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Early Weaning of HIV-Exposed Uninfected Infants and Risk of Serious Gastroenteritis: Findings from Two Perinatal HIV Prevention Trials in Kampala, Uganda

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Abstract

Objective—To assess serious gastroenteritis risk and mortality associated with early cessation of breastfeeding in infants enrolled in two prevention-of-maternal-to-child-HIV-transmission trials in Uganda.

Methods—We used hazard rates to evaluate serious gastroenteritis events by month of age and mortality among HIV-exposed uninfected infants enrolled in the HIVNET 012 (1997-2001) and HIVIGLOB/NVP (2004-2007) trials. HIV-infected mothers were counseled using local infant feeding guidelines current at the time.

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CONFLICT OF INTEREST STATEMENT J. Brooks Jackson as Principal Investigator for both trials has full access to all the data and has final responsibility for the decision to submit for publication. None of the authors have any conflicts of interest related to the conduct or reporting of this study.

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Results—Breastfeeding cessation occurred earlier in HIVIGLOB/NVP compared to HIVNET 012 (median 4.0 vs. 9.3 months, p<0.001). Rates of serious gastroenteritis were higher in HIVIGLOB/NVP (8.0/1000 child-months) compared to HIVNET 012 (3.1/1000 child-months; p < 0.001). Serious gastroenteritis events also peaked earlier at 3-4 and 7-8 months (16.2/1000 and 15.0/1000 child-months, respectively) compared to HIVNET 012 at 9 to10 months (20.8/1000 child-months). All cause-infant mortality did not statistically differ between the HIVIGLOB/NVP and the HIVNET 012 trials [3.2/1000 versus 2.0/1000 child-months respectively, (p=0.10)]

Conclusion—Early breastfeeding cessation seen in the HIVIGLOB/NVP trial was associated with increased risk of serious gastroenteritis among HIV-exposed uninfected infants when compared to later breastfeeding cessation in the HIVNET 012 trial. Testing interventions which could decrease HIV transmission through breastfeeding and allow safe breastfeeding into the second year of life are urgently needed.

Keywords

HIV; infants; breastfeeding cessation; serious gastroenteritis; mortality; Uganda

INTRODUCTION

Mother-to-child transmission of human immunodeficiency virus (HIV) accounts for nearly 90% of all pediatric HIV-infections [1]. More than 95% of HIV-infected children live in resource-poor settings where breastfeeding is the norm. Without prophylaxis, the overall risk of HIV transmission in breastfeeding populations is estimated to be between 30-45% and is due to perinatal infection and postnatal exposure through breast milk [2]. The latter contributes 12-16% of the cumulative absolute risk of acquisition among infants through 2 years of age [3-6]. This cumulative risk is considerably lower if exclusive breastfeeding is practiced for the first 3-6 months followed by weaning at 6 months of age; the estimated risk of HIV transmission through breast feeding after 6 weeks of age is less than 1% per month [4, 7, 8].

This low but cumulative risk of HIV transmission through breast milk must be balanced against the high morbidity and mortality risk due to malnutrition and infectious diseases seen among infants in resource-limited settings who are not breastfed. In resource-poor settings, increased rates of infant gastroenteritis have consistently been associated with early replacement feeding, likely due to lack of clean water, unsafe food preparation and early introduction of contaminated weaning foods [9]. Worldwide, diarrheal diseases account for a significant proportion (13-21%) of all deaths among infants and children less than 5 years of age with many of them suffering from frequent episodes which significantly impact development, growth and overall nutritional status [10-12].

The optimal time for an HIV-infected woman in resource-limited settings to cease breastfeeding is dependent on both her individual circumstances and locally available resources. Exclusive replacement feeding or early weaning are neither feasible nor safe options for the majority of HIV-infected women in resource-poor settings like Uganda. The ability to provide safe replacement feeding and the risks associated with it cannot be easily assessed, and adequate resources to ensure clean water and weaning foods are rarely

available for the majority of mothers. In addition, formula and animal milk substitutes are expensive and failing to breastfeed is stigmatizing. Even in mid-level countries, water safety cannot always be guaranteed. An example of this was a severe diarrheal outbreak in Francistown and surrounding areas in Botswana in 2006, when heavy rains led to ground-water contamination and resulted in a severe outbreak of infant diarrhea with resultant high morbidity and mortality among HIV exposed infants who had received government provided formula milk and were not being breastfed [13].

In these analyses from Kampala, Uganda, we assessed the risk of infant morbidity from serious gastroenteritis and mortality using data from two large perinatal HIV clinical trials -- the HIVNET 012 trial and the HIVIGLOB/NVP trial, both of which were conducted at the same site but at different time periods. The two studies reflect differing World Health Organization (WHO) infant feeding recommendations for HIV-infected women that were current at the times of the two trials.

METHODS

Parent Trial Study Design and Participants

These analyses were based on serious gastroenteritis and mortality data from two perinatal HIV transmission trials, HIVNET 012 and HIVIGLOB/NVP, both of which enrolled HIV-infected women and their children from the Mulago National Referral Hospital located in Kampala, Uganda.

The HIVNET 012 trial was a phase IIB randomized perinatal HIV prevention trial conducted from November 1997 to January 2001, with the primary goal of comparing the efficacy of intrapartum and neonatal single-dose (SD) nevirapine (NVP) versus zidovudine (ZDV) in reducing maternal-to-child-transmission. Pregnant women were consecutively recruited from Mulago Hospital antenatal clinics. The women were counseled and HIV tested; those who were HIV-seropositive, met eligibility criteria and gave informed consent were enrolled into the study. Mother-infant pairs were randomly assigned to receive SD NVP (200mg) at labor onset and NVP (2mg/kg) was given to the infant within 72 hours of birth or ZDV (600 mg) at the onset of labour, then 300 mg every 3 hours until delivery and to the newborn infant 4mg/kg ZDV twice daily for 7 days. The duration of follow up was through 18 months. Detailed description of the study design, methods and results are provided elsewhere [14, 15].

The HIVIGLOB/ NVP trial was a phase III randomized, three arm, partially blind trial comparing the efficacy of the SD NVP regimen with the addition of polyclonal HIV hyperimmune globulin (HIVIGLOB) or extended infant NVP dosing daily until 6 weeks of age compared with the SD NVP regimen alone for the prevention of perinatal and breast milk associated HIV transmission. HIVIGLOB, which is an experimental intravenous HIV hyperimmune globulin preparation containing antibodies against HIV (and others) was prepared by collecting the blood units from donors who were found to be HIV-1 antibody positive at the Nakasero Blood Bank in Kampala, Uganda. The HIVIGLOB product was tested in Sweden and Uganda in a phase I/II study and found to be safe [16]. The 200 mg/kg dosage for the mothers was based on a dosage chosen for a US perinatal trial (PACTG 185)

and the 400mg/kg dosage for the infants was based on findings from the phase I/II study where it was associated with a lower transmission rate of HIV at 6 weeks of age and significant increases in HIV-1 p24 antibody titer following infusion in women and their infants [16, 17].

Participant enrollment into the Phase III trial occurred between July 2004 and July 2006 and follow-up was completed in July 2007. Women were recruited from HIV-seropositive pregnant mothers participating in the Mulago Hospital PMTCT program. Following infant feeding counseling, those HIV-infected women who chose to breastfeed were referred to the study for enrollment. Mothers who met the eligibility criteria and gave informed consent were enrolled in the trial between 32-35 weeks of gestation. All women were given a SD NVP tablet (200mg) to take at the onset of labor and their newborns were given NVP syrup (2mg/kg) within 72 hours of birth. In arm one, mothers were randomized to receive a single intravenous (IV) infusion of 240 ml or 12 gm (approximately 200mg/kg) of HIVIGLOB at 36-37 weeks gestation and the infants born to these mothers received a single IV infusion 24 ml or 1.2 gm (approximately 400mg/kg) of HIVIGLOB preferably within 18 hours of birth. In arm 2, infants received extended NVP prophylaxis (5mg) daily from week one through six weeks of age while in arm 3, infants received only SD NVP after birth. All infants received multivitamins once daily from week one through six weeks of life. The HIVIGLOB/NVP trial was originally approved to follow up infants through 18 months. However, in April 2007, by which time most infants had completed their scheduled study exit at 18 months, funding constraints by the sponsor necessitated that the length of follow up be reduced to12 months for the remaining few that had not completed their original study scheduled exit.

Cotrimoxazole Prophylaxis

In the HIVNET 012 trial, HIV exposed infants did not routinely receive cotrimoxazole prophylaxis as there were no policy guidelines regarding this at the time. All infants in the HIVIGLOB/NVP trial were given prophylactic treatment with cotrimoxazole from 6 weeks of life to the time of confirmed negative HIV status following breastfeeding cessation as per the Ugandan Ministry of Health (MOH) guidelines [18]. Children who were confirmed as HIV infected continued with cotrimoxazole prophylaxis after breastfeeding cessation.

Counseling Regarding Breastfeeding

In HIVNET 012, women were counseled according to Ugandan MOH/WHO guidelines at the time of the trial which recommended exclusive breastfeeding for at least 6 months and to wean as early as possible thereafter [19]. Women were not counseled to discontinue breastfeeding at any specific time point and many continued through the first year of life and some into the second year.

In contrast, mothers in the HIVIGLOB/NVP trial were counseled according to revised Ugandan MOH PMTCT guidelines at the time which recommended exclusive breastfeeding for 3-6 months, with early and abrupt weaning over a 2 week period to minimize mixed feeding, if an HIV-infected woman had to breastfeed because of social or economic reasons.

[20, 21]. However, initiation of weaning was modified based on the mother's ability to provide adequate locally available nutritious foods and the nutritional status of the baby.

Determination of Infant HIV Infection

In HIVNET 012, qualitative HIV-1 RNA PCR assays were done at age 1-3 days, 6 weeks, 14 weeks, and 12 months. If HIV-1 RNA was detected, a second sample was obtained at the earliest opportunity possible or at the next scheduled visit for confirmation by HIV-1 RNA PCR or HIV-1 culture. Infants were tested using HIV-enzyme immunoassay (EIA) with western blot confirmation if reactive at 18 months. Diagnosis of HIV infection in infants was based on a positive qualitative HIV-1 RNA PCR assay confirmed by either quantitative HIV-1 RNA assay or HIV-1 culture on a second blood sample. In the case of an infant death where there was only one positive RNA assay on the sample preceding death, the infant was considered to be infected.

In the HIVIGLOB/NVP trial, infants were tested at birth, at weeks 2, 6, 14 and at 6 and 12 months using qualitative DNA or quantitative RNA PCR. Infant HIV infection status was based on at least two positive PCRs (DNA or RNA) on separate infant specimens. At 18 months, HIV-uninfected infants were tested using HIV EIA with western blot confirmation if reactive. Infants who died or were lost to follow up after only one positive PCR or antibody test were classified as infected. Infants were included in data analysis for this paper if they remained HIV-uninfected through to 18 months or at the last visit.

Measurements of Serious Adverse Events

Gastroenteritis was defined as an episode of diarrhea (the passage of 3 or more loose or watery stools within a 24 hour period) with or without vomiting. For purposes of this analysis, we defined serious gastroenteritis events as those diarrheal events in the infant, as described above, which resulted in a hospitalization or death.

Data on serious adverse events (including serious gastroenteritis), HIV infection and infant survival were collected systematically throughout the course of both the HIVNET 012 and HIVIGLOB/NVP trials. Adverse events grading was based on the NIH Division of AIDS (DAIDS) Toxicity Tables for Grading Severity of Adverse Experiences, April 1994 for paediatric events.

Medical history, clinical examination and routine laboratory tests were performed at scheduled protocol visits for both studies. For HIVNET 012, regular visits were scheduled at weeks 1, 6, 10, 14 and then at 6, 9, 12 and 18 months. In the HIVIGLOB/NVP trial, regular scheduled visits were weekly for the first 6 weeks, then at weeks 10, 14 and at 6, 12 and 18 months. In addition to the scheduled visits, mothers in both studies were encouraged to bring their infants to the clinic at any time in case of illness. At all visits, information on illnesses, hospitalizations and vital status were collected. In the HIVNET 012 study, questions were asked about breastfeeding cessation only, while in the HIVIGLOB/NVP trial, standardized questions were asked about exclusive breastfeeding, mixed and replacement feeding and timing of weaning. The complete cessation of breastfeeding was recorded as the date the mother or primary caretaker reported that the infant no longer received any breast milk.

Statistical Analyses

These analyses focused on rates and timing of gastroenteritis events that resulted in hospitalization or death as well as all cause mortality among HIV-uninfected exposed infants in the HIVIGLOB/NVP and the HIVNET 012 trials.

Baseline comparisons of continuous variables are presented using means, standard deviations, medians and inter quartile range (IQR). Means were evaluated using a two-sided sample t-test. Medians for duration of breastfeeding were based on Kaplan-Meier estimates to account for censoring over time. Categorical variables were compared using proportions and the corresponding cross-table Pearson chi-squared test (with Yates continuity correction). Event rates were computed as the sum of the number of events of interest occurring for children seen within a one-month age-interval band divided by the person-time at risk within that corresponding age-interval band, where risk time within each interval is indicated by the time during the interval that the child was still HIV uninfected. Thus, a child was dropped from the risk set for all the time within a specified age-band that follows HIV infection and for all that child's subsequent follow-up age intervals, but was considered for the length of time within the age-interval when they were still regarded as HIV uninfected. Otherwise, each child contributes to events and person-time as indicated until their termination from the study. Associated 95% confidence intervals (CIs) were calculated under the assumption that the observed numbers of events follow a Poisson distribution, and by use of Byar's approximation of Poisson exact limits which allow for asymmetric distribution of low event counts [22, 23].

Graphs were generated depicting the event rates (per 1000 child-months) in each age-interval band as well as associated curves depicting 95% CIs. P-values computed were based on Fisher's exact two-sided test which were obtained by performing a uniformly most powerful (UMP) unbiased test on the ratio of rates of two Poisson counts (divided by corresponding time at risk for each count) and defining the two-sided p-values as either 1 or twice the minimum of the one-sided p-values ensuring internally consistent p-values [24, 25].

Comparison of cumulative mortality between studies was based on the corresponding Kaplan-Meier (Aalen-Nelson) based cumulative hazard estimates of mortality over the follow up period. Gastroenteritis-associated and overall mortality rates were also estimated using the overall number of events divided by the group respective child-months over an 18 month period. Statistical significance for all tests was evaluated against the 5% alpha critical level. All the analyses were done using R statistical program and Stata [26, 27].

Ethical Considerations

IRB approval was obtained from Uganda and United States IRBs prior to each study commencement. Written informed consent was obtained from all study participants prior to enrollment after careful explanation of the studies.

RESULTS

In the HIVNET 012 study, 645 HIV-infected women were enrolled of whom 623 gave birth to HIV—uninfected babies whereas in the HIVIGLOB/NVP trial of the 722 women enrolled, 684 women gave birth to 698 HIV-uninfected babies who contribute risk time to the analysis. HIV-uninfected multiple births were included in this analysis.

Baseline Characteristics and Breastfeeding Cessation

Characteristics of mothers who gave birth to HIV-uninfected babies in the two trials are summarized in Table 1. The mothers in the HIVIGLOB/NVP trial were slightly older than mothers in HIVNET 012 by about a year. Parity of the mothers in the two trials was comparable with a median number of 3 children delivered (not including the study child). Marital status across the two studies was comparable with the majority of the women being married or in a stable union. The HIVNET 012 trial had a significantly higher proportion of women with less than secondary education (64.7% versus 56.2%, ($p = 0.002$)) and more women who described themselves as housewives (79.4% versus 69.6%, ($p = 0.001$)). Of importance there was a significant difference between the two trials with regard to breastfeeding cessation, with HIV-uninfected infants in the HIVIGLOB/NVP trial weaned at a median age of 4 months compared to 9.3 months in the HIVNET 012 trial ($p < 0.001$). Overall, the mothers were moderately immunosuppressed as evidenced by the absolute CD4 cell counts and viral load at enrollment which were comparable between the two studies (Table 1).

Rates of Serious Gastroenteritis

Table 2 presents age specific rates of serious gastroenteritis events per 1000 child-months by month of age through 18 months of life among HIV-uninfected infants in the HIVIGLOB/NVP and HIVNET 012 trials. In the HIVIGLOB/NVP trial, the highest rates of serious gastroenteritis events were 16.2 events per 1000 child-months at 3 - 4 months and 15.0 events per 1000 child-months at 7 - 8 months and thereafter remained relatively high up to 17 months. In the HIVNET 012 trial serious gastroenteritis events increased at 7 - 8 months with a rate of 10.3 events per 1000 child-months, peaked at 9 - 10 months of age with a rate of 20.8 events per 1000 child-months and then remained relatively low to 18 months of age.

Across all age specified intervals, except the 9 - 10 month age interval, the rates of serious gastroenteritis were generally higher among infants in the HIVIGLOB/NVP trial when compared to the HIVNET 012 trial and reached statistical significance at 3 - 4 months (16.2 events per 1000 child-months versus 0 events per 1000 child-months, $p= 0.005$). Serious gastroenteritis events in the HIVIGLOB/NVP trial notably began at younger ages compared with infants in the HIVNET 012 trial (Fig 1).

Overall rates of serious gastroenteritis events were highest in the HIVIGLOB/NVP trial at 8.0 events per 1000 child-months (95% CI 6.4 - 9.8) whereas the HIVNET 012 trial rates were 3.1 events per 1000 child-months (95% CI 2.1 - 4.4) which was statistically significant ($p < 0.001$).

Infant Mortality

The cumulative mortality for HIV negative infants was consistently although not statistically higher through 18 months of age in the HIVIGLOB/NVP trial when compared to the HIVNET 012 trial. The Kaplan-Meier (Aalen-Nelson) cumulative mortality hazard rates for the two studies are shown in Figure 2. The corresponding overall mortality rates in the HIVNET 012 trial versus the HIVIGLOB/NVP trial were 2.0 (95% CI 1.2 - 3.0) versus 3.2 (95% CI 2.1 - 4.6) per 1000 child-months respectively, (p=0.10). In the course of follow up, there were 6 gastroenteritis - associated deaths in the HIVIGLOB/NVP trial and only 1 gastroenteritis - related death in the HIVNET 012 trial. The overall gastroenteritis- related death rates in the HIVIGLOB/NVP trial versus the HIVNET 012 trial were 0.6 (95% CI 0.2 - 1.2) per 1000 child-months and 0.1 (95% CI 0.1 - 0.5) respectively, (p=0.18).

DISCUSSION

We compared rates of serious gastroenteritis events and death among HIV-exposed uninfected children from two trials in Uganda conducted at the same clinic but at periods of time where there were different infant feeding counseling guidelines in place. Prior to the HIV/AIDS era, Ugandan women were encouraged to exclusively breastfeed their children for 6 months and to continue thereafter to 2 years of age. This is still the practice among HIV-uninfected mothers in Uganda. In the HIVIGLOB/NVP trial, infants were weaned at a median age of 4 months with almost all ceasing breastfeeding by 6 months of age compared to the HIVNET 012 trial where weaning was at a median age of 9.3 months; and with some infants breastfeeding into the second year of life. Breastfeeding duration among HIV exposed infants was much shorter in both the two studies when compared to the median duration of 19.9 months of any breastfeeding among children in the general Ugandan population born in the three 3 years preceding the Uganda Demographic Health Survey (2000) [28].

Infant morbidity from serious gastroenteritis and mortality for HIV-uninfected infants was consistently higher in the HIVIGLOB/NVP trial with early breastfeeding cessation (4 months) than the HIVNET 012 trial with a later (9 months) median age of breastfeeding cessation. Likewise the serious gastroenteritis rates were significantly higher at 3 to 4 months of age in HIVIGLOB/NVP compared to HIVNET 012 around the time of early breastfeeding cessation. These results are highly concerning considering the fact that mothers in the HIVIGLOB/NVP study generally had higher levels of education and employment as compared to the mothers in the HIVNET 012 trial; and that cotrimoxazole prophylaxis which has been found to be beneficial against diarrhea was given to all HIV-exposed infants in the HIVIGLOB/NVP trial from 6 weeks of life to the time of confirmed negative HIV status following breastfeeding cessation [29]. Based on these two facts, we would have anticipated that the infant rates of serious gastroenteritis events in the HIVIGLOB/NVP trial would be lower than in the earlier HIVNET 012 trial- but this was not the case. These findings are consistent with recent reports from perinatal HIV prevention trials in Blantyre, Malawi and Kisumu, Kenya, which also reported higher rates of serious gastroenteritis events and/or infant mortality for HIV-exposed uninfected infants around the time of early breastfeeding cessation when compared to historical controls at the same sites

but where breastfeeding went into the second year of life [30, 31]. The early breastfeeding cessation for HIV exposed infants at the time of the trials was based on MOH and 2000 WHO guidance on HIV and infant feeding which were in effect during the trials [20, 21].

Recent data from the MITRA Study in Tanzania and the PEPI trial in Malawi show that HIV- free survival through 6 and 9 months respectively, is significantly associated with extended prophylactic antiretroviral treatment of the infant during the breastfeeding period [32, 33]. However, the Zambia Exclusive Breastfeeding study (ZEBS) reported no overall difference in HIV-free survival for infants who were randomized to abrupt weaning at 4 months versus continued exclusive breastfeeding to 6 months with gradual introduction of complementary foods thereafter and complete cessation of breastfeeding on average by 16 months. Of importance in that study, for healthier women with CD4 cell counts over 350/μL and who comprise the majority of HIV-infected mothers in most settings, HIV-free survival of the infants was significantly better for women who continued breastfeeding into the second year of life [34]. Likewise, data from the MASHI trial in Botswana found significantly higher overall infant mortality through 7 months for infants who were formula-fed from birth compared to those who were breastfed and prophylaxed with daily infant ZDV; but with no difference in overall HIV-free survival by intervention arm at 12 or 18 months [35].

While HIV-free survival is generally one of the most important endpoints to assess, overall all-cause survival is also important in the context of resource limited settings and is closely linked to duration of breastfeeding. The general child-survival literature has consistently shown the protective effects of breastfeeding against early infant mortality with a 3-6 fold decreased risk of mortality in the first 6 months of life and a 1.4-1.8 fold protective effect of breastfeeding against mortality in the second 6 months of life [36]. There may be a number of reasons for the protective effects of breastfeeding during the first year of life. First, breast milk is known to be rich in immunoglobulin A (IgA) which has a protective mechanism against enteropathic gut infections, along with other innate immune protection and nutritional benefits provided by breast milk. The babies in the HIVIGLOB/NVP study stopped breastfeeding at an early age before they were likely to have high levels of protective IgA along their epithelial linings [37-39]. Mixed-feeding prior to complete breastfeeding cessation could also have contributed to the early onset episodes of serious gastroenteritis that is seen in the HIVIGLOB/NVP trial through introduction of contaminated weaning foods when the infant’s natural immunity was not yet sufficient to control the infections. Recent data from studies conducted in Uganda and South Africa, also found formula- feeding was associated with a six- and almost four-fold higher infant mortality risk respectively, when compared to breastfeeding among infants of HIV-infected mothers. These findings reinforce the crucial role of extended breastfeeding in promoting overall infant survival including among HIV exposed infants living in resource limited settings [40, 41].

In both the HIVIGLOB/NVP and HIVNET 012 trials, the rates of serious gastroenteritis events were higher around the time of breastfeeding cessation when compared to the rate of serious gastroenteritis prior to stopping breastfeeding. This is consistent with other studies which show that the timing of introduction of weaning foods is frequently associated with

increased gastroenteritis morbidity/ mortality events. This increase in severe gastroenteritis is presumed to be precipitated by poor hygienic practices and use of contaminated water around the time of weaning [42, 43]. It has also been documented that the weaning foods usually selected by mothers in Uganda are inadequate to provide caloric, nutritional and immunological needs of the infant [44]. This in turn predisposes infants to immunological compromise which can lead to increased episodes of serious gastroenteritis especially without the immunologic protection provided by breast milk.

Both the HIVNET 012 and HIVIGLOB/NVP studies had some limitations relevant to the above analyses. The HIVIGLOB/NVP did not have HIV-free survival to compare to the HIVNET 012 data. Secondly, the HIVNET 012 findings are based on historical data and there may be unknown biases and temporal trends that could have contributed to the differences in rates of serious gastroenteritis events noted in the two studies. Given the relatively low mortality events, the analyses were underpowered to assess statistical differences in mortality between the two studies. There were also significant differences in the length of breastfeeding between the trials with infants in the HIVIGLOB/NVP trial stopping on average at about 4 months of age. The shorter period of breast feeding placed the HIVIGLOB/NVP group at increased risk of severe gastroenteritis morbidity/mortality when compared to most infants in HIVNET 012 who generally breast fed till about 9 months of age. In balance, the HIVIGLOB/NVP infants also had access to more potent recent antibiotics, as well as more consistent cotrimoxazole prophylaxis which would have reduced their risk of severe gastroenteritis compared to the earlier HIVNET 012 study infants. This would tend to mitigate any differences in severe gastroenteritis between the groups (i.e. bias the magnitude of effect towards the null). However, in spite of this bias towards the null, the results demonstrated a significantly higher rate of serious gastroenteritis for the HIVIGLOB/NVP group compared to the HIVNET 012 group which we attribute in large part to the early breastfeeding cessation.

Inherent strengths of the analyses include that the data on adverse events were consistently and carefully captured by site clinicians using the same standardized DAIDS/NIH Toxicity Tables; and that there was excellent follow up of participants in both trials so that late infant outcomes were well documented. The results are likewise consistent with findings from other recent trials in Malawi and Kenya; as well as reflecting the negative effects of early breastfeeding cessation reported in the general child survival literature.

These data from the HIVIGLOB/NVP trial in Uganda as well as the PEPI Malawi trial and the Kisumu, Kenya trials raise concerns that early breastfeeding cessation among HIV-infected women in resource-limited settings may lead to increased rates of serious gastroenteritis adverse events among infants and to increased overall infant mortality when compared to more prolonged breastfeeding of HIV exposed infants [30, 31]. In October 2006 WHO refined its HIV and infant feeding guidance and in February 2007, released revised guidelines to help policy makers and programme managers clarify earlier recommendations [45]. The findings from HIVIGLOB/NVP as well as the studies in Malawi (PEPI), Kenya (KIBS) and Zambia (ZEBS) led to revised recommendations for most HIV-infected women to exclusively breastfeed for the first six months and then to continue breastfeeding through the first year of life with introduction of complementary foods in

situations where safe nutritional alternatives are not readily accessible [30, 31, 34]. Further data are needed on HIV-free survival outcomes at 18-24 months in relation to infant feeding choices; with careful assessment of competing causes of infant mortality associated with early breastfeeding cessation.

The ultimate goal of infant feeding strategies for HIV-infected women should be to develop interventions which allow longer, safer breastfeeding in order to provide optimal infant nutrition and to reduce the risk of severe infant gastroenteritis and mortality; while at the same time decreasing the risk of post-natal HIV transmission in order to maximize the lifesaving protective benefits of breast milk.

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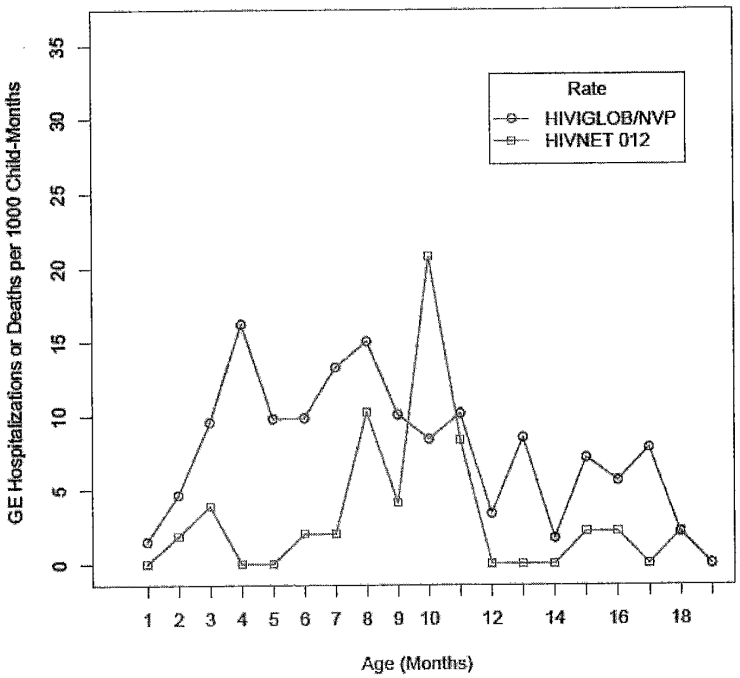

Figure 1.
Rates of Serious Gastroenteritis Events by month for the HIVNET 012 and HIVIGLOB/NVP Trials

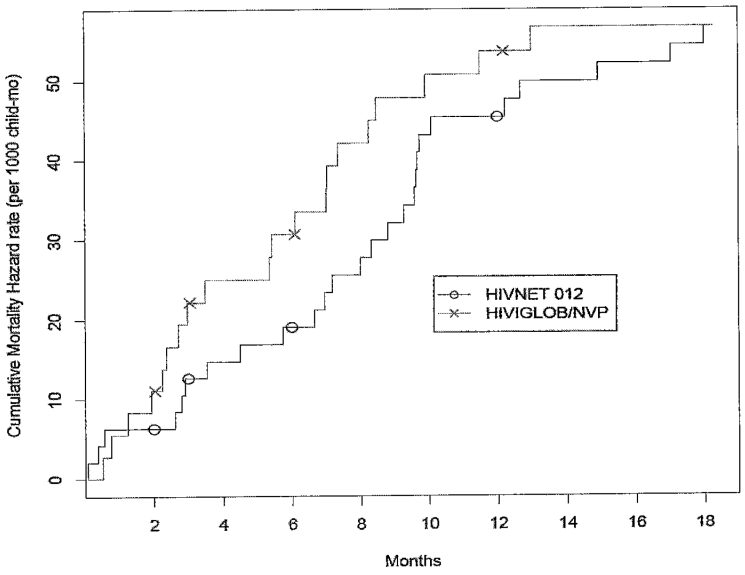

Figure 2.

Kalpan Meier Estimates of Mortality among Infants in the HIVNET 012 and HIVIGLOB/NVP Trials

Table 1

Baseline Characteristics for Ugandan HIV-Infected Mothers of Infants born HIV-Uninfected in the HIVNET 012 and HIVIGLOB/NVP Trials

Mothers with HIV-Uninfected Infants	**HIVNET 012 TRIAL** N= 623	**HIVIGLOB/NVP TRIAL** N= 684	**p-value**
Age Mean (years) (SD)	24.6 (4.3)	25.8 (4.9)	<0.001*
Parity Median (IQR)	3.0 (2 - 4)	3.0 (2 - 4)	0.26*
Marital status			
Married/stable union	535 (85.9 %)	587 (85.8%)	
Single (i.e., never married, separated, widowed, divorced)	88 (14.1 %)	97 (14.2%)	0.96†
Education			
Primary or less	400 (64.7 %)	384 (56.2%)	
Secondary or more	218 (35.3 %)	299 (43.8%)	0.002†
Occupation			
House wife	477 (79.4 %)	476 (69.6%)	
Employed, Other	124 (20.6 %)	208 (30.4%)	0.001 †
Breastfeeding Cessation (Age of Infant)			
Median(months) (IQR)	9.3‡(6.1 – 15.0)	4.0 ‡(2.8 – 6.0)	<0.001§
Baseline Absolute CD4 cell counts			
Median (IQR)	450 (278 - 646)	424 (279 -587)	0.25*
Baseline Viral Load			
Median (IQR)	25090 (7367 - 73797)	34222 (8388 -119711)	0.052*

*Based on the 2-sample t-test

†Based on Pearson's Chi-squared Test with Yates Continuity correction

‡Based on a Kaplan-Meier estimate

§[Reference 46]

Table 2

Age Specific Rates of Serious Gastroenteritis Events in the HIVNET 012 and HIVIGLOB/ NVP Trials

	HIVNET 012		HIVIGLOB/NVP		
Age Interval (months)	**Person Time of Follow-up (child-months)**	**Rate per 1000 child-mos (95% CI)**	**Person Time of Follow-up (child-months)**	**Rate per 1000 child-mos (95% CI)**	**p-value**
0-1	570	0 (0 - 4.3)	666	1.5 (0.1 - 7.0)	1.00
1-2	539	1.9 (0.2 - 8.7)	642	4.7 (1.3 - 12.5)	0.76
2-3	512	3.9 (0.8 - 12.5)	627	9.6 (4.0 - 19.7)	0.44
3-4	504	0 (0 - 4.9)	617	16.2 (8.3 - 28.8)	0.005**
4-5	499	0 (0 - 4.9)	612	9.8 (4.1 - 20.2)	0.056
5-6	499	2.0 (0.2 - 9.4)	610	9.8 (4.1 - 20.3)	0.21
6-7	493	2.0 (0.2 - 9.5)	603	13.3 (6.3 - 25.0)	0.08
7-8	488	10.3 (3.9 - 22.5)	599	15.0 (7.4 - 27.4)	0.68
8-9	487	4.1 (0.8 - 13.2)	596	10.1 (4.2 - 20.8)	0.44
9-10	482	20.8 (10.7 - 36.8)	594	8.4 (3.2 - 18.5)	0.15
10-11	479	8.4 (2.8 - 19.9)	592	10.1 (4.2 - 20.9)	1.00
11-12	477	0 (0 - 5.2)	590	3.4 (0.7 - 10.9)	0.61
12-13	469	0 (0 - 5.3)	587	8.5 (3.2 - 18.7)	0.11
13-14	463	0 (0 - 5.3)	579	1.7 (0.2 - 8.1)	1.00
14-15	461	2.2 (0.2 - 10.1)	559	7.2 (2.4 - 17.0)	0.51
15-16	461	2.2 (0.2 - 10.1)	534	5.6 (1.6 - 15.0)	0.74
16-17	461	0 (0 - 5.3)	511	7.8 (2.6 - 18.6)	0.15
17-18	460	2.2 (0.2 - 10.1)	473	2.1 (0.2 - 9.9)	1.00
18-19	451	0 (0 - 5.5)	163	0 (0 - 15.1)	1.00

** Significant at p-value of 0.05

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