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Cytomegalovirus and Epstein-Barr Virus in Breast Milk are Associated with HIV-1 Shedding but Not With Mastitis

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Abstract

Background—Breast milk HIV-1 load is associated with clinical and subclinical mastitis, and both milk viral load and mastitis are associated with increased mother-to-child-transmission of HIV-1 (MTCT) through breastfeeding. Bacterial infections may cause clinical mastitis, but whether other co-pathogens common in HIV-1 infection are associated with subclinical mastitis or HIV-1 shedding is unknown.

Design—A cross-sectional study of HIV-1 infected breastfeeding women in Zimbabwe was performed to examine the relationship between a wide range of breast co-infections, mastitis, and HIV-1 shedding.

Methods—Breast milk was cultured for bacteria and fungi, and tested by PCR for mycobacteria, mycoplasmas, human herpesvirus 6 (HHV-6), HHV-7, HHV-8, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and HIV-1 RNA and DNA. Symptoms of clinical mastitis were documented, and subclinical mastitis was identified by breast milk sodium concentration (Na⁺) and leukocyte counts.

Results—Co-infections of milk were not associated with clinical or subclinical mastitis in the 217 women studied. Detection of HIV-1 RNA, but not DNA, in breast milk was associated with CMV

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Roles of the authors.

The study was conceived by SG, AKS, DAK, and LMF. JM, GM, GW, and LSZ contributed to the study design and data collection. SG, JC, XQ, and JM assisted with laboratory assays. The statistical analyses were performed by KDS and SG. The manuscript was drafted by SG and LMF. All authors critically reviewed the manuscript and approved the final version.

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concentration (OR 1.8, $p=0.002$) and detection of EBV (OR 3.8, $p=0.0003$), but not other co-infections in multivariate analysis.

Conclusions—Co-infection of breast milk with bacteria, fungi or herpes viruses was not associated with mastitis. The associations between shedding of CMV and EBV with HIV-1 in milk suggest a local interaction between herpes virus infection and HIV-1 independent of mastitis. CMV and EBV infections may impact HIV-1 shedding in breast milk and the risk of MTCT.

Keywords

MTCT; breastfeeding; mastitis; CMV; EBV; HHV-6; HHV-7; HHV-8; mycobacteria; mycoplasmas

Introduction

The risk of mother-to-child-transmission of HIV-1 (MTCT) via breastfeeding is correlated with the concentration of HIV-1 RNA and HIV-1 DNA in breast milk, and with the duration of exposure (reviewed in [1]). Early weaning and artificial formula feeding of HIV-1 exposed infants in resource-poor communities frequently increases mortality due to diarrheal or respiratory infections [1]. Reducing HIV-1 shedding in breast milk may provide infants the health benefits of breastfeeding while reducing the risk of MTCT.

Mastitis, an inflammatory process of the breast, is recognized as an important risk factor for MTCT (reviewed in [2]). Subclinical (asymptomatic) mastitis has been defined by an elevated milk sodium (Na^+), sodium to potassium ratio ($\text{Na}^+:\text{K}^+$), or total leukocyte count, and is highly prevalent among HIV-1 infected breastfeeding women [1-3]. The mechanism by which mastitis increases the risk of transmission is likely due, at least in part, to the associated higher concentrations of HIV-1 RNA in breast milk [1-3].

Breast infections are often suspected of causing mastitis, but few studies have attempted to define the role of infectious organisms among breastfeeding women [2]. There is extensive research in dairy animals, however, describing associations between mastitis and a multitude of bacterial, mycobacterial, mycoplasmal, fungal, and viral pathogens [4]. In women, mastitis is associated with suboptimal breastfeeding techniques as well as deficiencies in micronutrients that are important for immune function, such as vitamin E [1,2]. It is plausible that these phenomena and infection are interdependent, with retained milk or impaired immune responses, respectively, predisposing to bacterial growth and infection of the breast.

The potential infectious causes of clinical and subclinical mastitis in women with HIV-1 infection, who may be susceptible to a much wider range of pathogens, have not been systematically studied. A better understanding of the mechanisms that result in mastitis and HIV-1 shedding in breast milk may lead to interventions to reduce MTCT. Here we evaluated the breast milk of HIV-1 infected women for a broad range of infectious agents, indicators of mastitis, and shedding of HIV-1 RNA and DNA.

Participants and methods

Participants and data collection

HIV-1 infected breastfeeding women attending clinics in the Zimbabwean towns of Chitungwiza and Epworth who were 6 to 16 weeks postpartum were enrolled in a cross-sectional study, which has been described previously [3]. A single milk specimen from each breast, blood, and demographic and clinical data were obtained from consenting women. Counselors administered questionnaires that captured symptoms of breast inflammation (pain, swelling, warmth or redness) as well as use of antiretroviral or other antimicrobial medications.

The Medical Research Council of Zimbabwe, and committees regulating research on human subjects at Seattle Children's Hospital and Stanford University approved this study.

Specimen collection and processing

Breast milk (minimum 5 mL, maximum 20 mL) was collected using an aseptic technique akin to a clean-catch urine specimen. Women were first assisted in a 2-minute scrub of their hands and breasts using povidone-iodine soap. Milk was then manually expressed, and, after discarding the first several drops, was collected into sterile tubes, one for each breast. Blood (6 mL) was collected by venipuncture. Blood and milk specimens were refrigerated immediately and processed within 4 hours of collection.

Fresh whole milk was cultured for bacteria and fungi, and then aliquots were stored at -70°C for additional studies (see below). The remaining whole milk was centrifuged at $1000 \times g$ for 15 minutes to separate the lipid, lactoserum, and cell pellet. The lipid layer was discarded, lactoserum was stored at -70°C , an aliquot of milk cells were counted manually [3] with the remainder frozen at -70°C . Blood plasma was separated by centrifugation and stored at -70°C .

Breast milk cultures

Fresh whole milk (1 mL) was used to inoculate 9 mL of tryptone soy nutrient broth, which was incubated at 37°C . In addition, 10 μL of each whole milk specimen were plated onto one chocolate, one MacKonkey, one Sabouraud, and two blood agar plates using sterile loops. The MacKonkey, Sabouraud, and one of the blood agar plates were incubated aerobically, with the chocolate agar plate in 5% CO_2 , and the second blood agar plate anaerobically, all at 37°C . Cultures were checked at 24, 48, and 72 hours, and 2 weeks. Positive cultures were sub-cultured, followed by identification using morphology and basic biochemical testing according to routine methods [5]. Bacterial growth on directly inoculated plates was quantified by the number of colony-forming units (CFU) per mL of whole milk. Fewer than 10^3 CFU of coagulase-negative staphylococci, diphtheroids, *Bacillus* spp. or *Propionibacterium acnes* were considered to be contamination from the skin and excluded from our analysis.

Electrolyte measurement

Na^+ and K^+ in lactoserum were measured using a Roche/Hitachi 902 Autoanalyzer (Roche Diagnostics, Basel, Switzerland), as described previously [3].

Quantification of HIV-1

HIV-1 RNA in lactosera and plasma specimens was quantified using the AMPLICOR™ Monitor kit, version 1.5 (Roche Diagnostics Basel, Switzerland). The detection limit for plasma was 400 copies/mL using the standard protocol, and for lactosera was 50 copies/mL using the ultra-sensitive protocol. HIV-1 DNA was quantified in breast milk as previously described [3]. Briefly, DNA was extracted from frozen breast-milk cell pellets, and a 129-bp region of HIV-1 gag was amplified using real-time PCR. A standard curve was included in each assay and consisted of 10-fold serial dilutions of a plasmid template from 10^5 to 10^0 copies/reaction. The detection limit was between 1 and 10 copies of HIV-1 DNA per reaction. β -Globin was also amplified from each sample to test for inhibitors of PCR. Negative controls without DNA were included in each assay.

DNA amplification of other infectious agents

Aliquots (2 mL) of previously frozen whole milk were centrifuged at $5000 \times g$ for 10 minutes to pellet mycobacteria. DNA was extracted from these pellets and separately from the corresponding supernatants using QiaAmp Mini Kit (Qiagen, Hilden, Germany) with 50 μL

of elution buffer. PCR to detect mycobacterial DNA was performed using the milk pellet DNA from either the left or right breast milk specimen randomly selected from each woman. Assays to detect CMV, EBV and HHV-7 were prioritized such that all specimens with sufficient amounts of milk supernatant DNA were tested for all three viruses. PCR for HHV-6 and HHV-8 DNA were each performed on 70 randomly selected milk supernatants. Mycoplasma PCR testing was performed on all milk specimens with sufficient DNA remaining from the milk supernatant.

Mycobacteria were detected by real-time PCR using primers specific for the internal transcribed spacer sequences between 16S and 23S rRNA genes of *Mycobacterium* species: SP-1 (5'-ACCTCCTTTCTAAGGAGCACC-3') and SP-2 (5'-GATGCTCGCAACCACTATCCA3') [6]. Each specimen was assayed in 50 µL reactions containing 10 µL of milk pellet DNA, 0.5 µM of each primer, and SYBR Green supermix (BioRad Laboratories, Hercules, CA). Cycling conditions were 2 minutes at 95° C, followed by 45 cycles of 30 seconds at 94° C, 40 seconds at 62° C and 40 seconds at 72° C, followed by a melting curve from 62–95° C with steps of 0.5°C every 10 seconds. The template for the standard curve in each run was DNA extracted as described above from serial dilutions of cultured *M. fermentans* (ATCC number 19709). The lower limit of detection was 10–100 copies/reaction.

Mycoplasmas were detected by real-time PCR using primers that amplify a region of the 16S rRNA gene conserved across *Mycoplasma* species: MGSO 5'-CCATCTGTCACTCTGTAAACCGC-3' and my-ins 5'-GTAATACATAGGTCGCAAGCGTTATC-3' [7]. Each specimen was assayed in 25 µL reactions containing 5 µL of milk supernatant DNA, 0.8 µM of each primer, and SYBR Green super mix (BioRad Laboratories, Hercules, CA). Cycling conditions were 3 minutes at 95°C, followed by 45 cycles of 30 seconds at 95°C alternating with 1 minute at 60°C, and a melt curve from 60–95°C with steps of 0.5 every 10 seconds, using the iCycler iQ real-time Detection System (BioRad Laboratories, Hercules, CA). The template for the standard curve in each run was serial dilutions of *M. hominis* DNA (ATCC number 23114D). The lower limit of detection was <10 copies/reaction.

Amplicons from mycoplasma and mycobacterial PCR were purified by treatment with ExoSAP-IT (Amersham Biosciences, Piscataway, NJ). Bidirectional sequencing was performed with the primers used for PCR amplification (MGSO and my-ins, or SP-1 and SP-2, respectively) and AB PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit, version 1.1 (Applied Biosystems, Foster City, CA). Sequences were aligned and checked using Sequencher, version 4.5 (Gene Codes Corp, Ann Arbor, MI). Species identification was performed by comparison of sequences with those in the GenBank database using BLAST [8].

Testing for CMV, EBV, HHV-6, HHV-7, and HHV-8 in milk supernatants was performed at the University of Washington Clinical Virology Laboratory by TaqMan PCR assays using Sequence Detector 7700 (Applied Biosystems, Foster City, CA) as previously described [9–11].

Statistical analysis

Analyses of dichotomous outcomes were performed with a Generalized Estimating Equation (GEE) version of logistic regression using Stata v. 9.2 software (StataCorp, College Station, TX). Odds ratios, p-values for tests of association, and accompanying confidence intervals, were generated using robust variance estimation and data clusters defined by individual women. A multivariate logistic GEE model was constructed to predict detection of HIV-1 RNA or DNA in milk. The model included plasma HIV-1 RNA concentration, symptoms of mastitis,

elevated milk Na^+ and total leukocyte count, and the detection of co-infections in breast milk. Plasma HIV-1 RNA and breast milk CMV concentrations were log-transformed and included as continuous variables and the other predictors as binary variables. EBV, HHV-7, mycoplasma and mycobacteria were modeled as binary variables because of the large proportion of specimens in which the respective pathogen was undetectable. Specimens with undetectable levels of HIV-1 or CMV were assigned a concentration equal to half the lower limit of detection.

Spearman's rank correlation (ρ) was used to evaluate the strength of the relationship between two continuous variables. Since the Spearman analysis assumes independent observations, Monte Carlo simulations were used to evaluate the impact of assuming independence between right and left breast milk samples from the same woman. These simulations calculated the ρ statistic using only one randomly selected breast milk specimen per woman, and were assessed for 10,000 replications using Stata v. 9.2 software. These analyses confirmed that in all cases the median of the ρ distribution in the simulation set matched the overall ρ to at least two decimal places. Confidence intervals for the Spearman rank correlation statistics were generated using StatXact v. 7 software (Cytel Inc., Cambridge, MA).

Results

Participants and specimens

Two-hundred and seventeen breastfeeding women with HIV-1 infection completed questionnaires and provided blood, and 216 women provided 428 breast milk specimens. Four women were not able to express at least 5 mL of milk from one breast, and one woman could not provide an adequate volume from either breast. Difficulty with milk expression was attributed to recent feeding of their infant, and not to breast symptoms or breastfeeding problems. One hundred eighty-five women (85%) reported receiving single-dose nevirapine for prevention of MTCT, but no other antiretroviral drug exposure was elicited. Seven women (3%) reported use of an antibiotic within the preceding week (2 amoxicillin, 4 ceftriaxone, 1 erythromycin), although not for treatment of a breast-related condition.

Prevalence of mastitis

One or more symptoms consistent with clinical mastitis were reported at the time of enrollment by 17 of 217 (8%) of women, in 22 of 434 (5%) breasts, as described previously [3]. Subclinical mastitis was detected in 63 (15%) of 407 of milk specimens from 53 (25%) of 212 women when defined by milk $\text{Na}^+ > 12$ mM. A similar proportion of specimens was classified as having subclinical mastitis using alternative definitions ($\text{Na}^+:\text{K}^+ > 1$ or total leukocyte count $> 10^6/\text{mL}$ of milk), as described previously [3].

Detection of bacterial and fungal co-infections

One or more potential bacterial or fungal pathogens were isolated from 50 (12%) of 428 breast milk cultures (Table 1). The most common bacterial isolate was *S. aureus* ($n=27$; 6%), followed by *E. coli* ($n=7$; 2%), Group A *Streptococci* ($n=4$; 1%), *Pseudomonas* ($n=4$; 1%) and other Gram negative bacilli ($n=4$; 1%). Fungi cultivated from milk specimens consisted of *Candida* ($n=3$; $<1\%$) and *Aspergillus* ($n=2$; $<1\%$). One specimen grew both *S. aureus* and *Candida*.

Mycoplasmas were amplified from 2 (1%) of 197 specimens, and were identified as *M. microti* and *M. moatsii* by sequence analysis. Testing for mycobacteria by PCR yielded amplicons from 97 (46%) of 211 breast milk samples, all at low concentration (≤ 100 copies/mL). Sequence analysis of 35 randomly selected amplicons revealed environmental mycobacterial bacterial species in 22 (63%), of which only 2 were identical (*M. gilvum*). The

remaining 13 sequences were determined to be *Nocardia* species (n=2), or were unreadable due to multiple genotypes (n=11).

Detection of viral co-infections

CMV was detected in nearly all (99%) breast milk specimens (Table 1), with a median concentration of 1.1×10^4 copies/mL (range 0 – 8.4×10^6). EBV was detected in 188 (45%) of 418 milk specimens (range 0 – 820 copies/mL), and HHV-7 was detected in 22 (6%) of 355 milk specimens. HHV-6 and HHV-8 were not detected in the first 70 specimens tested and thereafter were not evaluated to conserve specimen and minimize cost. A within-woman correlation between the right and left breast milk viral load was observed for both CMV ($\rho=0.9$, $p<0.001$) and EBV ($\rho=0.59$, $p<0.001$).

Co-infections as related to mastitis

The prevalence of one or more infectious agent (excluding HIV-1) in milk did not differ with respect to the presence or absence of mastitis (Table 1). Results were similar when subclinical mastitis was defined using Na^+ , $\text{Na}^+:\text{K}^+$ or total milk leukocyte count. In addition, the distribution of specific infectious agents (e.g. *S. aureus*) across milk specimens appeared unrelated to clinical or subclinical mastitis. Nor were HHV-7, CMV or EBV concentrations associated with mastitis assessed by milk Na^+ as a continuous or as a binary ($\text{Na}^+ >$ or ≤ 12 mM) variable.

Co-infections as related to HIV-1 RNA and DNA in breast milk

HIV-1 RNA was detected in 239 (58%) of 409 lactoserum specimens, and HIV-1 DNA was detected in 170 (85%) of 201 breast milk cell pellets [3]. The HIV-1 concentration in milk correlated between right and left breasts; both the RNA ($\rho=0.79$ ($p<0.001$)) and the DNA ($\rho=0.60$, $p<0.001$). Of the pathogens evaluated, only the detection of EBV and the CMV concentration were associated with detection of HIV-1 RNA in milk by PCR (Table 2). These relationships persisted after adjustment for plasma concentration of HIV-1 RNA, symptoms of mastitis, elevated milk Na^+ , milk leukocyte count, and the presence of other co-infections of milk. The respective odds ratios and confidence intervals for relating CMV and EBV to detection of breast milk HIV-1 RNA were similar whether milk leukocytes were modeled as: total leukocytes $>$ or $\leq 10^6/\text{mL}$ milk; milk leukocyte count as a continuous variable; or milk neutrophils and lymphocytes included separately as continuous variables. In addition, results of the multivariate model were similar whether the outcome used was detection of milk HIV-1 RNA or a threshold concentration of milk HIV-1 RNA $>10^3$ copies/mL. The concentrations of CMV and EBV also correlated with HIV-1 RNA concentration in milk, with Spearman rho values of 0.49 ($p<0.0001$) and 0.47 ($p<0.0001$), respectively (Figure 1, panels A and B).

Breast milk CMV and EBV concentrations were each weakly correlated with the concentration of HIV-1 DNA in milk when these variables were examined in isolation (Figure 1, panels C and D). Detection of HIV-1 DNA in milk by PCR was associated with the concentration of CMV but not with detection of EBV in the univariate model (Table 2). The association between CMV concentration and detection of HIV-1 DNA in breast milk, however, did not persist in the multivariate model (Table 2).

Discussion

Among the HIV-1 infected women we studied, co-infections in breast milk were not associated with mastitis. The evaluation of co-infections included testing for aerobic and anaerobic bacteria, mycobacteria, mycoplasma, fungi, CMV, EBV, HHV-6, HHV-7 and HHV-8. Because screening for every infectious agent is impractical, mastitis in these women might have been caused by an agent for which we did not test. Given our use of sensitive screening

methods for a broad range infectious agents, however, we think it is more likely that mastitis was not due to a co-infection.

WHO guidelines for the management of mastitis in breastfeeding women recommend antibiotic treatment of prolonged or severe clinical mastitis [2]. These recommendations are based in part on studies of otherwise healthy women with clinical mastitis, in which bacteria were cultured from approximately half of milk specimens. However, as bacterial culture has had a low sensitivity and specificity for predicting clinical mastitis, uncertainties persist regarding the role of infection and the use of antibiotics in clinical mastitis [2].

Few studies have investigated the infectious etiologies of mastitis in women with HIV-1 infection. The role of bacterial infections in subclinical mastitis was assessed by cultures of milk from HIV-1 infected Malawian women [12]. *S. aureus* was isolated from 30% of milk with $>10^6$ leukocytes/mL. However, the proportion of milk specimens with lesser leukocyte counts that grew *S. aureus* was not reported. Additionally, breast milk HIV-1 RNA concentrations were similar in these women before and after antibiotic treatment [13], suggesting that culturing bacteria from milk and mastitis may not have been causally linked. Interestingly, there appeared to be a very low prevalence of bacteria in the milk of Zambian HIV-1 infected and uninfected women [14], with no bacteria isolated from 168 breast milk cultures, of which an unspecified number had an elevated Na+:K+ ratio indicative of subclinical mastitis. These data suggest that the methodology for detecting bacteria in milk may vary and affect the study conclusions.

Fungal infections, particularly with *Candida* spp., have been postulated to cause mastitis in breastfeeding women [2], as can occur in dairy animals [4]. However, there is little evidence for candidal mastitis in women [2]. Among HIV-1 infected women we studied, whom we would expect to be more susceptible to *Candida*, fungi were rarely isolated from breast milk, and when detected, were not associated with mastitis.

Environmental mycobacteria and *M. tuberculosis* cause disease preferentially in individuals with HIV/AIDS. Mastitis due to mycobacteria has been reported in humans [15,16], although non-tuberculous mycobacterial infection of the breast is primarily associated with trauma or surgery [16]. Our frequent detection of environmental mycobacteria in breast milk by PCR, irrespective of mastitis or HIV-1 load, suggests either colonization or contamination of the nipple that was not removed by antiseptic scrubbing. PCR contamination appears unlikely, as only two of the 22 mycobacteria were the same species by sequence analysis.

HHV-6 and HHV-8 were not detected in the breast milk of women we studied, and HHV-7 and mycoplasmas were detected in only a small minority. These observations are consistent with other studies, in which these agents were rarely found in breast milk [17-19], and in which no significant transmission to infants through breastfeeding was identified [17,19].

CMV and EBV are frequently shed in breast milk [20,21]. In our cohort CMV or EBV were not associated with mastitis, despite the plausibility that these viruses could cause, or be reactivated by, inflammation. Unlike the other infectious agents we evaluated, CMV and EBV were independently associated with HIV-1 RNA concentration in breast milk after adjustment for indicators of mastitis and plasma HIV-1 RNA concentration.

Shedding of CMV and HIV-1 RNA from the genital tract have been correlated, and these associations appear to be independent of CD4 cell count, plasma HIV-1 load and other potential confounders [22-24]. Interestingly, CMV and HIV-1 each enhance replication of the other *in vitro* [25,26]. Production of HIV-1 virions may be induced indirectly through CMV-mediated T-cell activation [26], or directly by a chemokine receptor homologue generated by CMV that acts as a co-receptor for HIV-1 cell entry [27]. EBV also increases HIV-1 production *in*

vitro, through transactivation of the HIV-1 long terminal repeats by Epstein-Barr nuclear antigen 2 [28,29]. Inhibition of another human herpesvirus, herpes simplex virus type 2, with the antiviral valacyclovir decreases HIV-1 RNA levels in plasma as well as the genital tract [30]. As with sexual transmission of HIV-1, a greater understanding of the interaction between herpes viruses and HIV-1 may suggest novel methods to reduce HIV-1 transmission through breastfeeding.

Efforts to reduce breastfeeding MTCT include programs to support exclusive breastfeeding, and provision of antiretroviral treatment to breastfeeding mothers or prophylaxis for infants [1]. Based on our results, antibacterial or antifungal therapy for mastitis is unlikely to reduce HIV-1 concentrations in breast milk. However, the observed associations of CMV and EBV with greater HIV-1 milk concentrations suggests that suppression of CMV and EBV replication with antiviral drugs might decrease HIV-1 shedding, and potentially reduce MTCT through breastfeeding. Importantly, the safety of such drugs for the breastfeeding infant would need to be established before undertaking efficacy trials. A greater understanding of the mechanisms resulting in HIV-1 transmission through breast milk should facilitate interventions to reduce MTCT and maximize the benefits of breastfeeding.

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Figure 1.
CMV and EBV concentrations in breast milk correlate with those of HIV-1 RNA and DNA.

Table 1

Co-infections in breast milk are not associated with clinical or subclinical mastitis

Infectious agent	Proportion (%) of breast milk specimens with co-infection detected ^a		
	All	Clinical mastitis ^b	Subclinical mastitis ^c
Bacteria	46/428 (11%)	2/22 (9%)	9/59 (15%)
Fungi	5/428 (1%)	0/22 (0%)	0/59 (0%)
Mycoplasma	2/197 (1%)	0/7 (0%)	0/22 (0%)
Mycobacteria	97/211 (46%)	3/12 (25%)	20/36 (56%)
CMV	413/418 (99%)	22/22 (100%)	59/59 (100%)
EBV	188/418 (45%)	12/22 (55%)	30/59 (51%)
HHV-6	0/70 (0%)	0/5 (0%)	0/11 (0%)
HHV-7	22/355 (6%)	1/17 (6%)	4/47 (9%)
HHV-8	0/70 (0%)	0/8 (0%)	0/12 (0%)

^a No infectious agent had a statistically significant difference in prevalence between specimens with and without either clinical or subclinical mastitis, using GEE.

^b Clinical mastitis defined by report of any symptoms of breast inflammation using a standardized questionnaire.

^c Subclinical mastitis defined by milk Na⁺ >12 mM [3].

Table 2

Plasma HIV-1 RNA load, mastitis parameters and milk co-infections as related to detection of HIV-1 RNA or DNA in breast milk

Predictor	Unadjusted odds ratio (95% CI) ^a		Adjusted odds ratio (95% CI) ^{a, b}	
	HIV-1 RNA: OR (CI)	HIV-1 DNA: OR (CI)	HIV-1 RNA [n=326]	HIV-1 DNA [n=170]
Plasma HIV-1 RNA log copies/mL	5.4 (3.5, 8.3) [n=407]	2.7 (1.5, 4.8) [n=201]	6.2 (3.7, 10.2)	2.4 (1.3, 4.5)
Symptoms of mastitis	2.0 (0.7, 5.7) [n=409]	0.2 (0.03, 1.6) [n=201]	1.6 (0.3, 8.5)	0.3 (0.04, 2.3)
Milk Na ⁺ > 12 mM	2.7 (1.3, 5.6) [n=398]	0.9 (0.3, 3.1) [n=194]	6.2 (1.7, 23.0)	1.3 (0.3, 5.3)
Milk leukocytes > 10 ⁶ /mL	1.9 (1.04, 3.6) [n=408]	0.6 (0.3, 1.4) [n=201]	1.7 (0.7, 4.2)	0.4 (0.1, 1.2)
Bacteria or fungi detected in milk	1.6 (0.8, 3.3) [n=406]	2.8 (0.6, 12.5) [n=199]	1.3 (0.4, 4.0)	4.3 (0.4, 50.3)
Mycoplasmas detected in milk	0.8 (0.1, 13.8) [n=192]	Not applicable ^c	Not done	Not done
Mycobacteria detected in milk	1.2 (0.7, 2.0) [n=204]	0.9 (0.3, 2.8) [n=104]	Not done	Not done
HHV-7 detected in milk	0.5 (0.2, 1.4) [n=341]	1.2 (0.1, 10.3) [n=179]	0.5 (0.1, 2.6)	0.9 (0.1, 5.7)
CMV log copies/mL milk	2.3 (1.7, 3.1) [n=403]	1.9 (1.2, 2.8) [n=199]	1.8 (1.2, 2.6)	1.5 (0.95, 2.5)
EBV detected in milk	5.5 (3.4, 9.0) [n=403]	1.6 (0.7, 3.8) [n=200]	3.8 (1.8, 7.6)	1.0 (0.4, 2.4)

^aDetection of HIV-1 RNA at ≥ 50 copies/mL lactoserum, and HIV-1 DNA at $\geq 1-10$ copies/cell pellet from 1 to 14 mL whole milk [3].

^bGEE logistic model adjusted for all variables in the first column, except mycoplasma and mycobacteria. Mycoplasma detection was too rare to include in the multivariable modeling. Mycobacteria were not included because their detection likely represents contamination from the skin rather than infection within the breast tissue.

^cModel does not converge due to a proportion estimate of 0.0 or 1.0 in one group.