



Clinical and biological factors associated with early Epstein-Barr Virus infection in HIV-Exposed Uninfected Infants in Eastern Uganda

Ana Montoya-Ferrer, Armen Sanosyan, Alexis Fayd'Herbe de Maudave, Amandine Pisoni, Karine Bolloré, Jean-Pierre Moles, Marianne Peries, Thorkild Tylleskär, James Tumwine, Grace Ndeezi, et al.

► To cite this version:

Ana Montoya-Ferrer, Armen Sanosyan, Alexis Fayd'Herbe de Maudave, Amandine Pisoni, Karine Bolloré, et al.. Clinical and biological factors associated with early Epstein-Barr Virus infection in HIV-Exposed Uninfected Infants in Eastern Uganda. *Clinical Infectious Diseases*, 2020, 72 (6), pp.1026-1032. 10.1093/cid/ciaa161 . hal-02489845

HAL Id: hal-02489845

<https://hal.umontpellier.fr/hal-02489845>

Submitted on 2 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Clinical and biological factors associated with early Epstein-Barr Virus
infection in HIV-Exposed Uninfected Infants in Eastern Uganda

Ana MONTOYA-FERRER¹, Armen SANOSYAN¹, Alexis FAYD'HERBE DE MAUDAVE¹,
Amandine PISONI¹, Karine BOLLORE¹, Jean-Pierre MOLÈS¹, Marianne PERIES¹,
Thorkild TYLLESKAR², James K TUMWINE³, Grace NDEEZI³, Miguel GORGOLAS⁴,
Nicolas NAGOT¹, Philippe VAN DE PERRE¹ and Edouard TUAILLON¹

1. Pathogenesis and Control of Chronic Infections, INSERM,
Etablissement français du Sang, University of Montpellier, CHU
Montpellier, Montpellier, France
2. Centre for International Health, University of Bergen, Bergen, Norway
3. Department of Paediatrics and Child Health, School of Medicine,
College of Health Sciences, Makerere University, Kampala, Uganda
4. Department of Infectious Diseases, Hospital Fundación Jiménez-Díaz,
University Autonoma, Madrid, Spain

Corresponding author:

Dr. MONTOYA FERRER Ana

Department of Infectious and Tropical diseases

CHU de Montpellier - Hôpital Gui de Chauliac

80 av. Augustin Fliche

34295 Montpellier Cedex 5

Tel : 04 67 33 79 42

a-montoyaferrier@chu-montpellier.fr

Summary:

We studied the onset of EBV infection in the first year of life of HIV-exposed uninfected infants from Uganda. EBV infection was determined by HIV and EBV maternal factors and EBV DNA was higher among infants with clinical adverse events.

ABSTRACT

Background. Immune control of Epstein-Barr virus (EBV) infection is impaired in HIV-infected individuals. We explored maternal factors associated with EBV acquisition in HIV-exposed uninfected (HEU) infants and the relationship between EBV infection and serious adverse events (SAE) during the first year of life.

Methods. Two hundred and one HEU infants from Uganda enrolled in the ANRS12174 trial were tested for anti-viral capsid antigen (VCA) antibodies at week 50 of life. The date of infection was estimated by testing of EBV DNA at weeks 1, 6, 14, 26, 38 and 50 postpartum on dried blood spot (DBS).

Results. Eighty-seven (43%) infants were tested positive for anti-VCA IgG at week 50. Among the 59 infants positive for EBV DNA, 25% were infected within the first 26 weeks. Almost half of them (12%) were infected before week 14. Shedding of EBV in breast milk was associated with EBV DNA in maternal plasma ($P=.009$), HIV RNA detection ($P=.039$), lower CD4 count ($P=.001$) and was correlated with plasma EBV DNA levels ($P=.002$). EBV infant infection at week 50 was associated with shedding of EBV in breast milk ($P=.009$) and young maternal age ($P=.029$). Occurrence of a clinical SAE, including malaria and pneumonia, was associated with higher levels of EBV DNA in infants ($P=.010$).

Conclusions. By assessing EBV infection in HEU infants we observed that infection during the first year of life is determined by HIV and EBV maternal factors and that EBV DNA levels was higher among infants with clinical SAE.

Keywords: EBV; primary infection; HIV-exposed uninfected infants; Africa; herpes viruses

1.0. INTRODUCTION

The population of HIV-exposed uninfected (HEU) infants is growing steadily due to effective prevention programs of mother-to-child transmission (MTCT) [1]. However, several studies suggest that HEU infants have increased morbidity and mortality of infectious origin [2] and reports about growth and neurodevelopment are still concerning [3-5].

EBV infection commonly occurs during early childhood in Africa and nearly half of infants from Western and Eastern countries, have acquired the infection by one year of age [6,7]. More recent studies conducted in Eastern African countries have reported similar rates of EBV infection among HIV-exposed uninfected infants (HEU) at one year of life [8,9].

Biological factors associated with early EBV infection remain to be explored in HEU infants. Immunosuppression and overactivation of lymphocytes impair the host control over EBV infection in HIV-infected subjects [10,11]. EBV is essentially transmitted by saliva [12], and EBV oral shedding is increased in HIV-infected mothers [9]. Although breastfeeding is not considered a risk factor for EBV transmission [9,13,14], infectious potential of EBV in breast milk has been recently suggested [15]. Furthermore, presence of EBV in breast milk may enhance MTCT of HIV [16]. HEU infants might also be more vulnerable to EBV infection due to impaired transplacental transfer of protective maternal antibodies [17,18].

Primary EBV infection is usually asymptomatic when it occurs during early age [19,20]. However, early EBV infection has been suggested as a risk factor for EBV

related malignancies, such as the endemic Burkitt lymphoma (eBL), the most prevalent pediatric cancer in Africa [6-8,21]. Geographic distribution of eBL is overlapped with high endemic malaria regions, highlighting the role of malaria and EBV interactions in eBL genesis [22].

In this study, we aimed to determine the onset of EBV infection in HEU infants during the first year of life in Africa and maternal factors associated with early EBV infection. We also assessed the relationship between EBV infection, growth and serious adverse events recorded during the follow-up period.

2.0. METHODS

2.1. Study population and samples

This longitudinal study included infants born from HIV-positive mothers enrolled in the ANRS12174 trial (NCT00640263). The ANRS12174 trial is a randomized multicenter controlled trial held in Burkina Faso, South Africa, Uganda, and Zambia from 2009 to 2012 [23]. HEU breastfed infants born from HIV-1-infected mothers not eligible for antiretroviral therapy (CD4 count >350 cells per μ L) according to national and international guidelines at that time, were randomized to either lopinavir–ritonavir or lamivudine infant prophylaxis to prevent postnatal MTCT of HIV up to 50 weeks of breastfeeding.

The study population was enrolled at Mbale Regional Referral Hospital, a semi-rural site in Eastern Uganda. Infants and maternal samples were stored locally at -80°C and then sent to INSERM UMR-1058 Montpellier research unit. Plasma HIV RNA was

tested using the GENERIC HIV Charge Virale assay (Biocentric, Bandol France), as previously described [24]. The lower limit of detection was 300 copies/ml.

2.2. Diagnoses of EBV infection in mothers and HEU infants

EBV infection was diagnosed by detecting IgG antibodies directed against viral capsid antigen (VCA) in 201 maternal plasma and dried blood spots (DBS) samples collected in infants at week 1 and week 50 respectively, using an automated immunoassay according to the manufacturer's instructions (LIAISON® VCA IgG, DiaSorin, Saluggia, Italy). Results were expressed as signal to cut off index (S/CO: sample signal (RLU)/mean levels of anti-VCA negative controls + 3 standard deviation). The technique was validated in DBS samples (Supplementary Figure 1).

The index of maternally-transferred anti-VCA IgG antibodies were only tested in a subgroup of 150 infants at week 1. The decay of the index after birth is shown in Supplementary Figure 2.

2.3. EBV DNA quantification

DBS samples from 87 infants detected positive for anti-VCA IgG at week 50 were sequentially tested for EBV DNA in all sampling points of the follow-up (weeks 1, 6, 14, 26, 38 and 50).

A DBS disc of 6 mm was punched into 1.5 mL sample tubes. Nucleic acid elution was performed in 200 µL according to the method previously described [25]. EBV DNA was also tested in 188 lactoserum and 197 maternal plasma samples at week 1 postpartum.

EBV DNA was extracted using an automated QIAamp DNA Mini QIAcube Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany) and EBV DNA was amplified using R-gene EBV™ kit (Argene, Biomerieux, France). The results were expressed as EBV DNA copies/10⁶ cells in DBS samples and as copies/mL in plasma and breast milk samples. Human cell count in the DBS was determined using β globin qPCR as described [26]. The detection limit of R-gene EBV™ kit was set at 182 copies/mL according to the manufacturer [27].

The highest level of EBV DNA quantified in DBS during the follow-up was considered as the peak of EBV DNA.

2.4. Malaria exposure

Asymptomatic malaria was assessed by detection of *Plasmodium falciparum* and *Plasmodium vivax* DNA, which is considered an appropriate method to estimate malaria prevalence in low transmission settings [28]. DNA was eluted and extracted from DBS samples in 177 and 197 infants at week 26 and 50, respectively (as described above). Detection of *Plasmodium falciparum* and *vivax* DNA was done by a nested PCR [29].

2.5. Clinical assessments

Serious adverse events (SAE) grade III or IV were recorded at any time between week 1 and 50 [23,30]. We distinguished SAE as biological - abnormal laboratory blood test (e.g. anemia); or as clinical (e.g. pneumonia, see Supplementary Table 1). Malaria infection was considered as a clinical SAE based on clinical symptoms and thick blood smear independently of detection of *Plasmodium falciparum* DNA on DBS samples at week 26 or 50.

Growth at one year of age was evaluated as weight-for-length (WLZ), weight-for-age (WAZ) and length-for-age (LAZ) Z-scores using the WHO child growth standards and the free WHO program (<http://www.who.int/childgrowth/training/en/>)[31]. Growth was assessed among 158 infants who had completed the final visit. WLZ, WAZ and LAZ were considered severely-moderately abnormal when Z score was less than -2.

2.6. Statistical analysis

The cumulative incidence of primary EBV infection was calculated using the Kaplan–Meier method. Characteristics were compared between EBV infected and EBV uninfected infants at week 50 using the χ^2 test for categorical variables, or Fisher's exact test if the numbers were small. Continuous data were assessed with Student's t test or Wilcoxon Mann–Whitney test when their distributions were non-normal. Spearman's rank correlation coefficient was used to describe the correlation between maternal EBV DNA levels in plasma and breast milk. EBV DNA and HIV RNA levels were log transformed and undetectable viral load (VL) were transformed to logarithmic zero for analyses. All tests were 2-tailed with $\alpha = .05$.

A logistic regression model with a backward selection was used to identify maternal and infant risk factors associated with EBV infection at week 50 and to estimate the risk factors of a WLZ score less than -2 SD in infants at one year of life. Variables with a *P* value less than .20 in univariate analysis were included in the multivariate model. The prophylactic antiretroviral (ARV) regimen was also included in the multivariate model since prophylactic regimen based on lopinavir-ritonavir might impair growth in HEU infants [4].

Statistical analyses and graphs were performed using IBM SPSS statistics 20 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA).

3.0. RESULTS

3.1. Baseline characteristics of study population

Out of 273 mother-infant pairs enrolled in the ANRS12174 trial in the Ugandan study site, 201 were selected for the present sub-study since they had completed the follow up visits and had available baseline specimens (Figure 1). Baseline sociodemographic and biological characteristics were not significantly different between mother-infant pairs included and not included (data not shown).

The majority of mothers included presented with WHO clinical HIV-1 stage I [32] and a median of CD4 above 500 cells per μL . One third of mothers had detectable levels of HIV RNA in plasma. The median breastfeeding duration was 40 weeks (Table 1). All mothers were anti-VCA IgG positive .

3.2. Dynamics of EBV infection in HEU infants

Anti-VCA IgG antibodies were detected in 87 out of 201 infants (43%) at week 50 of life. Among them, a median of six DBS samples per infant (interquartile range: IQR: 4-6) were retrospectively tested for EBV DNA. EBV DNA were detected at least once in 59 infants (68%): seven out of 59 (12%) at week 6 and 14, eight (14%) - at week 26 and 34 (58%) - at week 38 and 50 (Supplementary Figure 3). No sample was detected positive for EBV DNA at week 1 postpartum. The median values and IQR of peak of EBV DNA was 713 copies/ 10^6 cells (261-2.739).

3.3. Factors associated with EBV infant infection during the first year of life

3.3.1 Maternal factors

EBV infection at week 50 was associated with a young maternal age ($P=.029$) and detectable levels of HIV RNA in plasma ($P=.035$) (Table 2). Educational level, maternal parity and breastfeeding duration were not associated with EBV infection at week 50 in univariate analysis (Table 2).

Breast milk and plasma EBV DNA were detectable in 19% (35/188) and 8% (15/197) of mothers at week 1 post-delivery, respectively. EBV infection at week 50 was more frequent among infants born from mothers who tested positive for EBV DNA in breast milk (22/80, 27.5%) compared with those born from mothers who tested negative in breast milk (13/108, 12%) ($P=.009$) (Table 2). EBV infection at week 50 also tended to be more frequent among infants born to mothers with detectable EBV DNA plasma samples compared with those with undetectable EBV DNA (10/84, 11.9% vs 5/113, 4.4%; $P=.059$) (Table 2).

3.3.2. Infant factors

All infants had detectable anti-VCA IgG antibodies at week 1 of life without differences in anti-VCA IgG index between EBV infected and uninfected infants at week 50 (Table 2). Infants who acquired EBV infection before or at week 14 tended to have a lower index of anti-VCA IgG at week 1 than infants infected beyond week 14 ($P=.153$) and uninfected infants ($P=.119$) (see Supplementary Figure 4).

Plasmodium falciparum DNA was detected in 12 out 177 infants (6.8%) at week 26 and in 21 out of 197 (10.9%) at week 50. Only one infant out 177 tested positive for

Plasmodium vivax DNA at week 26 and two infants out 197 at week 50. The frequency of EBV infection at week 50 was not associated neither with *Plasmodium* DNA detection nor with occurrence of SAE during the first year of life (Table 2). The univariate analysis did not demonstrate any difference in EBV infection at week 50 between the two prophylactic ARV regimens (Table 2).

3.3.3. Risk factors for EBV infection during the first year of life

EBV DNA shedding in breast milk at week 1 and young maternal age remained significantly associated with EBV infection at week 50 in multivariate analysis (Table 2).

3.4. Factors associated with presence of EBV DNA in breast milk at week 1

Seven out of 35 (20%) mothers tested positive for EBV DNA in breast milk were also tested positive for EBV DNA in plasma compared to only 8 out of 152 (5.3%) mothers tested negative for EBV DNA in breast milk but positive in plasma ($P=.009$; data not shown). When detected, EBV DNA levels were higher in breast milk than in maternal plasma samples (ranging from 75 to 34,200 copies/mL compared to 2 to 342 copies/mL, respectively) and were correlated in both compartments ($Rho = 0.228$; $P=.002$) (data not shown). EBV DNA in breast milk were more frequently detected among mothers with detectable plasma HIV RNA compared to mothers who tested negative for plasma HIV RNA (48.6% vs 30.3%, $P=.039$) (data not shown). Presence of EBV in breast milk was also significantly associated with a lower median (IQR) CD4 cell count (451 cells per μL [391-550] vs. 549 cells per μL [451-638]; $P=.001$) (Figure 2). Levels of EBV DNA in plasma were not correlated with plasma HIV RNA.

3.5. Factors associated with higher EBV viral load in infants

Among the 10 EBV infected infants diagnosed with a clinical SAE, half of them experienced an episode of malaria. The peak of EBV DNA was higher among infants who presented a clinical SAE during follow-up comparing with those without SAE (median: 1887 copies/ 10^6 cells; IQR 362-76138 vs. 27 copies/ 10^6 cells; IQR: 0-920; $P=.010$) (Figure 3). The peak of EBV DNA was detected after the occurrence of the clinical SAE in eight out of ten infants. In three out of four infants for whom a diagnosis of malaria was made, EBV DNA was detected in the week following the probable malaria episode (data not shown).

A higher peak of EBV DNA was not associated neither with the presence of *Plasmodium falciparum* or *vivax* DNA in infants DBS at week 26 or 50 nor with the prophylactic ARV regimen (lopinavir-ritonavir vs. lamivudine) (data not shown).

3.6. Association between EBV infection and infant anthropometric status

A severe-moderate WLZ (Z score less than -2) at week 50 was more frequently found among EBV infected infants compared to uninfected infants: 10/15 (66.7%) vs 5/15 (33.3%) ($P=.024$) (Table 3). A lower gestational age and the occurrence of at least one SAE during the follow-up period were also associated with a WLZ less than -2 at week 50 (Table 3). In multivariate analysis, EBV infection at week 50 remained associated with a WLZ less than -2, independently of gestational age or the occurrence of a SAE during follow-up (Table 3).

No differences in WAZ and LAZ at week 50 were found between EBV infected and uninfected infants (data not shown).

4.0. DISCUSSION

Our results suggest that the shedding of EBV in maternal breast milk can be considered as a marker of immunosuppression in HIV mothers. Together with the transfer of less protective maternal antibodies, this may facilitate EBV infection in infants. Furthermore, serious clinical conditions including malaria and pneumonia were associated with higher EBV DNA levels.

In this cohort, almost half of the HEU infants acquired EBV infection during the first year of life. Previous studies from Kenya and also Uganda have shown comparable rates ranging from 40-47% at one year of life [8,9]. Of note, primary EBV infection at one year was found in a similar percentage before the start of the HIV epidemic among African infants from urban Ghana and Uganda [6,7]. Early age at EBV acquisition has been described among non-Hispanic black children in the United States [14]. According to the authors, genetics and family environmental factors might be associated with the acquisition of EBV infection [14].

We detected EBV infection within the first 26 weeks in at least a quarter of HEU infants and in some of them before week 14. As previously suggested, transfer of less protective maternal antibodies may be responsible for EBV infection before 6 months among HIV exposed infants (HEI and HEU) [8,9,33,34] or infants living in high endemic malaria regions [21,35]. EBV infection has been diagnosed as early as 6 weeks of life in 13% (33 out of 257) of HEI infants participating in a Zimbabwean study, with a rate of 2% of congenital EBV infection [33] while it reached 14% of HEI in the first trimester of life in a Kenyan study [34].

In this study, infants born from mothers with EBV shedding in breast milk were more frequently infected by EBV during the first year of life. EBV DNA is encapsidated in the breast milk [36], - by contrast with EBV DNA released from lysed infected cells -, and therefore consists of potentially infectious virus particles. Infectivity of EBV has been recently reported *in vitro* using breast milk samples from lactating women living in a malaria-endemic region [15]. However, previous studies had not identified breast milk as a potential infectious vehicle for EBV [9,16,37]. Actually, our results do not demonstrate that breast milk is a route for EBV transmission but rather that EBV shedding in breast milk reflects host-virus interplay in mucosal-associated tissues, such as lactating mammary gland. EBV shedding is frequent in breast milk, probably fueled by EBV replication in infected B cells, and ultimately limited by the immune response [36]. By contrast, the presence of EBV DNA in plasma is very infrequent, and when detected, this implies primary infection or an impairment of the immunosurveillance against EBV [38].

Infants participating in ANRS12174 trial were randomized to receive either lopinavir-ritonavir or lamivudine ARV prophylaxis. Previously, Slyker *et al.* found that HEI infants with primary EBV infection suppressed EBV replication faster when they were treated with lopinavir-based ARV treatment comparing to infants treated with nevirapine [34]. However, we did not observe a lower incidence of EBV infection, nor a lower level of the EBV DNA in the infant group receiving protease inhibitor compared to the lamivudine group.

We observed a relationship between occurrence of clinical SAEs, – including malaria–, and the peak of EBV DNA which was generally detected after the occurrence of clinical SAEs. Symptomatic primary EBV infection can occurs in both healthy [39] and infants born from HIV mothers [8,33]. In a previous study, mild

clinical symptoms in HEU infants, - and more severe in HEI-, were concomitantly observed with peaks of EBV viral load, leading authors to suggest that EBV infection may be an important cause of morbidity [8]. However, serious clinical conditions as well as stress can favor EBV reactivations [40]. Clinical SAE are likely to be important enough to disrupt immune responses against EBV allowing to higher viral load.

Malaria exposure alone, however, as measured by *Plasmodium* DNA detection, was neither related to EBV seroconversion at one year of life, nor to higher levels EBV DNA level. Infants living in high endemic malaria regions acquire EBV earlier and present higher levels of EBV DNA, as compared to infants from low endemic areas [21].

Finally, we observed that abnormal growth at one year was more prevalent among infants who acquired EBV infection along first year of life, independently of gestational age and presence of a SAE. Mild growth impairment related to EBV infection has been reported in a cohort of HEI early infected by EBV [33]. Our results suggest that EBV infection may be one of the multiple infectious agents associated with early HEU morbidity and growth retardation. To our knowledge, no association between EBV infection and infant growth have been reported before the start of HIV epidemic.

In conclusion, EBV infection in HEU was determined by maternal factors associated to EBV immunological control. Shedding of EBV in breast milk likely reflected impaired EBV control in mucosal compartment and the risk of EBV infection in infant. Higher levels of EBV DNA in infants was associated with SAEs and both EBV infection and SAEs might negatively affect the infant growth. EBV infection should be considered in further studies exploring the health outcomes of HEU children.

ACKNOWLEDGEMENTS

We would like to thank all the mothers who agreed to be part of the trial together with their infants. Our acknowledgements also go to the different ANRS 12174 sites staff for their dedicated effort in this research. We specially thank the UMR 1058, pathogenesis and control of chronic infections, Montpellier, France, from which we received great support to accomplish this work.

CONFLICT OF INTEREST

We declare no competing interests.

REFERENCES

1. Global guidance on criteria and processes for validation : elimination of mother-to-child transmission of HIV and syphilis, 2nd edition. World Health Organization, 2017. Available at <http://www.who.int/iris/handle/10665/25951> . Accessed 7 mai 2019.
2. Arikawa S, Rollins N, Newell M-L, Becquet R. Mortality risk and associated factors in HIV-exposed, uninfected children. *Trop Med Int Health* 2016; 21:720–734.
3. le Roux SM, Abrams EJ, Nguyen K, Myer L. Clinical outcomes of HIV-exposed, HIV-uninfected children in sub-Saharan Africa. *Trop Med Int Health* 2016; 21:829–845.
4. Blanche S, Tylleskar T, Peries M, et al. Growth in HIV-1-exposed but uninfected infants treated with lopinavir-ritonavir versus lamivudine: a secondary analysis of the ANRS 12174 trial. *Lancet HIV* 2019;
5. Debeaudrap P, Bodeau-Livinec F, Pasquier E, et al. Neurodevelopmental outcomes in HIV-infected and uninfected African children. *AIDS* 2018; 32:2749–2757.
6. Biggar RJ, Henle W, Fleisher G, Böcker J, Lennette ET, Henle G. Primary epstein-barr virus infections in african infants. I. Decline of maternal antibodies and time of infection. *Int J Cancer* 1978; 22:239–243.

7. de-The G. Epstein-Barr virus behavior in different populations and implications for control of Epstein-Barr virus-associated tumors. *Cancer Res* 1976; 36:692–695.
8. Slyker JA, Casper C, Tapia K, et al. Clinical and virologic manifestations of primary Epstein-Barr virus (EBV) infection in Kenyan infants born to HIV-infected women. *J Infect Dis* 2013; 207:1798–1806.
9. Gantt S, Orem J, Krantz EM, et al. Prospective Characterization of the Risk Factors for Transmission and Symptoms of Primary Human Herpesvirus Infections Among Ugandan Infants. *J Infect Dis* 2016; 214:36–44.
10. Petrara MR, Cattelan AM, Zanchetta M, et al. Epstein-Barr virus load and immune activation in human immunodeficiency virus type 1-infected patients. *J. Clin. Virol.* 2012; 53:195–200.
11. Ouedraogo DE, Tuailon E, Rubbo P-A, et al. Close relationship between immunoglobulin secreting-cells and Epstein–Barr virus reservoir in patients infected with HIV. *J Med Virol* 2014; 86:30–37.
12. Cederberg LE, Rabinovitch MD, Grimm-Geris JM, et al. Epstein-Barr Virus DNA in Parental Oral Secretions: A Potential Source of Infection for Their Young Children. *Clinical Infectious Diseases* 2019; 68:306–312.
13. Kusuhara K, Takabayashi A, Ueda K, et al. Breast Milk Is Not a Significant Source for Early Epstein-Barr Virus or Human Herpesvirus 6 Infection in Infants: A Seroepidemiologic Study in 2 Endemic Areas of

Human T-Cell Lymphotropic Virus Type I in Japan. Microbiology and Immunology 1997; 41:309–312.

14. Condon LM, Diseases LCI, 2014. Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. Clin Infect Dis. 2014;59(4):501-8.
15. Daud II, Coleman CB, of NSTJ, 2015. Breast milk as a potential source of Epstein-Barr virus transmission among infants living in a malaria-endemic region of Kenya. J Infect Dis. 2015;212(11):1735-42.
16. Viljoen J, Tuaillon E, Nagot N, et al. Cytomegalovirus, and possibly Epstein-Barr virus, shedding in breast milk is associated with HIV-1 transmission by breastfeeding. AIDS 2015; 29:145–153.
17. Ruck C, Reikie BA, Marchant A, Kollmann TR, Kakkar F. Linking Susceptibility to Infectious Diseases to Immune System Abnormalities among HIV-Exposed Uninfected Infants. Front Immunol 2016; 7:757.
18. Jones CE, Naidoo S, De Beer C, Esser M, Kampmann B, Hesselning AC. Maternal HIV Infection and Antibody Responses Against Vaccine-Preventable Diseases in Uninfected Infants. JAMA 2011; 305:576–584.
19. Biggar RJ, Henle G, Böcker J, Lennette ET, Fleisher G, Henle W. Primary Epstein-Barr virus infections in African infants. II. Clinical and serological observations during seroconversion. Int J Cancer 1978; 22:244–250.

20. Fleisher G, Henle W, Henle G, Lennette ET, Biggar RJ. Primary infection with Epstein-Barr virus in infants in the United States: clinical and serologic observations. *J Infect Dis* 1979; 139:553–558.
21. Piriou E, Asito AS, Sumba PO, et al. Early age at time of primary Epstein-Barr virus infection results in poorly controlled viral infection in infants from Western Kenya: clues to the etiology of endemic Burkitt lymphoma. *J Infect Dis* 2012; 205:906–913.
22. Molyneux EM, Rochford R, Griffin B, et al. Burkitt's lymphoma. *The Lancet* 2012; 379:1234–1244.
23. Nagot N, Kankasa C, Tumwine JK, et al. Extended pre-exposure prophylaxis with lopinavir–ritonavir versus lamivudine to prevent HIV-1 transmission through breastfeeding up to 50 weeks in infants in Africa (ANRS 12174): a randomised controlled trial. *The Lancet* 2016; 387:566–573.
24. Mouinga-Ondeme A, Mabika-Mabika A, Alalade P, et al. Significant impact of non-B HIV-1 variants genetic diversity in Gabon on plasma HIV-1 RNA quantitation. *J Med Virol* 2014; 86:52–57.
25. Viljoen J, Gampini S, Danaviah S, et al. Dried blood spot HIV-1 RNA quantification using open real-time systems in South Africa and Burkina Faso. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2010; 55:290–298.
26. Orozco AF, Jorgez CJ, Horne C, et al. Membrane Protected Apoptotic Trophoblast Microparticles Contain Nucleic Acids: Relevance to

Preeclampsia. The American Journal of Pathology 2008; 173:1595–1608.

27. Fafi-Kremer S, Morand P, Barranger C, et al. Evaluation of the Epstein-Barr virus R-gene quantification kit in whole blood with different extraction methods and PCR platforms. The Journal of Molecular Diagnostics 2008; 10:78–84.
28. Mogeni P, Williams TN, Omedo I, et al. Detecting Malaria Hotspots: A Comparison of Rapid Diagnostic Test, Microscopy, and Polymerase Chain Reaction. J Infect Dis 2017; 216:1091–1098.
29. Bharti AR, Saravanan S, Madhavan V, et al. Correlates of HIV and malaria co-infection in Southern India. Malar J 2012; 11:306.
30. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014]. Available at: <https://rsc.niaid.nih.gov/sites/default/files/daids-ae-grading-table-v2-nov2014.pdf>. Accessed 7 May 2019.
31. WHO child growth standards and the identification of severe acute malnutrition in infants and children. Geneva, World Health Organization, 2009. Available at: <https://www.who.int/nutrition/publications/severemalnutrition>. Accessed 7 May 2019.

32. World Health Organization. Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance: African region. Switzerland: World Health Organization; 2005.
33. Gumbo H, Chasekwa B, Church JA, et al. Congenital and Postnatal CMV and EBV Acquisition in HIV-Infected Zimbabwean Infants. PLoS ONE 2014; 9:e114870.
34. Slyker JA, Casper C, Tapia K, et al. Accelerated Suppression of Primary Epstein-Barr Virus Infection in HIV-Infected Infants Initiating Lopinavir/Ritonavir-Based Versus Nevirapine-Based Combination Antiretroviral Therapy. Clinical Infectious Diseases 2014; 58:1333–1337.
35. Ogolla S, Daud II, Asito AS, et al. Reduced Transplacental Transfer of a Subset of Epstein-Barr Virus-Specific Antibodies to Neonates of Mothers Infected with Plasmodium falciparum Malaria during Pregnancy. Clin Vaccine Immunol 2015; 22:1197–1205.
36. Sanosyan A, Rutagwera DG, Moles J-P, et al. Increased Epstein-Barr virus in breast milk occurs with subclinical mastitis and HIV shedding. Medicine (Baltimore) 2016; 95:e4005.
37. Junker AK, Thomas EE, Radcliffe A, Forsyth RB, Davidson AGF, Rymo L. Epstein-Barr Virus Shedding in Breast Milk. The American Journal of the Medical Sciences 1991; 302:220–223.
38. Ouedraogo DE, Makinson A, Vendrell J-P, et al. Pivotal role of HIV and EBV replication in the long-term persistence of monoclonal

gammopathy in patients on antiretroviral therapy. *Blood* 2013; 122:3030–3033.

39. Sumaya CV, Ench Y. Epstein-Barr virus infectious mononucleosis in children. I. Clinical and general laboratory findings. *Pediatrics* 1985; 75:1003–1010.
40. Kerr JR. Epstein-Barr virus (EBV) reactivation and therapeutic inhibitors. *J Clin Pathol* 2019; 72:651–658.

FIGURES LEGENDS

Figure 1. Diagram of study procedures. Flowchart summarizing the identification of EBV-infected infants

* Follow-up visits missing or samples not available

Figure 2. Number of CD4 T cell according to EBV shedding in breast milk (BM)

Middle lines and end bars represent mean and standard deviation of maternal CD4 T cell count. Differences between groups were analyzed using the Mann–Whitney test.

Figure 3. Levels of EBV DNA in EBV infected infants according to occurrence of serious adverse events (SAE)

The peak of EBV DNA (as the highest level of EBV DNA recorded over the study period) were compared between infants having neither experienced biological (abnormal laboratory blood test, e.g. anemia) nor clinical SAE (N=39) and having experienced biological (N=38) or clinical SAE (e.g. pneumonia) (N=10). Middle lines and end bars represent mean and standard deviation of EBV DNA levels compared by the Mann–Whitney test.

Table 1. Baseline socio-demographic, clinical, and immunovirological characteristics of 201 mother-infant pairs

	Median (IQR) or n (%)
	N=201
Maternal characteristics	
Age, years	
18-22	50 (24.9%)
23-26	54 (26.9%)
27-30	63 (31.3%)
31-40	34 (16.9%)
Education level completed	
None	11 (5.5%)
Primary	125 (62.2%)
Secondary or tertiary	65 (32.3%)
Parity	3 (2-5)
PMTCT regimen	
During pregnancy	166 (82.6%)
During labour	200 (99.5%)
WHO clinical HIV-1 Staging	
Staging I	182 (90.5%)
Staging II, III	19 (9.5%)
CD4 cell count (cells per μL)*	525 (436-624)

Detectable plasma HIV-1 RNA (>300 copies per mL)*	68 (33.8%)[200]
Breastfeeding duration, weeks	40.4 (36.3-43)
Infants characteristics	
Gestational age, weeks	40 (39-40) [200]
Birth weight, kilograms	3 (2.8-3.3)
Male sex	102 (50.7%)

* At week 1 post-partum

Data are number (%) of subjects or median value (interquartile range: IQR). For variables with missing data, the number of participants with available data is shown in square brackets.

Abbreviations: PMTCT, Prevention of mother-to-child transmission **Table**

2. Maternal and infant factors associated with EBV infant infection at week 50
(univariate and multivariate analysis)

	Median (IQR) or n (%)		Crude estimates ^a			Adjusted estimates ^b		
	EBV infected (N = 87)	EBV not infected (N = 114)	OR	95% CI	P value	OR	95% CI	P value
Maternal factors								
Age (years)								
18-22	29 (33.3%)	21 (18.4%)	0.73	0.56 - 0.97	.029	0.69	0.51 - 0.93	.014
23-26	22 (28.1%)	32 (25.3%)						
27-30	24 (34.2%)	39 (29.6%)						
31-40	12 (13.8%)	22 (19.3%)						
Education	57	68	0.7	0.41	.341			

[illegible]

Gestational	40 (39-41)	40 (39-	1.0	0.79	.985
age, weeks		41)	0	-	
				1.26	
Anti-VCA IgG	7.31	9.09	0.9	0.98	.880
index*	(5.79-	(5.43 –	9	-	
	11.68)	18.60)		1.02	
	[61]	[89]			
<i>Plasmodium</i>	16	16	1.3	0.65	.404
<i>falciparum/viv</i>	(14.1%)	(18.4%)	8	-	
<i>ax</i> DNA at				2.94	
week 26/50					
(at least once					
positive vs. no)					
SAE during the	48	59	1.1	0.65	.630
first year of	(55.17%)	(51.75	5	-	
life (at least		%)		2.01	
once vs. no)					
Type of	50	58	1.3	0.74	.353
prophylaxie	(57.5%)	(50.9%)	0	-	
(lopinavir-				2.29	
ritonavir vs.					
lamivudine)					

* at week 1 post-partum

^aCrude odds ratio using univariate analysis

^bAdjusted odds ratio using a logistic regression model with a backward selection (variables with a *P* value less than .20 in univariate analysis were included in the multivariate model).

Abbreviations: OR, odds ratio; CI, confidence interval; SAE, serious adverse events

Table 3. Factors associated with infant's abnormal WLZ at week 50 (univariate and multivariate analysis)

	WLZ		Crude estimates ^a			Adjusted estimates ^b		
	Z score	Z score	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
	> -2 SD	< -2 SD						
	(N=15)	(N=143)						
Age (years)	27 (20-34)	26 (19-33)	1.00	0.91-1.11	0.959			
CD4 cell count (cells per µL)*	523 (330-716)	538 (359-717)	1.00	0.99-1.00	0.453			
Detectable plasma HIV-1 RNA*	7 (46.7%)	47 (33.1%)	0.56	0.19-1.65	0.293			
Birth weight, kilograms	2.9 (2.8-3.2)	3 (2.8-3.3)	0.99	0.99-1.00	.361			
Gestational age, weeks	39 (38-40)	40 (39-40)	0.57	0.38-0.86	.008	0.54	0.33-0.89	.007
Breastfeeding duration, g	39.86 (33.14-)	40.57 (36.43-)	1.02	0.95-1.11	.554			

weeks	44.86)	43)						
Antiretroviral	8	68	1.26	0.43-	.670			
prophylactic	(53.3%	(47.6%)		3.66				
regimen)							
(lopinavir-								
ritonavir vs.								
lamivudine)								
EBV infection	10	50	3.72	1.20-	.022	5.27	1.47-	.011
by week 50	(66.7%	(35%)		11.48			18.94	
(yes vs. no))							
At least one	13	76	5.73	1.25-	.025	5.11	1.06-	.043
SAE ^c during	(86.7%	(53.1%)		26.32			24.75	
the first year)							
of life (yes vs.								
no)								

*At week 1

^aCrude odds ratio using univariate analysis.

^bAdjusted odds ratio using a logistic regression model with a backward selection (variables in bold were included in the multivariate model).

^cSAE: all serious adverse events grade III or IV (including clinical and biological)

Abbreviations: WLZ, weight for length; SD, standard deviation; OR, odds ratio; CI, confidence interval; SAE, serious adverse events.

Figure 1

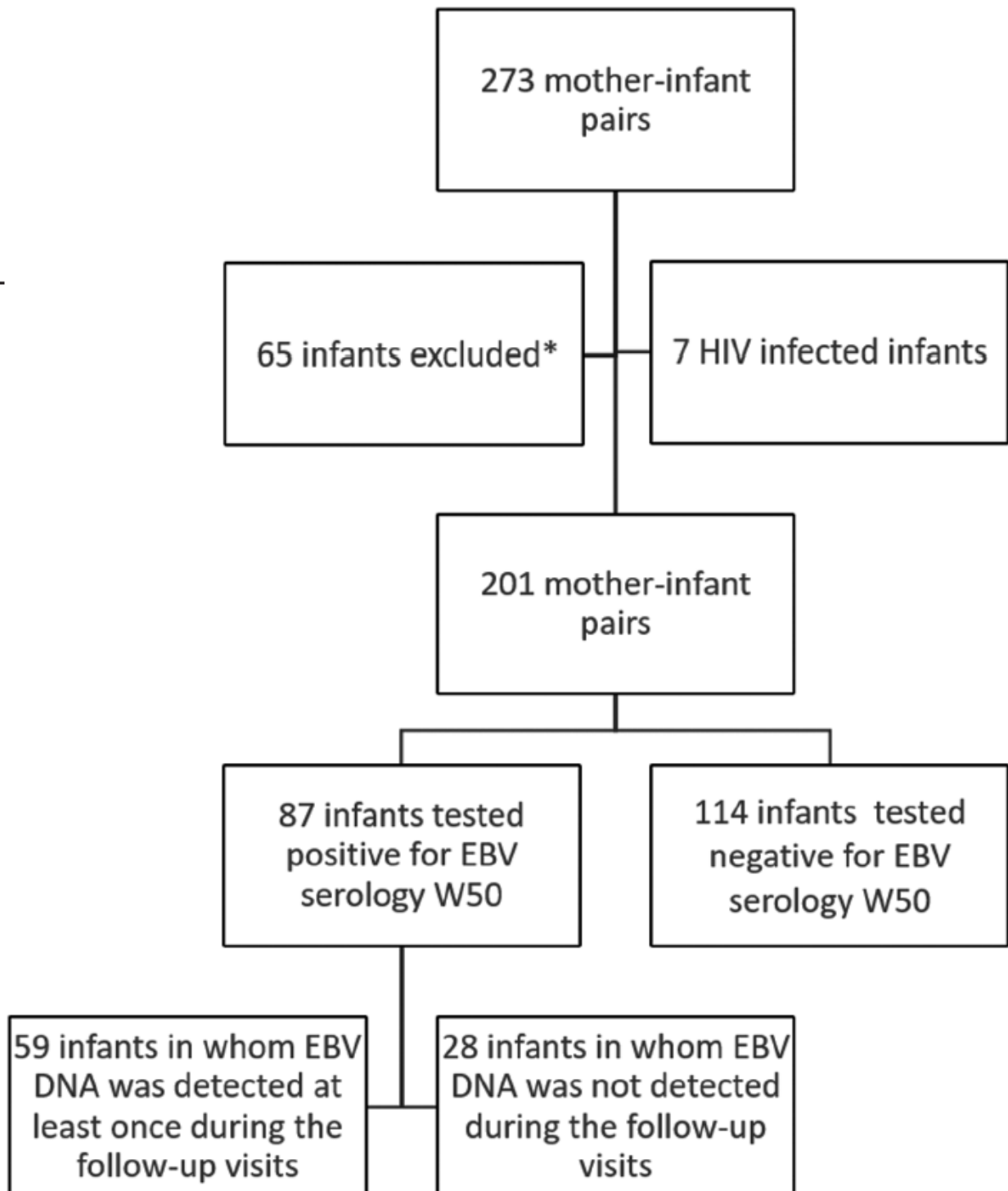


Figure 2

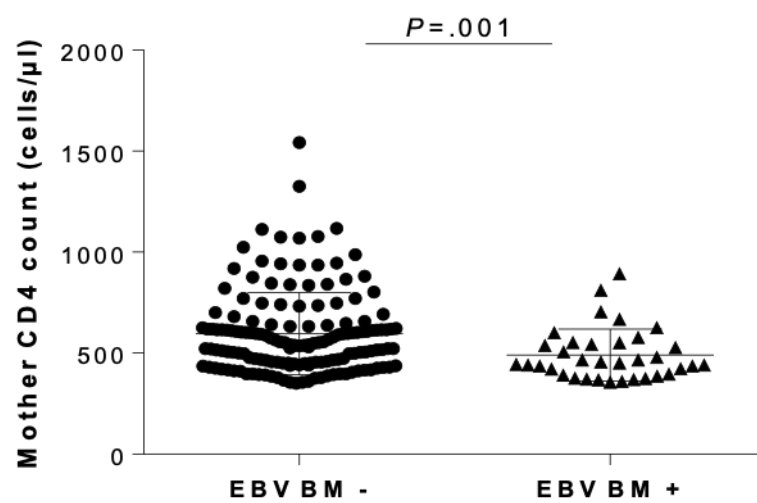


Figure 3

