milk, and recommended that mothers with possible or confirmed ZIKV infection or exposure continue to breast feed.[14] However, because data about long-term outcomes associated with ZIKV infection in infants and young

children are limited, it is important to elucidate any evidence of human milk transmission of ZIKV.

We conducted a systematic literature review to summarize the currently available evidence about the presence of flaviviruses in human milk and the risk of flavivirus transmission from mother to child through human milk as well as vertical transmission through nonhuman (animal) milk, specifically focusing on seven flaviviruses: ZIKV, JEV, TBEV, POWV, WNV, DENV, and YFV. Our objectives were to assess studies regarding the presence and transmission of flaviviruses through human milk, and to evaluate animal studies regarding flavivirus transmission through milk.

Methods

Search Strategy

A systematic review was conducted using Medline (OVID), Embase (OVID), Global Health (OVID), CINAHL (Ebsco), Cochrane Library, Scopus, Popline, Virtual Health Library, and WorldCat. We included articles published before June 12, 2017 (database start dates varied, see Appendix 1). The literature search strategy used the combination of terms developed by the authors (Appendix 1). Additionally, reference sections of all articles identified through the database search strategy and included in this review were manually searched to identify any additional relevant articles.

Study Selection and Eligibility Criteria

One author (TM) performed the database search on June 12, 2017. Two authors (TM, TW) reviewed the database search results and removed duplicates and articles not related to flaviviruses and/or breast milk as based on title and abstract. Two authors (TM, TW) screened all remaining titles and abstracts for inclusion criteria. We included all animal and human studies published in English, Spanish, or Portuguese regarding ZIKV, JEV, TBEV, POWV, WNV, DENV, and YFV. Conference abstracts and guidance documents were excluded. Studies were included if they described: 1) Confirmed, probable, or possible cases of transmission from mother to child of these flaviviruses through human milk or vertical transmission through animal milk; or 2) the presence of the flavivirus genome in human milk or colostrum. Confirmed transmission through breast milk included cases where 1) laboratory testing indicated infection in the mother or she had been vaccinated, 2) testing indicated infection in the child, 3) time-order of maternal-infant infection was appropriate, 4) the infant was exposed to breast milk during the period of possible transmission, and 5) other routes of transmission were eliminated. Probable transmission through breast milk included cases where 1) laboratory testing indicated infection in the mother or she had been vaccinated, 2) testing indicated infection in the child, 3) time-order of maternal-infant infection was appropriate, 4) the infant was exposed to breast milk during the period of possible transmission, and 5) other routes of transmission were unlikely but could not be ruled out. Possible transmission through breast milk included cases where 1) laboratory testing indicated infection in the mother or she had been vaccinated, 2) testing indicated infection in the child, 3)time-order of maternal-infant infection was appropriate, 4) the infant could have been exposed to breast milk during the period of possible transmission, and 5)

other routes of transmission were considered equally likely. Results were limited to cases and studies of transmission from mother to child or mother to suckling within the same species, and results describing transmission of passive immunity through maternal antibodies were excluded. Two authors (TM, TW) assessed the abstracts and the full texts of all articles meeting inclusion criteria for eligibility. All but one article describing the same cases or cohorts were discarded unless they included new evidence or previously unidentified information of relevance (Figure 1). A third author (LH) independently evaluated the selected studies and resolved any disagreement on inclusion or eligibility. All references were maintained in an electronic library using EndNote software.

Data Synthesis

Included articles were reviewed for information of interest, and information was abstracted by two authors (TM, TW) using standardized abstraction forms developed for this review. A third author (LH) independently evaluated all studies and abstraction forms and resolved any disagreement on abstraction. Findings tables were constructed for each objective, stratified by individual flaviviruses and evidence of detection or transmission.

Results

Database searches yielded 915 results, and two further articles were identified through manual searches of the reference sections of articles meeting inclusion criteria (Figure 1). Of these 917 articles, based on screened titles and abstracts, 829 were either duplicates of the same article (n=287) or unrelated to flavivirus transmission through human or animal milk (n=542). The remaining 88 articles were screened, and 24 were excluded due to not meeting the inclusion criteria for language (n=13) or document type (n=11). The 64 remaining publications were assessed for eligibility. Of these, 17 descriptive studies were included in the findings tables; 47 articles were excluded as they described transmission of passive immunity through maternal antibodies (n=13) or were secondary sources citing included studies (n=34). Meta-analyses were not conducted because of limited data and heterogeneity of study designs.

Detection of Flaviviruses in Human Milk

Ten studies were identified that described detection of flavivirus genomic material (ZIKV, DENV, and WNV) in human milk (Table 1a). Five articles (three case reports and two case series) described six cases in which ZIKV ribonucleic acid (RNA) was detected in human milk, including four in which ZIKV was cultured.[15–19] In one case series (n=2), reverse-transcription polymerase chain reaction (RT-PCR) testing revealed ZIKV RNA in human milk samples collected five days after maternal symptom onset, but cell cultures were negative for infectious virus in both cases.[15] From two case reports and one case from a case series (n=4), three human milk samples collected at three to four days post onset of maternal illness were positive for ZIKV RNA by RT-PCR; cell culture demonstrated potential infectivity of the viral genomic material.[16–18] ZIKV RNA was not detected in breast milk samples collected from three other women included in the case series at four days or longer post-onset of symptoms.[17] Finally, in a case report, ZIKV RNA was found in colostrum at

14 days and milk samples at 23 and 32 days after onset, and infectious ZIKV particles were found on culturing the day 14 colostrum sample and the day 32 milk sample.[19]

DENV RNA was detected in milk samples from 10 women in two studies (one case report and one case series), including culturable virus in a sample from one woman.[20,21] In the case report, DENV RNA was detected by RT-PCR in milk samples collected on days six and eight after the woman's symptom onset, with infectious virus particles detected at both time points.[20] The case series (n=12) described DENV RNA detection in nine (75%) cases, with the longest observed period of detection being 14 days after maternal illness onset.[21] All DENV RNA positive samples were assessed for infectivity but were negative in cell culture.[21]

Three studies (one case report and two cross-sectional studies) described confirmed or possible detection of WNV RNA in human milk or colostrum.[22–24] WNV RNA was detected in human milk in one case, in colostrum in two cases, and there was an equivocal result in milk in one case; none of the studies demonstrated infectious virus particles.[22–24] In the case report, a milk sample tested at six days after maternal illness onset was positive for WNV RNA, but a subsequent sample collected on day 15 after onset was WNV PCR-negative; virus was not culturable.[22] In one cross-sectional study, WNV RNA was detected in colostrum samples from two women 50 and 70 days after maternal illness onset, but was not detected on testing 30 colostrum samples and 13 mature human milk samples from 43 other women infected with WNV during pregnancy.[23] Virus isolation was not attempted due to low levels of RNA in the two positive samples.[23] Another cross-sectional examination of human milk samples collected from nine of 22 seropositive women among a cohort of 566 women living in an area with ongoing WNV transmission revealed one milk sample with equivocal results for WNV RNA by PCR.[24]

Human Milk Transmission of Flaviviruses

We identified nine studies (six case reports and three case series) that addressed potential transmission of ZIKV, DENV, WNV, and YFV through human milk (Table 1b). Potential ZIKV transmission was described in three cases from two studies (one case report and one case series), although other modes of transmission were considered more likely for two cases.[15,18] All of the women in these reports were symptomatic, and serum, saliva, and/or urine samples were ZIKV PCR-positive.[15,18] All infants were ZIKV PCR-positive, but only one became symptomatic.[15,18] In one case, both the viral genome isolated from milk and that found in the infant were shown to be 99% identical when sequenced.[18] Infection in mother-infant pairs was identified within the first week postpartum for two cases,[15] and at five months postpartum for the third.[18] Based on the timing of infant illness and/or positive test results, one case was considered possibly related to breast milk transmission,[18] and the remaining two cases were considered more likely to be perinatal infections.[15] Vector-borne transmission could not be excluded for two cases. Milk-borne transmission could not be confirmed in any of these cases.[15,18]

Two studies (one case report and one case series) reported possible DENV transmission through human milk in two cases.[20,21] In the case report, DENV was detected in both mother and infant by RT-PCR.[20] Cord blood was negative for DENV by RT-PCR; however,

perinatal transmission could not be ruled out.[20] In the other study, maternal onset was two days after delivery and infant onset was five days later.[21] A cord blood specimen was negative for DENV RNA, but perinatal and vector-borne transmission could not be excluded.

Two studies (one case report and one case series) described four cases of probable, possible, or potential human milk transmission of WNV.[22,23] In the case report, the mother received a transfusion of blood products the day after delivery from the same donor who provided a donation that had resulted in WNV transmission after transfusion into a liver transplant patient.[22] Maternal symptoms began shortly after the transfusion, and the infant, though asymptomatic, tested positive for WNV-specific IgM about two weeks later, suggesting probable transmission by breast feeding. In a case series of mother-infant pairs, one breast feeding infant became ill at 8 months of age and was WNV IgM-positive.[23] The mother did not become ill until one month later, with IgM detected by serology.[23] The time-order of symptom onset of mother and child suggests vector-borne transmission was most likely. In two other cases, both women became symptomatic about one week antepartum and tested positive by serology.[23] One infant was born with a maculopapular rash and was WNV IgM-positive when tested at two months after birth. The other infant became symptomatic at 16 days after maternal onset (10 days after birth) and was WNV IgM-positive in both serum and cerebrospinal fluid (CSF); however, cord blood specimens were negative for WNV antibodies by serology.[23] The timing of maternal and infant symptom onset makes exclusion of perinatal transmission difficult in both cases, and vector-borne transmission could not be ruled out.

Three studies (all case reports) described confirmed or probable transmission of the vaccine strain of YFV through breasfteeding.[25–27] In the first case report, the mother received yellow fever vaccine 15 days after delivery, and had symptoms of headache, malaise, and fever at 20–22 days postpartum.[25] The mother breast fed from delivery through the infant symptom onset at eight days after maternal vaccination. Infant CSF was YFV PCR-positive and the infant's serum and CSF were YFV IgM-positive. Although the mother-infant pair lived in an area with an ongoing YFV epidemic, MTCT was confirmed by nucleotide sequencing, which matched the viral RNA detected in the infant's CSF to the vaccine strain. The other two case reports described mothers who received yellow fever vaccine and had infants who developed encephalitis three to four weeks later.[26,27] YFV-specific IgM was detected in infant serum and CSF in both cases.[26,27] Both reported cases included possible exposure in endemic regions, from either residence or travel (although no outbreaks were reported at the locations of either infant);[26,27] thus, vector-borne transmission could not be ruled out.

Animal Milk Transmission of Flaviviruses

Possible vertical transmission of DENV, POWV, and WNV through animal milk was evaluated in four studies (Table 2). In the DENV study, six lactating hamsters underwent intraperitoneal inoculation, after which 32 suckling hamsters were allowed to nurse without interference for the subsequent six days.[28] Of the 32 subjects, four were determined to be DENV-positive.[28] In the POWV study, a lactating goat underwent intramuscular inoculation at 74 days postpartum and was subsequently allowed to nurse without restriction.[29] The kid

remained asymptomatic, but both the mother and the kid were serologically positive for POWV neutralizing antibody. Milk samples were positive for both POWV and POWV-specific neutralizing antibody.[28] In a WNV study of mice, inoculation of the foster mother mice occurred prior to initiation of nursing in a subset of animal subjects.[30] Subsequently, brain tissue samples from five (17%) of 29 of the suckling mice were WNV PCR-positive, of which four (80%) were infectious.[30] In a different WNV study in hamsters, three mother hamsters underwent intraperitoneal inoculations postpartum and nursed 18 offspring.[31] Thirteen (72%) of 18 offspring had symptoms referable to the central nervous system and presence of WNV was confirmed.[31]

Comment

Principal Findings

The reports included in this review provide limited evidence of the possible transmission of certain flaviviruses through human and animal milk. The presence of viral RNA in human milk was confirmed for DENV,[20,21] WNV,[22,23] and ZIKV,[15–19] and was found to be potentially infectious in five of 20 cases (4 ZIKV, 1 DENV).[16–20] For ZIKV specifically, infectious virus was detected as late as 32 days post-onset of maternal illness.[19] Of the 12 reported cases of potential postnatal MTCT of the selected flaviviruses in humans, [15,18,20–23,25–27] non-breast feeding-related routes of transmission could not be ruled out in all but one case involving vaccine strain YFV.[25] In our review of animal studies, we found evidence of milk-related transmission of three flaviviruses (DENV, POWV, WNV).[28–31] The studies published to date are limited to individual case reports, case series, and cross-sectional studies with small study populations. The small number of cases reported prohibits any determination of the actual risk of transmission associated with human milk. The small number of published studies meeting our eligibility criteria coupled with the low-quality evidence limits generalizability and hindered our ability to make strong conclusions or to conduct meta-analyses.

Interpretation

The limitations of currently published studies and the difficulty in determining specific route of transmission in areas with mosquito-borne transmission of flaviviruses hinder our ability to make definite conclusions about the transmission of flaviviruses through milk. While flavivirus genomic material may be detected in human milk, it is unknown how often the virus is replication-competent and/or of sufficient quantity to be infectious. In some of the included studies, the presence of virus-specific IgM and/or IgG in human milk was detected. [22–24] However, the implications of IgG or IgM presence in human milk are unclear, and these antibodies may confer passive immunity to the infant. Despite animal studies demonstrating the potential for transmission through milk,[28–31] concerns remain as to whether the results of animal studies can predict human transmission and exposure.[33,34] Many of the human observational studies involved maternal exposure during the perinatal period, and, in several case reports, the timing of MTCT could not be definitively determined, making confirmation of transmission through human milk (versus perinatal transmission) infeasible.[15,18,20,21,23] Further, in endemic areas, it is challenging to eliminate the possibility of mosquito vector transmission.[15,16,19–21,25–27] Though closely related to the

other selected viruses, ZIKV demonstrates distinct viral behavior,[6–10] which limits the validity of using data on other flaviviruses to address Zika-specific clinical and research questions. Insufficient data exist to counter current guidance at this time, but the scarcity of data highlights the many questions that remain unanswered: 1) How long are these viruses detectable in milk?; 2) Is human milk transmission of ZIKV or other flaviviruses possible?; 3) What quantity of virus (viral load) is required to produce infection in breast feeding children?; 4) Is human milk transmission of ZIKV or other flaviviruses associated with adverse outcomes in infants?; and 5) How does the timing of maternal infection affect the transmissibility of the viruses? The answers to questions such as these could greatly inform breast feeding guidelines, both for regions with endemic flaviviruses and areas susceptible to flavivirus outbreaks.

Strengths of the study

Our review adds to the knowledge previously gathered by Colt et al. regarding possible ZIKV transmission through human milk,[32] contributing additional ZIKV data and including information on possible transmission of other flaviviruses through human milk. We included seven flaviviruses and both human and animal studies in our review, whereas Cole et al. focused solely on ZIKV in humans. Despite including more viruses, our findings were similar to those of Cole et al., namely that there is limited evidence of vertical transmission of these viruses through milk but current data is inadequate to draw definitive conclusions.

Limitations

Despite the value added by our review, several limitations exist. Although we included a wide range of search terms, used several well-known databases, and searched references and citations of included studies, we may have missed some relevant studies. Our use of the search term ‘human milk’ may have increased the sensitivity of the search strategy for human studies relative to animal studies. Further, our review included only papers published in English, Spanish, or Portuguese languages, and excluded conference abstracts. As with any systematic review, publication bias is also possible, especially if studies are unpublished because of negative findings.

Conclusions

Although evidence shows that flaviviruses can occasionally be present in human milk, there is very limited evidence of risk of flavivirus transmission through human milk to infants. For ZIKV specifically, the World Health Organization recommends that mothers with possible or confirmed ZIKV infection or exposure continue to breast feed as the health advantages of breast feeding are considered greater than the potential risk of transmission.[14] Should postnatal ZIKV infection in infancy and early childhood be causally linked to adverse health or developmental outcomes, more rigorous studies will be necessary to determine the level of risk and develop updated recommendations.

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Abbreviations

CSF	cerebrospinal fluid
DENV	dengue virus
IgG	immunoglobulin G
IgM	immunoglobulin M
JEV	Japanese encephalitis virus
MTCT	mother-to-child transmission
PRNT	plaque reduction neutralization test
POWV	Powassan virus
RNA	ribonucleic acidl
RT-PCR	reverse transcription polymerase chain reaction
TBEV	tick-borne encephalitis virus
WHO	World Health Organization
WNV	West Nile virus
YFV	yellow fever virus
ZIKV	Zika virus

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Appendix 1. Search Strategy

Database	Strategy	Run Date
Medline (OVID) 1946-	flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV AND Breast* OR colostrum OR “human milk” OR lactation	6/12/2017
Embase (OVID) 1996-	flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV AND Breast* OR colostrum OR “human milk” OR lactation	6/12/2017
Global Health (OVID) 1910-	flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV AND Breast* OR colostrum OR “human milk” OR lactation	6/12/2017
CINAHL (Ebsco) 1982-	flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV AND Breast* OR colostrum OR “human milk” OR lactation	6/12/2017
Cochrane Central Register of Controlled Trials (CENTRAL) No date restrictions	(flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV) AND (Breast* OR colostrum OR “human milk” OR lactation)	6/12/2017
Scopus 1960-	TITLE-ABS-KEY((flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV) AND (Breast* OR colostrum OR “human milk” OR lactation)) AND NOT INDEX(medline)	6/12/2017
Popline 1827-	(flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV) AND	6/12/2017

Database	Strategy	Run Date
	(Breast* OR colostrum OR “human milk” OR lactation)	
Virtual Health Library – WHOLIS, PAHO, LILACS, IBECS 1998-	(flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV) AND (Breast* OR colostrum OR “human milk” OR lactation)	6/12/2017
WorldCat	(flavivirus* OR flaviviridae OR arbovirus* OR “Japanese encephalitis” OR “tick-borne encephalitis” OR “Powassan virus” OR “West Nile virus” OR dengue OR “Yellow Fever” OR Zika OR ZIKV) (Breast* OR colostrum OR “human milk” OR lactation)	6/12/2017

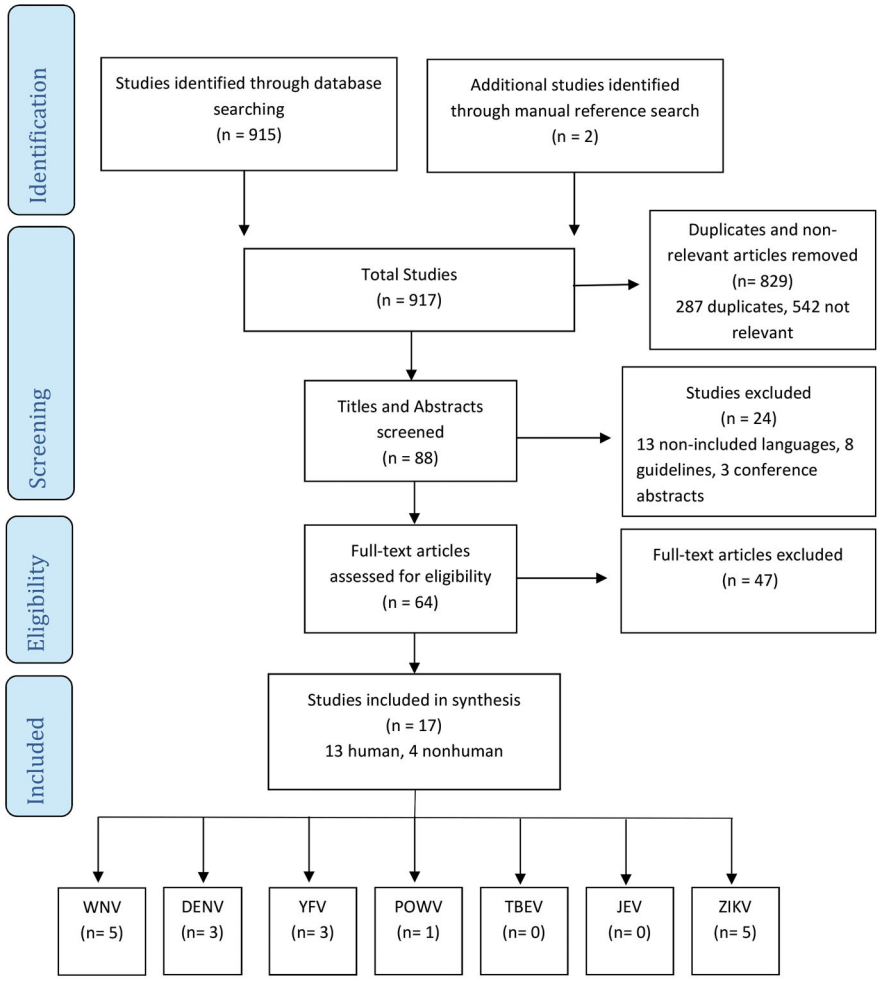

Figure 1.
Flowchart for identification of studies for inclusion in review

Table 1a

Detection of flaviviral ribonucleic acid (RNA) and virus in human milk

Author	Case Number for cases with flaviviral RNA detected	RNA detected (days post maternal illness onset)*	Virus cultured (days post maternal illness onset)*
Zika Virus			
Besnard 2014[15] (n=2)	Case 1	Yes (5)	No (5)
	Case 2	Yes (5)	No (5)
Dupont-Rouzeyrol 2016[16]	Single Case	Yes (4)	Yes (4)
Cavalcanti 2017[17] (n=4)	Case 1	Yes (3)	Yes (3)
Blohm 2017[18]	Single Case	Yes (3)	Yes (3)
Sotello 2017[19]	Single Case	Yes (14[b],23,32)	Yes (14[b],32)
Dengue Virus			
Barthel 2013[20]	Single Case	Yes (6,8)	Yes (6,8)
Arragain 2016[21] (n=12)	Case 1	Yes (3)	No (3)
	Case 2	Yes (2–10)	No (2–10)
	Case 3[a]	Yes (1,3–7)	No (1,3–7)
	Case 4[a]	Yes (4,6–8,10)	No (4,6–8,10)
	Case 5[a]	Yes (7–9,12)	No (7–9,12)
	Case 6[a]	Yes (2–11,14)	No (2–11,14)
	Case 7[a]	Yes (3)	No (3)
	Case 8[a]	Yes (1–6)	No (1–6)
	Case 9[a]	Yes (9)	No (9)
West Nile Virus			
CDC 2002[22]	Single Case	Yes (6)	No (6)
Hinckley 2007[23] (n=45)	Case 1	Yes (50[b])	Not attempted due to low viral load
	Case 2	Yes (70[b])	Not attempted due to low viral load
Paisley 2016[24] (n=9)	Case 1[a]	Equivocal (Not reported)	Not reported

RNA= ribonucleic acid,

*Day 0 considered day of maternal illness onset unless otherwise indicated,

[a]case number assigned differs from the case number reported in cited study due to excluded cases,

[b]Colostrum sample

Table 1b

Cases of possible, probable, or confirmed transmission of a flavivirus or flaviviral vaccine virus by breast feeding

Author	Case Number for cases with evidence of transmission	Certainty of transmission through breast feeding	Maternal illness onset	Maternal PCR (days post maternal illness onset or vaccination)*	Maternal serology (days post maternal illness onset or vaccination)*	Period of infant exposure to potentially contaminated milk*	Cord blood test results	Infant's illness onset*	Infant PCR (days post infant illness onset)**	Infant serology (days post infant illness onset)**
Zika Virus										
Besnard 2014[15] (n=2)	Case 1	Unlikely (Infant only breast fed 1 day prior to positive test). Perinatal transmission more likely, vector-borne transmission not excluded. *Endemic region*	Day 2 BD	Serum+ (4) Saliva+ (4)	Not reported	Days 2–5	Not reported	Asymptomatic	Serum+ (5) *, Saliva + (5) *	Not reported
	Case 2	Unlikely (Infant only breast fed 1 day prior to onset). Perinatal infection not excluded. *Endemic region*	Day 3 AD	Serum+ (−2,2) Urine+ (4)	Not reported	Days 0–1	Not reported	Day 1	Serum+ (0,3) *b*, Urine + (4)	Not reported
Blohm 2017[18]	Single Case	Possible. Vector-borne transmission not excluded. *Endemic region*	~ Month 5 AD	Serum+ (3) Urine+ (3)	Not reported	Days 0–3	Not applicable	Asymptomatic	Serum+ (3) *, Urine + (3) *	Not reported
Dengue Virus										
Barthel 2013[20]	Single Case	Possible. Cord blood was negative by PCR, but perinatal infection not excluded. *Endemic Region*	Day 2 BD	Blood+ (2–8)	IgM+ (8), IgG+ (2)	Days 4–6	PCR−	Day 6	Serum+ *b* (0–9)	IgM+ (21)
Arragain 2016[21] (n=16)	Case 1	Possible. Cord blood was negative by PCR, but perinatal and vector-bome transmission not excluded. *Endemic Region*	Day 2 AD	Serum+ (−4, 1, 4, 6, 8)	Not reported	Day −2–4	PCR−	Day 5	Serum+ (−3,−1,3, 5)	Not reported
West Nile Virus										
CDC 2002[22]	Single Case	Probable. Perinatal and vector-borne transmission not excluded, although unlikely. *Endemic region*	Day 10 AD (Day 9 after receiving WNV contaminated blood)	Not reported	IgM+ in CSF	Days −10–6	Not reported	Asymptomatic	Not reported	IgM+ (15) *
Hinckley 2007[23] (n=8)	Case 1 *a*	Unlikely. Not supported by time-order of mother and infant symptom onsets. Vector-borne transmission likely. *Endemic region*	~ 1 month after infant onset (Month 9 AD)	Not reported	IgM+ (Not reported)	Infant onset prior to maternal onset	Not applicable	Month 8 *c*	Not reported	IgM+ (~14)
	Case 2 *a*	Unlikely. Perinatal and vector-borne transmission not excluded. *Endemic region*	Day 5 BD	Not reported	IgM+ (7)	Not reported	Not reported	Day 5 (Bom symptomatic)	Not reported	IgM+ (~60, ~240), NT+ (~60; ~240, titer 4-fold higher)
	Case 3 *a*	Possible Perinatal and vector-borne transmission not excluded. *Endemic region*	Day 6 BD	Serum-(12)	IgM+ (12), IgG+ (12), NT+ (12)	Not reported	IgM−, IgG−, NT−	Day 16	Not reported	IgM+ (0) in serum and CSF
Yellow Fever Virus										
CDC 2010[25]	Single Case	Confirmed. YFV vaccine virus detected in CSF by PCR	Yellow fever vaccine day 15 AD; symptom onset days 5 days after vaccination	Not reported	Not reported	Day 0–8	Not reported	Day 8	CSF+ (4)	IgM+ (4) in serum and CSF

Author	Case Number for cases with evidence of transmission	Certainty of transmission through breast feeding	Maternal illness onset	Maternal PCR (days post maternal illness onset or vaccination)*	Maternal serology (days post maternal illness onset or vaccination)*	Period of infant exposure to potentially contaminated milk*	Cord blood test results	Infant's illness onset*	Infant PCR (days post infant illness onset)**	Infant serology (days post infant illness onset)**
Traiber 2011[26]	Single Case	Probable. Vector-borne transmission not excluded. *Endemic region*	Yellow fever vaccine ~day 13 AD	Not reported	Not reported	Days 0–24	Not reported	Day 24	Not reported	IgM+ in serum and CSF
Kuhn 2011[27]	Single Case	Probable. Vector-borne transmission not excluded. *Visited endemic region*	Yellow fever vaccine day 10 AD	Not reported	Not reported	Day 0–~28	Not reported	~ Day 28	CSF− (2)	IgM+ (2) in serum and CSF, NT+ (2)

PCR=polymerase chain reaction, WNV= West Nile virus, YFV= Yellow Fever virus, CSF= cerebrospinal fluid, NT= neutralizing antibodies, BD= Before Delivery, AD=After Delivery,

*Day 0 considered day of maternal illness onset or vaccination unless otherwise indicated,

**Day 0 considered day of infant illness onset unless otherwise indicated,

[a]case number assigned differs from the case number reported in cited study,

[b]Serum samples collected before infant onset were negative,

[c]Day 0 considered day of birth

Table 2

Animal milk transmission of flaviviruses

Author	Study type	Study details	Maternal Viremia (Days after delivery)	Infant Viremia (Days after delivery)	Maternal serology (Days after delivery)	Infant serology (Days after delivery)	Infant symptoms	Period of exposure to potentially contaminated milk	Milk Test Results (Days after delivery)
Dengue Virus									
Brueckner 1956[28]	Hamster model	6 lactating mother hamsters; intraperitoneal inoculation day 12 postpartum; 32 suckling hamsters	Not reported	Confirmed[a] in 4 of 32 subjects	Not reported	Not reported	CNS involvement 4 of 4 viremic subjects	Days 12–18 after birth	Not reported
Powassan Virus									
Woodall 1977[29]	Goat model	Lactating goat; inoculation day 74 postpartum; 1 suckling goat	Serum-[a] (78–89)	Serum-[a] (82, 89)	NT+ (96, 103, 110)	NT+ (96, 103, 110)	Asymptomatic	Days 74–120 after birth	POWV+ (81, 82, 85–89), NT+ (110)
West Nile Virus									
Blazquez 2010[30]	Mouse model	29 Foster Swiss mice; inoculation before breast feeding initiated[b]	Not reported	5 of 29 (17%) PCR + (brain tissue)	Not reported	Not reported	Ruffled fur, hunching, hind limb weakness and paralysis.	Not reported	Not reported
Reagan 1956[31]	Hamster model	3 mother hamsters; inoculation postpartum day 12; 18 suckling hamsters	Confirmed in 3 of 3 mother hamsters[a]	Brain tissue from subjects pooled into 4 groups; all groups WNV+[a]	Not reported	Not reported	CNS symptoms in 13 of 18 subjects	Days 12–18 after birth	Not reported

CNS= central nervous system, NT= neutralizing antibodies, POWV= Powassan virus, WNV= West Nile virus,

[a]Intracerebral neutralization tests performed on Swiss albino mice,

[b]Other experimental subgroups involving prenatal exposure excluded