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## Cytomegalovirus shedding from breastmilk and mucosal sites in healthy post-partum women: a pilot study

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### Abstract

Mother-to-child cytomegalovirus (CMV) breastmilk transmission can occur in the postnatal period. In a pilot study, we measured daily CMV detection by PCR in breastmilk, vaginal, and saliva samples from 9 healthy CMV-seropositive postpartum women for 28 days. CMV was found in 7 of 9 women and 171 of 253 breastmilk samples (67.6%). In 4 women, all breast milk samples were positive. CMV was less frequently detected in the vagina (39 of 258, 15.1%) and saliva (53 of 258, 20.5%). Daily breastmilk, oral and genital collection is feasible and demonstrates high variability between women. Further study of the dynamics of CMV in distinct anatomic compartments is warranted.

### Keywords

cytomegalovirus (CMV); breastmilk; post-partum

### Introduction

Cytomegalovirus (CMV) has a high seroprevalence among reproductive-age women in the United States (50%) and globally (45–100%).<sup>1</sup> CMV establishes latent infection that can result in viral shedding at various epithelial sites, allowing for viral transmission via direct contact with body fluids.<sup>1</sup> Mother-to-child CMV transmission in the postnatal period occurs primarily via breastmilk.<sup>2</sup> While CMV acquisition in healthy infants is not associated with morbidity, infection in preterm or low-birth-weight infants can cause significant disease.<sup>2</sup>

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Studies using polymerase chain reaction (PCR) to detect CMV DNA have demonstrated that 40–96% of healthy CMV-seropositive women shed CMV in their breastmilk,<sup>2</sup> and that CMV quantity in breastmilk is positively correlated with mother-to-child transmission.<sup>3</sup> CMV is detectable in colostrum and by 2 weeks post-delivery in breastmilk.<sup>4,5</sup> The quantity of CMV in breastmilk peaks at 2–8 weeks and then declines 9–12 weeks post-delivery.<sup>4–6</sup> Increased or prolonged CMV shedding has been associated with immunosuppression, as seen in HIV-infected women, who may shed CMV in breastmilk up to 1 year postpartum.<sup>6</sup> The extent to which CMV detection in breastmilk reflects systemic reactivation is not known.

To assess the feasibility of daily CMV breastmilk and mucosal sampling to plan for future studies to characterize frequency, patterns, and associations of CMV shedding in breastmilk and other anatomic sites, we followed healthy breastfeeding post-partum women, who obtained daily samples of breastmilk, vaginal fluid, and saliva.

## Methods

### Participants and Clinical Procedures.

This pilot study was conducted at the University of Washington Virology Research Clinic from 2014 to 2015. Participants were recruited from the community. Eligible women were 18 years or older, breastfeeding an infant less than four months old, CMV-seropositive, HIV-seronegative, with a negative urine pregnancy test, and in good health. The University of Washington Institutional Review Board approved the study protocol and all participants provided written informed consent.

Demographic information and medical history were collected with standardized forms; follow up was every week for 8 weeks. Blood was collected weekly for plasma CMV DNA quantification. Participants were instructed on self-collection of 3 mL breastmilk, 1.5 mL urine, and oral and vaginal swabs. Samples were collected for 7 consecutive days every other week for the 8 week study period. All samples were stored at 4°C at home and at –20°C at clinic until testing.

### Laboratory Testing.

CMV serostatus was determined by immunoassay (Abbott Laboratories, Illinois). DNA was extracted from unfractionated breastmilk, oral and vaginal swabs, urine, and plasma. Real-time polymerase chain reaction (RT-PCR) was performed using specific primers to detect CMV.<sup>7</sup> Positive and negative controls were included on each PCR plate. CMV quantity was determined using a standard curve with known quantities of CMV. Samples with greater than 150 copies per milliliter (mL), (equivalent to 125 IU/mL) of CMV DNA were considered positive.

### Statistical Analysis.

CMV shedding rates were calculated as the number of days with positive results divided by the number of days with samples collected. The amount of virus shed per sample was quantified as log<sub>10</sub> copies/mL. Per-person and overall median values of CMV shedding rates and quantities were calculated. Generalized estimating equations (GEE) with an

autoregressive correlation structure (AR1) and a log link were used to determine associations between CMV shedding in different compartments.

## Results

Nine women were enrolled and completed the study. The median maternal age was 31.5 years (range 23–39 years). Participants self-reported as Caucasian (N=6), African-American (N=1), Asian (N=1), or mixed race (N=1). The women were a median 49 days postpartum at enrollment (range 35–104 days). All participants gave birth to full term infants (range 37–42 weeks gestation). Four participants delivered vaginally; five had cesarean sections. One participant had twins.

Breastmilk, vaginal fluid, saliva, and urine samples were self-collected for a median of 28 days (range 26–31 days). Of 1008 expected samples, 1026 were returned, indicating outstanding adherence to study procedures. Among the 9 participants, CMV was detected in 7 (78%) women in breastmilk, 3 (33%) women in vaginal swabs, and 2 (22%) women in oral swabs. The rates of breastmilk, vaginal, and oral shedding in each participant are shown in Tables 1A and 1B. Overall, CMV was found in most breastmilk samples (171 of 253, 67.6%), in particular among the 7 women with any CMV shedding detected (171 of 199, 88.9%). Four women had CMV detected in all breastmilk samples. CMV was less frequently detected in the vagina (39 of 258, 15.1%) and mouth (53 of 258, 20.5%) (Table 1A, Figure 1). The two women with no detectable CMV at any site were the furthest post-partum (103 and 104 days at study enrollment, respectively) among participants (Figure 1). CMV was not detected in the 257 urine samples collected, nor in the 81 weekly plasma samples collected.

The per person CMV shedding rates were a median of 100% (interquartile range 25–100) for breastmilk, 0% (range 0–90) for vaginal swabs, and 0% (range 0–100) for oral swabs (Table 1B). Among samples with detectable virus, median (range) quantities of CMV DNA were 3.6 (2.2–5.4)  $\log_{10}$  copies/mL in breastmilk, 2.8 (2.2–4.4)  $\log_{10}$  copies/mL in vaginal swabs, and 3.2 (2.2–5.1)  $\log_{10}$  copies/mL in oral swabs (Table 1B).

Vaginal CMV detection was significantly more likely to occur on days with oral CMV detection (RR = 26.1, 95% CI 3.9 to 176.0,  $p=0.0008$ ). However, oral CMV detection was not more likely to occur on days with vaginal CMV shedding (RR=1.1, 95% CI 1.0 to 1.1). Breastmilk CMV detection was not found to be more likely on days with oral CMV detection (RR 1.2, 95% CI=0.9–1.5) or vaginal CMV detection (RR= 1.4, 95% CI 0.9 to 2.4,  $p=0.17$ ). Having breastmilk CMV quantities greater than 3.6  $\log_{10}$  copies/mL was significantly associated with simultaneous oral CMV detection (RR=4.1, 95% CI 1.9 to 8.8,  $p=0.0002$ ) but not with vaginal CMV detection (RR=1.8, 95% CI=0.9 to 3.7,  $p=0.11$ ).

## Discussion

Through daily sampling, we found a high frequency and quantity of CMV in breastmilk among CMV-seropositive healthy women up to 17 weeks postpartum. In contrast, CMV shedding in vaginal fluid and saliva was infrequent, and was not found in urine and plasma. In this small cohort, oral CMV shedding was associated with increased risk of vaginal CMV shedding and increased quantities of CMV in breastmilk. These data are consistent with

earlier studies using viral culture which showed concurrent CMV reactivation at multiple sites in some postpartum women.<sup>8,9</sup> Intensive studies of CMV reactivation from multiple sites are feasible in healthy post-partum women, and suggest that immunologic control at each anatomic site differs. These preliminary findings also allow for assessment of required sample size calculations for novel intervention studies aimed at reducing viral replication with a long-term goal of preventing CMV transmission to the neonate via breastmilk.

This study extends what is known about CMV shedding patterns in healthy postpartum women, as most recently published studies of CMV shedding focused on women who gave birth to preterm or low birth weight infants<sup>3,4,10</sup> or women with HIV infection.<sup>6</sup> Most women in our study shed CMV in breastmilk, which is consistent with studies of CMV-seropositive women that report 66–88% of CMV-seropositive women have detectable CMV DNA in breastmilk.<sup>4,5,10</sup> We found the median CMV quantity in breastmilk to be similar to prior studies that show peak CMV DNA of 4–6 log<sub>10</sub> copies/mL.<sup>4,5</sup> The consistency of CMV presence and quantity in breastmilk in each woman suggests that weekly CMV DNA breastmilk quantification may be representative of daily values.

Our finding of persistent CMV shedding in breastmilk up to 17 weeks postpartum augments prior studies that followed women for shorter time periods.<sup>3–5,10</sup> One study showed similarly persistent CMV shedding in breastmilk (present up to one year after delivery) among healthy postpartum women.<sup>3</sup> Previous studies have shown that increased time postpartum (>100 days) has been correlated with decreased CMV shedding in breastmilk;<sup>4,5</sup> we hypothesize that this may account for the lack of CMV detection in breastmilk for 2 participants.

Among the seven women who shed CMV in breastmilk in our study, three also shed CMV in the vagina and two shed CMV in the mouth. These data are consistent with results from CMV seropositive postpartum women which showed CMV shedding by culture in 10–13% of women in the vagina and 2–9% of women in oral cavity.<sup>8,9</sup> Data on oral CMV shedding measured by PCR in healthy postpartum women is limited to one cross-sectional study, which showed CMV detection from a single saliva sample of 21% of participants.<sup>11</sup>

While prior studies have evaluated CMV shedding in multiple sites in healthy postpartum women, our study is the first to evaluate multisite CMV shedding daily and via quantitative PCR in this population.<sup>8,9</sup> We found that on days when oral shedding was present, vaginal detection was much more likely and that breastmilk quantities above median values also occurred at higher frequency. This suggests that CMV reactivates concurrently in several compartments. Slyker *et al.* showed that CMV shedding in the cervix is associated with higher quantity of CMV shedding in breastmilk in HIV-positive breastfeeding women.<sup>12</sup> Among HIV-infected pregnant women, peripartum urinary CMV shedding is associated with increased risk of congenital CMV infection in their infants.<sup>14</sup> The mechanism for CMV reactivation, particularly at multiple sites, in postpartum women may be related to CMV-specific immunosuppression during pregnancy and postpartum. The number of CMV-specific CD4+ T cell responses in pregnant women is significantly lower than age-matched non-pregnant women, and the decreased responses persist postpartum.<sup>13</sup> However, the differential effect of immunity in each compartment is puzzling. CMV was not identified in

the plasma in any of the participants, suggesting that control of replication occurs locally at each site. Understanding the patterns of shedding and predictors in each compartment may allow the development of strategies to suppress CMV shedding and prevent transmission to neonates.

A strength of our study is that we longitudinally collected samples from multiple compartments. Our study is limited by the small cohort of women with a range of days postpartum at study enrollment, especially given that time postpartum has been correlated with changes in CMV shedding patterns.<sup>4,5</sup> Although we studied a small number of participants, we structured our study to focus in depth on fewer women with longer duration of testing purposefully, as these types of natural history data for CMV breastmilk have not, to our knowledge, been published. Our data are also limited by unknown time of CMV acquisition and lack of data on CMV transmission to the infant. Prior data, however, show that quantity of CMV viral shedding, for example, in breastmilk has positively correlated with mother-to-child transmission.<sup>3</sup>

Our findings are relevant for the increasing practice of sharing raw breastmilk in the community, particularly given that women with preterm or low-birth weight infants are more likely to consider breastmilk sharing than those with full term infants.<sup>17</sup> A recent prospective study of postnatal CMV transmission in very low-birth-weight infants, found that CMV transmission from occurred in 29 of 539 infants (5.4%).<sup>18</sup> Among 29 CMV infected infants, 27 infections occurred in infants who received CMV-positive breastmilk; mortality rate among CMV-infected infants was high.<sup>18</sup>

In contrast to preterm or low-birth weight infants, full term infants who acquire CMV from breastmilk are almost always asymptomatic, as the infection may be modulated by maternal antibodies. These antibodies are lacking in premature infants who are at risk for severe CMV disease.<sup>2</sup> Whether a full term infant born to a CMV-seronegative woman is at risk for complications from CMV acquired from donated breastmilk is unknown.

In conclusion, we demonstrate that daily sampling of breastmilk and mucosal samples is feasible in post-partum women. The rate of breastmilk CMV detection is variable between women, but with substantially less variability within women over time. These data may be helpful to generate sample size calculations for interventional trials to measure the effect of an intervention on CMV detection in post-partum women.

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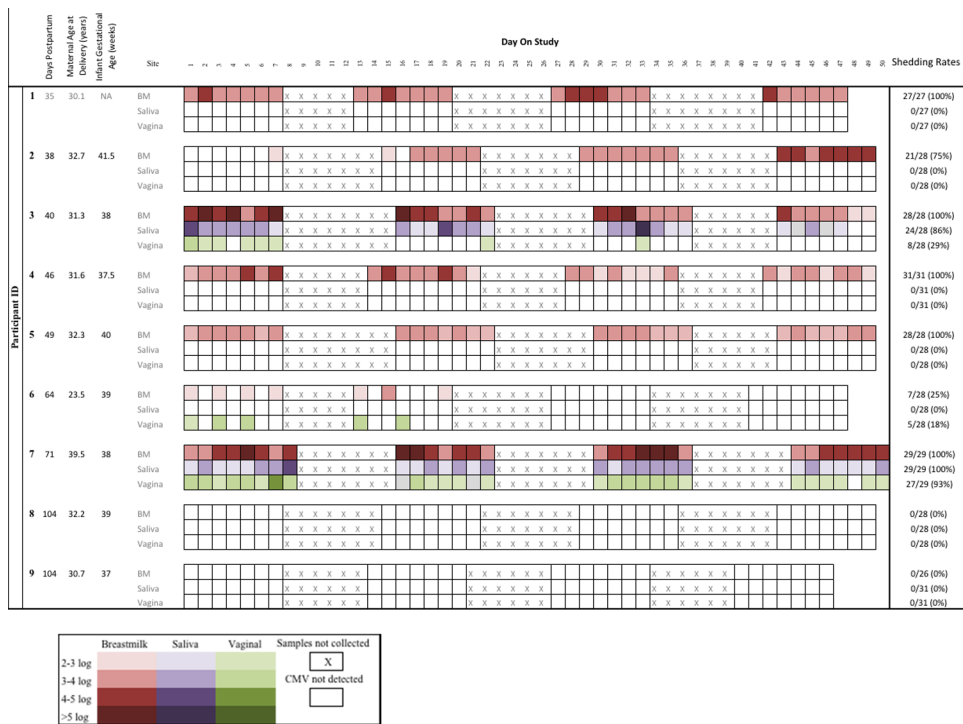
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**Figure 1: CMV presence and quantity in daily self-collected breastmilk samples, oral swabs, and vaginal swabs in 9 post-partum women.**

CMV PCR results from each woman are shown by the postpartum day of sample collection. Sample sites are shown in the following order for each participant: breastmilk (BM), saliva, vagina. Swabs with CMV detected are indicated by color (breastmilk in red, oral swabs in purple, and vaginal swabs in green) with viral quantity indicated by the heatmap. Days on which CMV were not detected are indicated by a blank box. Days on which samples were not collected are indicated by “X.” Participants 8 & 9 had samples collected every other week through day 155 and 153 respectively, and CMV was not detected from any site. The number of swabs with CMV detected/number of swabs collected and proportion of swabs with CMV detected by site for each participant is indicated under the header “Shedding Rate.” NA: Not available.



**Table 1A:**

Per Participant Frequency of CMV Shedding in Breastmilk, Vagina, and Oral Cavity

Participant	Days Post-Partum	Delivery Method	Infant Gestational Age (weeks)	Mother Age at Delivery (years)	# samples positive/ # samples collected (%)		
					Breastmilk	Vagina	Oral
1	35	Caesarean	NA	30.1	27/ 27 (100%)	0/27 (0%)	0/27 (0%)
2	38	Caesarean	41.5	32.7	21/ 28 (75%)	0/28 (0%)	0/28 (0%)
3	40	Vaginal	38	31.3	28/ 28 (100%)	8/ 28 (29%)	24/ 28 (86%)
4	46	Vaginal	37.5	31.6	31/ 31 (100%)	0/31 (0%)	0/31 (0%)
5	49	Vaginal	40	32.3	28/ 28 (100%)	0/28 (0%)	0/28 (0%)
6	64	Caesarean	39	23.5	7/ 21 (33%)	5/ 28 (28%)	0/28 (0%)
7	71	Caesarean	38	39.5	29/ 29 (100%)	27/ 29 (100%)	29/ 29 (100%)
8	104	Caesarean	39	32.2	0/28 (0%)	0/28 (0%)	0/28 (0%)
9	104	Vaginal	37	30.7	0/26 (0%)	0/26 (0%)	0/26 (0%)

**Table 1B:**

Frequency and Quantity of CMV Shedding in Breastmilk, Vagina, and Oral Cavity

	<b>Breastmilk</b>	<b>Vagina</b>	<b>Oral</b>
Days sampled per person, median (IQR)	28 (28–28)	28 (28–29)	28 (28–29)
Persons with any CMV detection, No. (%)	7 (77.8)	3 (33.3)	2 (22.2)
PCR-positive days/total PCR swabs, No. (%)	171/253 (67.6)	39/258 (15.1)	53/258 (20.5)
$\log_{10}$ viral copies, median (IQR) <sup>a</sup>	3.6 (3.1–4.1)	2.8 (2.5–3.3)	3.2 (2.8–3.7)
Per person CMV shedding rate, median (range) <sup>b</sup>	100 (0–100)	0 (0–90)	0 (0–100)

Abbreviations: NA: not available; IQR, interquartile range; PCR, polymerase chain reaction

<sup>a</sup> Among positive samples.

<sup>b</sup> Shedding rate is defined as percent days positive for CMV DNA by RT-PCR with a detection threshold of > 150 copies /ml.

Plasma was collected twice for each participant. Urine was collected on the same days as breastmilk samples by each participant. No CMV was detected in plasma or urine samples.