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Table: 1

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iii. Abstract and keywords

As breastfeeding is of utmost importance for child development and survival, identifying whether breast milk is a route of transmission for human viruses is critical.

Based on the principle of Koch's postulate, we propose an analytical framework to determine the plausibility of viral transmission by breast milk. This framework is based on five criteria: viral infection in children receiving breastmilk from infected mothers; the presence of virus, viral antigen or viral genome in the breast milk of infected mothers; the evidence for the virus in breast milk being infectious; the attempts to rule out other transmission modalities; and the reproduction of viral transmission by oral inoculation in an animal model. We searched for evidence in published reports to determine whether the 5 criteria are fulfilled for 16 human viruses that are suspected to be transmissible by breast milk. We considered breast milk transmission is proven if all 5 criteria are fulfilled, as probable if 4 of the 5 criteria are met, as possible if 3 of the 5 criteria are fulfilled and as unlikely if less than 3 criteria are met. Only five viruses have proven transmission through breast milk: human T-cell lymphotropic virus 1, human immunodeficiency virus, human cytomegalovirus, dengue virus and Zika virus. The other 11 viruses fulfilled some but not all criteria and were categorized accordingly.

Our framework analysis is useful for guiding public health recommendations and for identifying knowledge gaps amenable to original experiments.

Keywords**:** breast milk, viral transmission, plausibility, Koch's postulate, analytical framework

iv. Key Message

This report will inform pediatricians and immunologists on the existence of viral transmission by breast milk, alleviate public anxiety regarding potential transmission, identify knowledge gaps amenable to original experiments and enrich the debate on how to encourage best practice of infant feeding while preventing breastfeeding transmission of human viruses.

v. Main text

Introduction

Exclusive breastfeeding in the first six months of life and continued breastfeeding for at least 24 months, is the optimal feeding mode for infants and children. Breastfeeding not only provides optimal nutrition but also contributes significantly to child survival, lifelong health and development. [1] Breast milk contains a multitude of biologically active substances (including antibodies, cytokines, anti-infectious agents, cell growth factors, complex lipids, immunomodulating oligosaccharides and complement) and maternal cells that confer benefits to enable these outcomes. [2] It is now understood that the fragile neonatal immune system only becomes fully competent if it is complemented by components of the maternal immune system transferred through breastfeeding during the first few weeks postpartum so called “fourth trimester of pregnancy”. The interactions and intimacy between mother and infant through breastfeeding also support neuropsychological maturation and early childhood development.

However, in some very specific conditions, breast milk and breastfeeding can be important routes for viral transmission. For at least three human viruses – the human T-cell lymphotropic virus 1 (HTLV-I), the human immunodeficiency virus (HIV) and the human cytomegalovirus (CMV) – breastfeeding contributes to mother-to-child transmission. Several other human viruses have also been hypothesized to be transmitted through breast milk or by breastfeeding because of observations such as the presence of viral particles or viral genomes in breast milk or the acquisition of the infection by infants fed by mothers with confirmed infection. For many of these viruses, the experimental or observational data linked to actual transmission remain piecemeal and incomplete, rendering the causality of the relationship still elusive. Definitive proof of a causal link between the infant feeding modality and infectious risk is particularly difficult to ascertain and is presently not based on a consensus framework for the interpretation of evidence.

The portal of entry for milk-borne viruses in the breastfed infant remains to be fully clarified but may involve tonsils, pharyngeal mucosae and digestive tract mucosae, including enterocytes and Peyer’s patches. [3,4] Various mechanisms are used by human viruses to cross infant’s mucosae and establish infection, including direct translocation facilitated by breaches in the mucosal integrity, cell-to-cell transfer via virological synapses, transcytosis across M cells or enterocytes, or possibly by breastfeeding-induced microchimerism. [3-7]

Differentiating transmission through breast milk – as a result of ingesting milk containing the virus – from breastfeeding transmission which might also include other transmission routes (airborne, droplets, skin or mucous contacts, blood borne, vector borne) due to proximity

with the mother during feeding – is challenging. A recent example of such difficult and inconsistent interpretation of evidence was generated during the Coronavirus disease 19 (Covid-19) pandemic. Studies reported that severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) can be transmitted by approximately 10% of infected pregnant women to their offspring, in utero or in the first weeks of life. [8] Also, SARS-CoV-2 RNA has been detected in the breast milk of lactating mothers with confirmed SARS-CoV-2 infection and mild Covid-19 symptoms. [9] Whether breast milk and/or breastfeeding transmission of SARS-CoV-2 is possible and, if so, whether this transmission represents a significant threat to infant health remain to be demonstrated. This uncertainty has generated scientific questioning and also anxiety in the public and a significant threat to infant feeding practices worldwide. The World Health Organization has reviewed this evidence and released recommendations but other national authorities and professional associations have not always concurred with these guidelines. [10]

Here, we propose an analytical framework based on 5 criteria to help establish a causal relationship between breast milk exposure and acquisition of viral infections in breastfed infants. Based on revisiting the concept of Koch's postulate, this analytical framework should help refine health policies regarding infant feeding and infectious risk and stimulate research to fill the gaps in order to confirm or refute breast milk transmission of specific human viruses.

The analytical framework

The first Koch's postulate was proposed in 1876 by Robert Hermann Koch in a pioneering attempt to establish the causative relationship between a microbe and a disease. In its initial form (followed by many revisions) the postulate included the following four criteria:

- The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms;
- The microorganism must be isolated from a diseased organism and grown in pure culture;
- The cultured microorganism should cause disease when introduced into a healthy organism;
- The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent. [11]

The postulate implies that the demonstration of the presence of an infectious agent in a patient affected by the disease is not sufficient to infer a causal relationship. In that sense, Koch's postulate is considered as a founding concept of modern evidence-based medicine. Koch's postulate focused particularly on acute disease causation, as chronic viral infections were clearly not a concern at that time. One hundred and forty four years later, the principle of the postulate, – the fulfilment of criteria, each of them contributing to a final inference of

causality – remains however perfectly valid. Breast milk transmission of viruses is complex as it often involves a mother with no disease and an infant who may acquire infection with no signs of disease. The immense advantage today over the situation in 1876 results from spectacular advancements in the tools offered by medical research to ascertain evidence. Applying the underlying principles of Koch's postulate with a common framework for interpretation of evidence would help determine with confidence whether a given virus is transmissible by a specific route.

We propose that transmission of a human virus by breast milk is considered proven if the five following elements are all demonstrated:

1. **There is evidence for viral infection in infants receiving breastmilk from infected mothers**

Epidemiologic observations that substantiate transmission occurs from mother to child, possibly through breastfeeding, based on evidence of infant infection by means of direct or indirect assays.

2. **Virus, viral antigen or viral genome are present in the breast milk of infected mothers**

The presence of viral particle, viral antigen or viral genome or infected cells in breast milk may reflect either viral replication within breast milk or the mammary gland, extravasation of viruses from the vascular compartment, attraction of infected cells into the mammary gland or milk - as an effector site of the mucosal immune system –, clinical contamination during collection of breast milk or a laboratory contaminant. [4] In certain circumstances, local humoral response to the virus may be also interesting to explore as it may mitigate viral shedding. [12]

3. **The virus in breast milk is infectious**

The capacity for a virus identified in breast milk to cause infection can be confirmed if the virus can replicate *in vitro* in cell culture, in tissue explants or in organoïds. In case of highly diverging viruses (usually RNA viruses), viral infectiousness can be indirectly inferred if the virus present in breast milk and the virus isolated in the infant are indistinguishable (at least 95% genetically identical). Also, a cytotoxic response to viral epitopes of some viruses demonstrated in breast milk can also be considered as strongly suggesting local virus replication.

4. **Reasonable attempts have been made to rule out other relevant transmission routes (e.g. by transplacental, airborne droplets, arthropod bites and blood borne routes) potentially associated with breastfeeding**

Most frequently, this can be assessed in carefully described case reports, case series or cohorts. Other routes of mother-to-child transmission can be potentially ruled out by demonstrating the absence of virus detection in cord blood and/or the birth canal or by

demonstrating a risk reduction by avoidance of breastfeeding (strict replacement feeding) or by viral inactivation of expressed breast milk (pasteurization, freezing-thawing …).

5. **Transmission by breast milk can be reproduced by oral inoculation in an animal model**.

The animal model can convincingly contribute to the hypothesis of breast milk transmission if infection is demonstrated in new-born animals breastfed by infected mothers, although transmission through close contact with the mother can never be ruled out in this model, or after oral inoculation by means of milk containing the virus. Oral inoculation by means of culture supernatant or concentrated infected cells is less convincing, as it is not reproducing the complex composition of breast milk and its interactions with viable viruses.

In order to challenge this analytical framework, we searched for evidence in published reports to determine whether the 5 criteria are fulfilled or not for 16 human viruses that are suspected to be transmissible by breast milk. [6,8,9,13-78] According to the literature search, information can be found to validate or not the criteria for these viruses, although this information is sometimes scarce or incomplete. As an example, animal model exists for almost all 16 viruses but few of these models have been challenged by the oral route and a fortiori by breast milk. [13]

We considered breast milk transmission is proven if all 5 criteria are fulfilled, as probable if 4 of the 5 criteria are met, as possible if 3 of the 5 criteria are fulfilled and as unlikely if fewer than 3 criteria are met. If at least two criteria were not reported, viral transmission by breast milk was considered as insufficiently documented. According to this analytical framework (see Table), transmissibility through breast milk is proven for only five of the selected human viruses. Not surprisingly, three of them - HTLV-1, HIV and CMV - are generally considered as the prototypes of human viruses transmissible by breast milk.[13, 15, 16] However for the other two, dengue virus (DENV) and Zika virus (ZIKV) transmissibility through breast milk was not previously considered proven despite reasonably strong evidence to support transmission. Three human viruses – Ebola virus (EBOV), West Nile virus (WNV) and the recently studied Andes virus (ANDV), the only hantavirus transmitted between humans by close contacts [17] - are probably transmissible through breast milk. For each of them, the gap in knowledge that needs to be filled by experimental evidence is identified and discussed (see Table). Yellow fever virus (YFV, vaccine strain 17D), Epstein-Barr virus (EBV) and hepatitis E virus (HEV) are judged as only possibly transmissible by breast milk. For YFV, the virus or its genome has never been detected in breast milk and no animal model has been used so far to replicate oral challenge. For EBV, routes of transmission other than breast milk cannot be ruled out and the animal model for breast milk transmission is insufficiently convincing. [18] Two viruses – Chikungunya virus (CHIKV) and SARS-CoV-2 –

are considered as unlikely candidates for breast milk transmission. Finally, herpes simplex virus (HSV), hepatitis C virus (HCV) and hepatitis B virus (HBV) transmission by breast milk is insufficiently documented, as it may be for other human viruses than the 16 selected, such as Tick-Borne Encephalitis Virus. [19]

Conclusions

Over recent years, outbreaks of emerging or re-emerging viral infections have raised the question of transmission through breast milk and breastfeeding. As unknowns create anxiety and may impact feeding practices inappropriately, we believe our analytical framework contributes an important step in the process by which health policy for infant feeding is made in the context of human virus outbreaks. A drawback of our analytical framework may result from the 5 criteria having not equal weight in predicting transmission. For example, if substituting formula for breast milk clearly reduces the risk of transmission of a pathogenic virus – an effect measured in randomized clinical trials on very few viral infections so far, such as HIV [26] -, do we need tissue culture infection or an animal model before we can make public health decision? Also, viruses co-infecting breast milk may interfere with each other for viral shedding. In a study of HIV-1-infected breastfeeding women in Zimbabwe, breast milk CMV and EBV levels (reflecting local reactivation) were independently associated with detection of breast milk HIV-1 RNA. [79]

It has to be stressed that demonstration of breast milk transmission of a virus alone does not necessarily require or imply preventive interventions and does not justify that infection with this organisms is a contraindication to breastfeeding; recommendations on infant feeding must consider several other individual and societal-level factors such as the frequency and severity of the viral infection in infants, the social and environmental context (social norms, burden of infectious diseases, access to water and sanitation) and health and development cost of not breastfeeding for the individual child, and the background morbidity and mortality profiles within that context. For many of these viruses, even if breast milk transmission is confirmed, the risk of not breastfeeding largely outweigh the risk of transmitting the virus to the infant. Some of these viruses, such as CMV in non preterm infants, induce only asymptomatic or benign disease and do not therefore justify avoidance of breastfeeding or necessitate an alternative infant feeding practice. [80]

By pinpointing gaps in knowledge that urgently need evidence generation, our analytical framework is also important for decision making on scientific agendas in order to decipher mechanisms of transmission and confirm or refute breast milk transmission of viruses. Similar frameworks and exercises should be conceptualized and undertaken to ascertain the

level of evidence for other modes of viral transmission such as sexual transmission or horizontal transmission of specific viruses including HCV and arboviruses.

v. Acknowledgements

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viii. Table. Plausibility of viral transmission through breast milk: applying the framework to 16 human viruses

Human viruses	**Criteria**					**Plausibility of transmission through breast milk***
	1 Evidence for infection in infants receiving breastmilk from infected mothers	**2** Virus antigen or viral genome are present in breast milk from infected mothers	**3** The virus in breast milk is infectious	**4** Other transmission modalities can be ruled out	**5** Transmission by breast milk can be reproduced in an animal model	
Human T-cell lymphotropic virus 1	Yes Percher et al (2016)[6] Hino et al (2011)[13]	Yes Kinoshita et al (1984)[20]	Yes Kinoshita et al (1984)[20]	Yes, Absence of other risk factors, risk reduction by replacement feeding or freezing/thawing of breast milk Percher et al (2016)[6] Hino et al (2011)[13]	Yes : Marmoset Yamanoushi et al (1985) [21] Rabbit Uemara et al (1986)[22]	Proven
Human	Yes	Yes	Yes	Yes	Yes	Proven

immunodeficiency virus	Van de Perre et al (1991)[15]	Neveu et al (2011)[23] Ndirangu et al (2012)[24]	Thiry et al (1985)[25]	Mothers infected post partum Van de Perre et al (1991)[15] Risk reduction by replacement feeding Nduati et al (2000)[26]	Non human primates Ruprecht et al (1998)[27]	
Human cytomegalovirus	Yes Minamishima et al (1994)[16]	Yes Hamprecht et al (2017)[28] Moylan et al (2017)[29] Prendergast et al (2019)[3]	Yes, local cytotoxic response to CMV antigens Moylan et al (2017)[29]	Yes Risk reduction by pasteurization or freezing/thawing of breast milk Hamprecht et al (2017)[28]	Yes Rhesus monkey Kaur et al (2018)[30] Antoine et al (2014)[31]	Proven
Dengue virus	Yes Arrangain et al (2017)[32]	Yes Arrangain et al (2017)[32]	Yes Barthel et al (2013)[33]	Yes Absence of detectable virus in cord blood; infant breastfed by an infected wet-nurse Arrangain et al (2017)[32] Barthel et al (2013)[33]	Yes Mouse Lee et al (2016)[34] Hamster Brueckner et al (1958)[35]	Proven

Zika virus	Yes Hoen et al (2018)[36]	Yes Dupont-Rouzeyrol M et al (2016)[37] Sotelo et al (2017)[38] Cavalcanti et al (2017)[39] Blohm et al (2017)[40]	Yes Dupont-Rouzeyrol et al (2016)[37] Sotelo et al (2017)[38]	Yes Vector-borne transmission non excluded but 99% genetic identity between breast milk and infant's strains Blohm et al (2018)[40] Colt et al (2017)[41]	Yes Rhesus macaque Newman et al (2017)[42] Bradley et al (2017)[43]	Proven
Ebola virus	Yes Sissoko et al (2017)[44]	Yes Nordenstedt et al (2016)[45]	Yes Bausch et al (2007)[46] Logue et al (2019)[47]	Yes Transmission from an asymptomatic woman with long term EBOV shedding in breast milk Sissoko et al (2017)[44]	Model exists [Ferrets: de la Vega et al (2018)[48]] but no milk transmission experiment reported	Probable
West Nile virus	Yes CDC (2002)[49]	Yes CDC (2002)[49]	No CDC (2002)[49]	YES Vector-borne transmission non excluded but mother infected postpartum by blood product	YES Hamster Reagan et al (1956)[50] Mouse Blazquez et al	Probable

				transfusion CDC (2002)[49]	(2010)[51]	
Andes virus	Yes Ferres et al (2020)[17]	Yes Ferres et al (2020)[17]	Yes Ferres et al (2020)[17]	Not documented	Model exists [hamster: Witkowski et al (2017)[52]] but no milk transmission experiment reported	Probable
Yellow fever virus (vaccine strain 17D)	Yes CDC (2010)[53]	Not documented	Yes Genetic identity of infant and maternal viruses CDC (2010)[53]	Yes YFV vaccine virus in CSF by PCR CDC (2010)[53]	No animal milk transmission experiment reported	Possible
Epstein-Barr virus	Yes Junker et al (1991)[54] Daud et al (2015)[55]	Yes Sanosyan et al (2016)[56]	Yes Junker et al (1991)[54] Daud et al (2015)[55]	Not documented	Model exists [rabbit: Okuno et al (2010)[18]] but no milk transmission experiment reported	Possible
Hepatitis E virus	Yes Verghese et al (2014)[57]	Yes Rivero-Juarez et al (2016)[58]	Yes Reported in cows milk Huang et al	Not documented	Model exists [rabbit: Wang et al (2018)[60]] but no milk transmission	Possible

			(2016)[59]		experiment reported	
Chikungunya virus	Yes Gérardin et al (2008)[61]	Yes Campos et al (2017)[62]	No Campos et al (2017)[62]	No Vector-borne transmission non excluded	Models exist [mouse, non human primates: Haese et al (2016)[63]] but no milk transmission experiment reported	Unlikely
Severe acute respiratory syndrome coronavirus type 2	Yes Lackey et al (2020)[64] Walker et al (2020)[8]	Yes Groß et al (2020)[9] Costa et al (2020)[65]	No Chambers et al (2020)[66]	No Possible by respiratory, droplets; no higher risk if breastfeeding Walker et al (2020)[8]	Rhesus macaque and marmoset can be infected by oral inoculation of MERS-CoV Ruiz et al (2017)[14] Not reported for SARS-CoV2.	Unlikely but Insufficiently documented
Herpes simplex virus	Yes Dunkle et al (1979)[67]	Yes Kotrionas et al (1999)[68]	Not reported	No Sullivan-Bolyai et al (1983)[69]	Several models exist [guinea pig, mouse: Kollias et al (2016)[70]] but no milk transmission experiment reported in animal.	Unlikely but Insufficiently documented
Hepatitis C virus	Yes	Yes	No culture	No	Several animal	Unlikely but

	Polywka et al (1997)[71]	Ogasawara et al (1993)[72] Kage et al (1997)[73]	available	Breastfeeding not an identified risk factor Polywka et al (1997)[71]	models exist [chimpanzee, humanized mouse: Berggren et al (2020)[74]] but no milk transmission experiment reported	Insufficiently documented
Hepatitis B virus	Yes Beasley et al (1975)[75] Shi et al (2011)[76]	Yes Montoya-Ferrer et al (2015)[77]	No culture available	No Breastfeeding not an identified risk factor Shi et al (2011)[76]	Several animal models available [Guo et al (2018)[78]] but no milk transmission experiment reported	Unlikely but Insufficiently documented

***** Proven=all 5 criteria fulfilled; Probable=4 of the 5 criteria fulfilled; Possible=3 of the 5 criteria fulfilled; unlikely= less than 3 criteria; insufficiently documented=at least two undocumented criteria