



Risk of DENV vertical transmission during the perinatal period and through breastfeeding

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Dengue transmission

DENV = arboviruses (Flaviviruses, *Flaviviridae* family)

transmitted between human hosts by **mosquito-vectors** (*Aedes* females - blood meals)

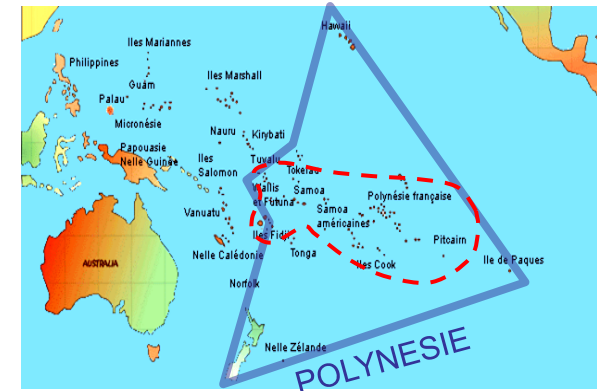
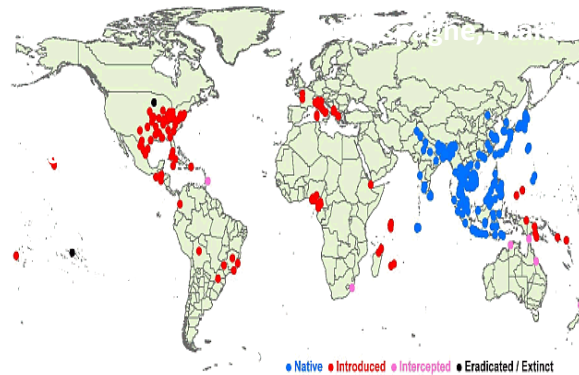
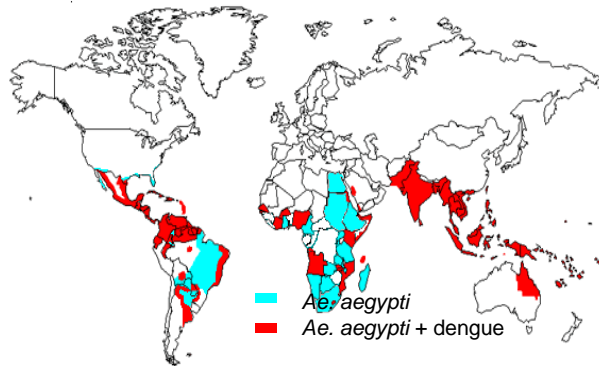
***Aedes aegypti* +++**



***Aedes albopictus* ++**



Aedes polynesiensis



Non vector-borne transmission modes of dengue :

- **blood** (needle-stick-related transmission, transfusion, transplantation)
- **mucocutaneous** contact (infected blood/eye, nose, mouth)
- **maternofoetal** transmission

Wiwanitkit V. J Infect Dev Ctries 2010

Chen et al. Clin Infect Dis 2004

Vertical transmission of DENV in humans

Few data, **most case reports and series from South East Asia and French Guyana (n=2 to 57 infected mothers)**

Viral transmission through the placenta ? => congenital infection

Explanations for this rarity: immunity of mothers, non diagnosed cases, asymptomatic newborns

➤ **First cases (IgM +/- viral cultures)**

Tahiti 1989, Thailand 1994, Malaysia 1997, Guadeloupe 2001

Mother IgM+, cord blood and infant RT-PCR + *Kerdpanich A et al. Southeast Asian J Trop Med Public Health 2001*

➤ **No or few neonatal cases (clinical evaluation), bad obstetrical and neonatal outcomes ?**

Maternal morbi-mortality, preterm delivery, low birth weight, neonatal infection/death

Basurko et al. Eur J Obstet Gynecol Reprod Biol 2009

French Guyana (1992-2006)

57 infected women during pregnancy

20 samples of cord blood at birth.

Mother consequences: premature labour (41%), premature birth (9.6%), haemorrhage during labour (9.3%) and retroplacental haematoma (1.9%)

Foetal consequences : prematurity (20%), foetal death in utero (3.8%), late miscarriage (3.8%), acute foetal distress during labour (7.5%), neonatal death (1.9%)

Maternal-foetal transmission (5.6%)

Tan et al. PLoS NTD 2012

Malaysia, prospective case control Study

“Recent dengue infections were more frequently detected in women presenting with miscarriage (up to 22 weeks of gestation) than in controls whose pregnancies were viable.”

6/115 (5.2%) DENV cases versus 5/296 (1.7%) controls

RR 3.1 (95% CI =1–10) p= 0.047.

What we know about arboviruses transmission through maternal breastfeeding ?

- Flaviviruses

Dengue fever

One case of DENV detected in breastmilk with results and chronological history compatible with breastfeeding transmission (New Caledonia 2012) *Barthel et al. CID 2013*

West Nile

WNV detected in breastmilk and one case of breastfeeding mother-to-child transmission (Michigan 2002) *CDC Morb Mortal Wkly Rep 2002*

Yellow fever

transmission of vaccine strain of yellow fever virus to three infants via breast milk

Kuhn et al. CMAJ 2011 *Traiber et al. J Pediatr 2011* (Brésil)

Recommandations to avoid breastfeeding 15 days after vaccination using live attenuated vaccine

Imbert et al. Med Trop 2010

- Alphaviruses

Chikungunya

No CHIKV detection in breastmilk (~ 30 negative samples during the 2005-2006 outbreak in La Réunion)

Gerardin (PLoS Medecine 2008)

Ross River ?

Case report

Vertical DENV transmission during the perinatal period
kinetics of viremia in the mother's and the newborn's blood
first reported detection of DENV in breastmilk

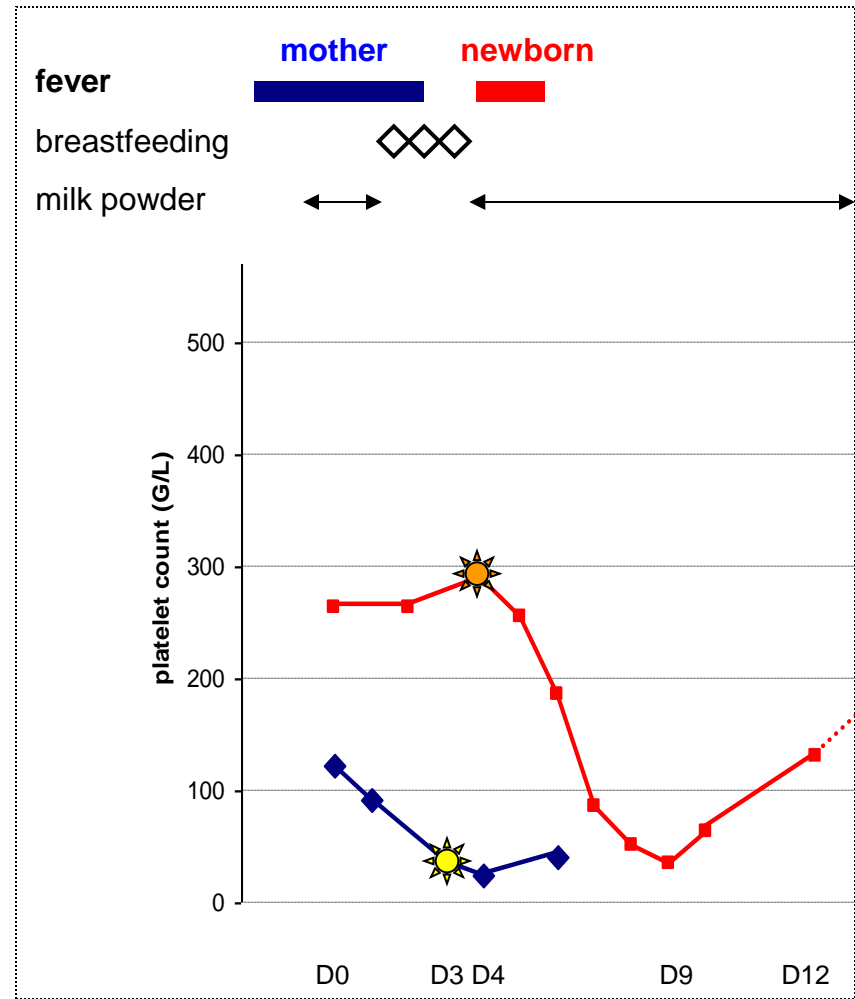
23 year old woman, Noumea's Hospital, July 2012
Preterm labor at 30 weeks + 4 days of gestation

Mother: fever since 2 days, delivery at Day 0,
thrombocytopenia (38 G/L) without bleeding on Day 3
=> RT-PCR DENV-1+ ☀

Expressed breast milk from Day 0 to Day 2
Breastfeeding (no breast lesion) from Day 2 to Day 4 ☀

Newborn: fever on Day 4 => RT-PCR DENV-1+ ☀
Thrombocytopenia (34G/L) on Day 9
without bleeding or severe symptoms

Favourable evolution for both (symptomatic treatment)



Breast Milk as a Possible Route of Vertical Transmission of Dengue Virus?

Anne Barthel,¹ Ann-Claire Gourinat,¹ Cécile Cazoria,² Corinne Joubert,⁴ Myrielle Dupont-Rouzeyrol,² and Elodie Descieux²

Virological investigations

were retrospectively and prospectively done after DENV detection in breast milk (Day 4)

- RT-PCR on sequential samples (mother and infant's blood, cord blood, breastmilk)

- Quantification by realtime RT-PCR

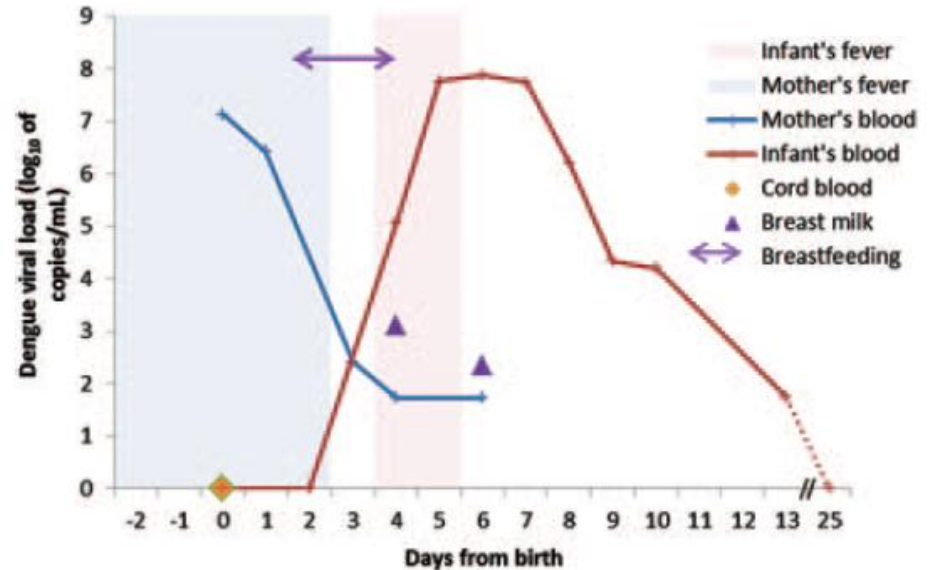


Figure 1. Mother and infant clinical symptoms and kinetics of viral loads from blood and breast milk samples in a case of vertical transmission of dengue virus.

Viral loads from mother (secondary infection) and newborn (primary infection) were high and similar

peak= 10^7 - 10^8 copies/mL

Viral kinetics of DENV in the newborn's blood

increasing viremia 3 days before symptoms

plateau phase during 2 days

decrease with prolonged viremia ≥ 10 days

Similar viral loads in breastmilk and mother's blood (Day 4 and 6)

$>10^2$ - 10^3 copies/mL

Cord blood negative for DENV (Day 0)

Infant's blood negative at Day 0 and Day 2

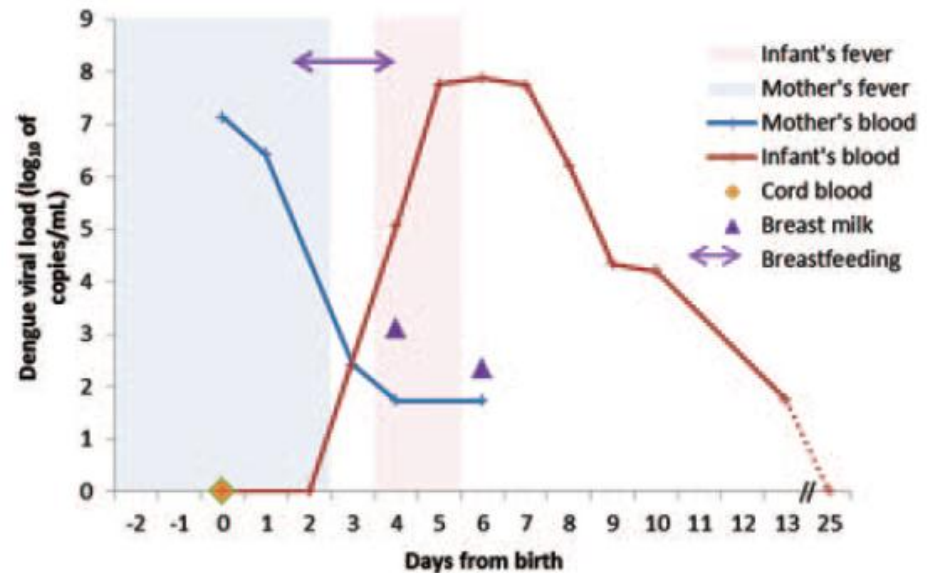
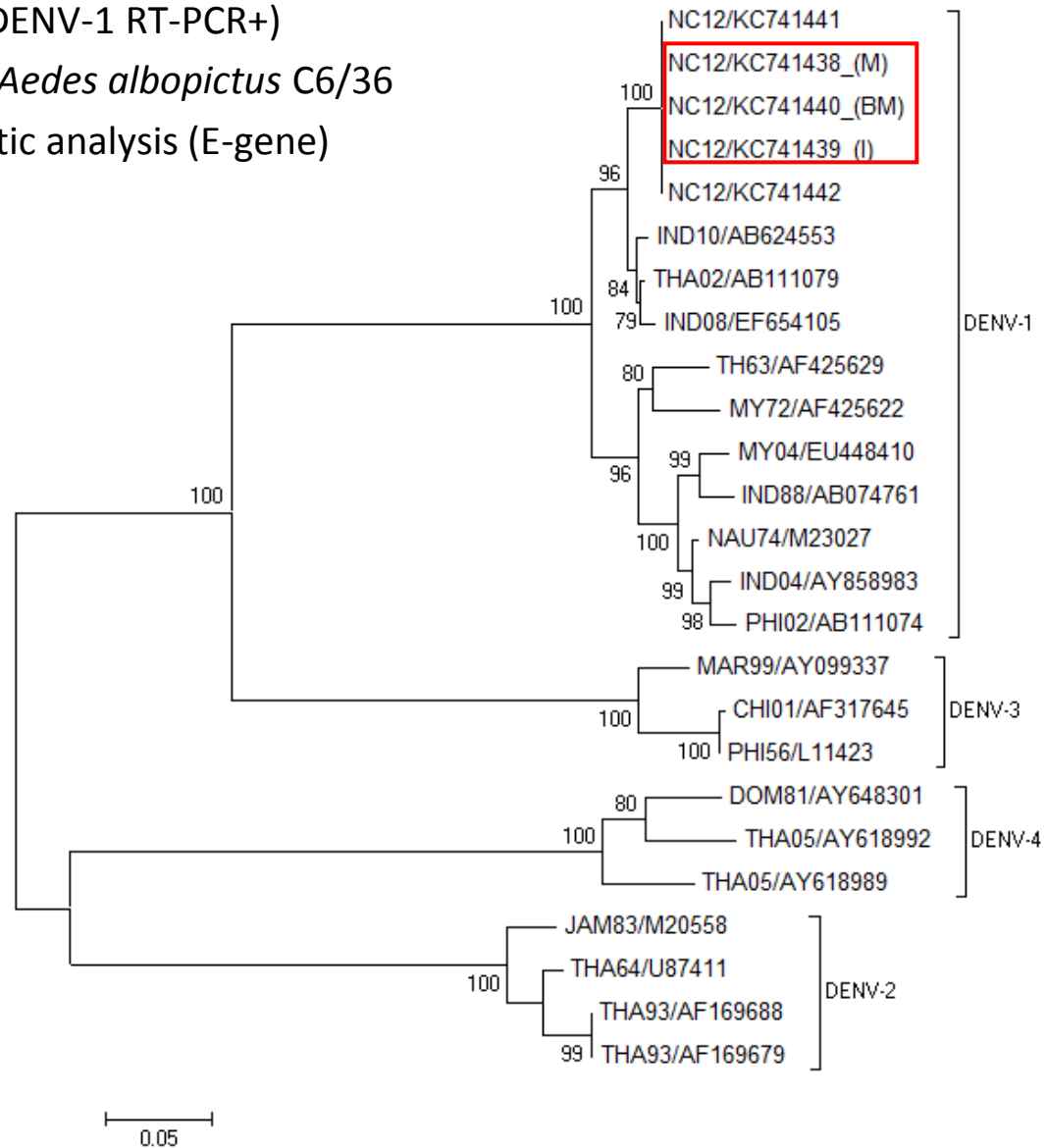


Figure 1. Mother and infant clinical symptoms and kinetics of viral loads from blood and breast milk samples in a case of vertical transmission of dengue virus.

DENV serotyping (specific DENV-1 RT-PCR+)

Viral cultures (breast milk) *Aedes albopictus* C6/36

Sequencing and phylogenetic analysis (E-gene)



100% homology between strains sequences of the mother's blood (M), breastmilk (BM) and infant's blood (I)

Vertical transmission of DENV through maternal breastfeeding ?

Our case provides evidence, for the first time to our knowledge, of the **presence of DENV in breast milk** during acute dengue infection.

Although prenatal or perpartum infection cannot be strictly excluded, our results suggest that **breast milk may be a possible route of DENV transmission from a mother to a child.**

Indeed, DENV was not detected from cord blood or from the infant's blood samples on Day 0 and Day 2.

Whereas the presence of a few viral copies on samples from Day 0 could fall under the technique's low detection limit, it seems unlikely that dengue viral load would still be undetectable 2 days later.

The contact time with milk during feeding and the large volume of intake compared to very small blood volumes reported as infectious make transmission through breastfeeding plausible.

The mechanism of infection of the newborn through breastmilk may be digestive or transmucous (sublingual?).

Should breast milk be the route of transmission, the short incubation period in our case might be related to a high infective dose.

DENNAT study, New Caledonia

CHT, DASS, IPNC, PMI network

Objectives = evaluate the risk of DENV vertical transmission during the perinatal period and through breastfeeding (transmission rate, routes)

Methods =

- Cases description of mothers +/- infants infected with DENV laboratory confirmed with RT-PCR or NS1 Ag
- all febrile mothers hospitalized at Noumea CHT 7 days before -2 days after delivery ([perinatal study](#))
- all breastfeeding mothers with DENV acute infection ([breastfeeding study](#))

Viral kinetic profiles of DENV in the mother's blood, breastmilk, placenta, cord blood, gastric fluid, infant's blood (sequential samples)

Serological status of the mothers and newborns (IgG-DENV)

Serotyping and viral quantification with RT-PCR

Viral cultures

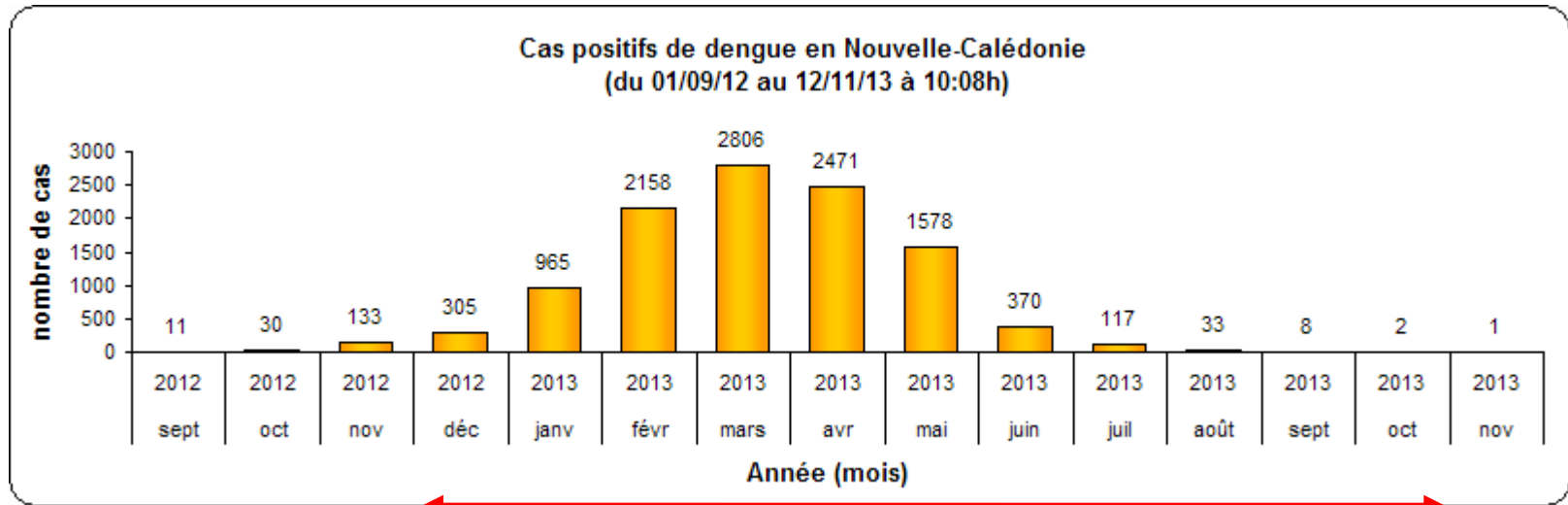
Sequencing and phylogenetic analyses

(E-gene +/- complete genome)

CCP DIRCI SOOM and CHT direction approvals



Study period during a major outbreak in NC (10 988 cases / ~250 000 inhabitants)



From December 2012 to October 2013 (10 813 DENV cases)

10 women with DENV-1 infection 7 days before to 2 days after delivery

1 premature delivery (36.42 SA)

3 maternal complications:

- 2 cases of delivery hemorrhage (1 maternal death / retroplacental hematoma + multiple visceral failure)
- 1 case of intraperitoneal hemorrhage after cesarian

All 3 required red cells transfusions, 1 required platelets transfusion

9 neonatal cases confirmed with RT-PCR (transmission rate= 90%)

all symptomatics (fever, discomfort, feeding difficulties), no severe Dengue case

1 baby required preventive platelets transfusion for severe thrombocytopenia

1 baby (mother dead) developed respiratory distress secondary to meconial fluid inhalation, mechanical ventilation, ischemic encephalopathy

DENNAT perinatal study, preliminary results

Table 1: Results of DENV RT-PCR in 10 mother-infant couples collected during the perinatal period

	mother blood	newborn blood	breastmilk	placenta	cord blood	gastric fluid
1	+	+	+	NA	NA	NA
2	+	+	+	NA	+	+
3	+	+	NA	+	inhPCR	+
4	+	0	NA	NA	0	NA
5*	+	+	+	+	0	0
6	+	+	NA	NA	NA	NA
7	+	+	NA	+	NA	+
8	+	+	+	NA	+	+
9	+	+	+	NA	NA	NA
10	+	+	NA	NA	+	NA

* artificial feeding

9/10 cases of vertical transmission

prolonged viremia in newborns (≥ 10 days)

5/5 breastmilk + in viremic mothers

prolonged presence of DENV in breastmilk ? (until 10 days after the 1st day of the symptoms)

lack of data but different modes of viral transmission can be suspected (cord blood, placenta (5), amniotic fluid, mother's blood contact/newborn's mucus membrane, breastmilk?)

=> Prospective study with sequential sampling, viral quantification blood/breastmilk until negativation

DENNAT breastfeeding study, preliminary results

30 breastfeeding women with DENV-1 acute infection

4 newborns tested positive (RT-PCR, NS1Ag)

3 symptomatics (no severe form)

early contamination (day 2 to 17 after delivery)

perpartum contamination and transmission through mosquito bites cannot be excluded

3 negative newborns

n? untested

8 breastmilk tested

6 +

2 - (late samples: day 9 and day 21 after the first days of the symptoms)

Conclusions and perspectives

High risk of vertical transmission during the studied perinatal period (90%)

Unknown mechanisms, lack of data

Evidence for DENV presence in maternal breastmilk

⇒ Prospective study in NC

collaborations (multisite study: French Guyana (Cécile Basurko),
Guadeloupe ?)

Recommendations to minimize the risk for vertical transmission?

(delivery mode/time, platelets infusion, newborn feeding mode)

Breastfeeding = possible route of vertical DENV transmission ?

Consequences in endemic regions and countries with large outbreaks ?

⇒ Prospective study, viral kinetics blood/breastmilk

Experimental studies ?

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