Clinical outcomes of HIV-exposed, -uninfected children in sub-Saharan Africa

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ABSTRACT

Objective: HIV-exposed but uninfected (HEU) children are widely considered at increased risk of mortality and morbidity. We critically reviewed reports of mortality/morbidity among HEU and HIV-unexposed children in sub-Saharan Africa.

Methods: We searched Medline, EMBASE, CINAHL, PsycINFO, Academic Search Premier, Global Health & Psychosocial Instruments databases, conference abstracts; reference lists for longitudinal studies from sub-Saharan Africa reporting mortality and clinical morbidity among HIV-uninfected children aged \( \leq 10 \) years, by maternal HIV status. Studies were appraised by Newcastle-Ottawa Scale and ACROBAT-NSRI. Due to substantial heterogeneity of study designs, populations and results (\( I^2=75\% \)), data were not synthesised.

Results: We included 37 reports (28 studies, 11,164 HEU children); methodological and reporting quality were variable. Most reports came from settings without universal access to maternal ART (n=35). Results were conflicting, with some studies indicating increased risk of mortality, hospitalization and/or under-nutrition among HEU children while others found no evidence of increased risk. In sub-analyses, improved maternal health, ART use and breastfeeding were strongly protective for all outcomes. Only 39\% (11/28) of studies adjusted for major confounders. Reports from settings using universal maternal ART with breastfeeding (n=2) found no differences in growth or development but did not report mortality or infectious morbidity.

Conclusions: The existing literature provides little insight into HEU child health under recently-adopted PMTCT strategies. There is a need for robust comparative data on HEU and HIV-unexposed child health outcomes under Option B+; optimizing breastfeeding practices and increasing maternal use of ART should be urgent public health priorities.
Keywords: Africa, sub-Saharan; Maternal Health; HIV; Prevention; infant

INTRODUCTION
In 2014, an estimated 13.8 million women of child-bearing age were living with HIV in sub-Saharan Africa (SSA). With the rapid global expansion of effective strategies to prevent mother-to-child transmission of HIV (PMTCT), an increasing proportion of HIV-exposed children are born uninfected (HEU) annually; during 2014 more than one million HEU infants were born in the region.(1) Historically, HEU children have been reported to have higher than expected risks of mortality and morbidity.(2-6) This association is hypothesized to be driven by a combination of biologic and socio-economic risk factors, including advanced maternal HIV disease and/or death,(5, 7) disruptions in family and socio-economic structures,(8) insufficient infant vaccine and other immunological responses,(9-12) altered child health care-seeking behaviour,(13) increased pathogen exposure in the home(14-16) and suboptimal infant feeding practices.(4, 17-22)

However, existing insights into the health of HEU children come predominantly from a previous era of HIV and PMTCT in Africa, when access to lifelong triple drug maternal antiretroviral therapy (ART) was limited, and breastfeeding by HIV-infected mothers often controversial.(20, 21) Since 2010, WHO has recommended ART for all pregnant and breastfeeding women with HIV, maintained at least for the duration of breastfeeding (“Option B”) or for life (“Option B+”).(23) (24) (25) Since 2015, WHO recommends universal ART for all HIV infected individuals, irrespective of disease stage, and in line with this, “Option B+” as the preferred PMTCT strategy.(26) Effective use of maternal ART results in viral suppression with subsequent immune restoration and protection of maternal health;(16, 27, 28) HEU infants born to mothers with lower HIV viral load and/or higher CD4 count are at significantly lower risks of death,(7, 29) hospitalization,(7) severe infection,(7) immune dysfunction (30, 31) and growth faltering (32, 33) than those born to women with untreated or advanced HIV disease.(29, 33)

Breastfeeding is well understood to reduce child morbidity and mortality.(34-37) However in the absence of any PMTCT interventions, breastfeeding, and particularly mixed feeding, contributes to overall MTCT risk.(38) Accordingly, WHO HIV and infant feeding guidelines between 2001 and 2009 placed a strong emphasis on replacement feeding for HIV prevention; recommended feeding options included commercial infant formula, home-modified animal milk, wet-nursing by an uninfected woman, heat-treated expressed breastfeeding, or exclusive breastfeeding (EBF) for 6 months followed by accelerated weaning over a short period of time (“rapid weaning”).(39, 40) However, by 2010 strong evidence had accumulated regarding the substantial mortality and infectious disease
risk associated with both replacement feeding and rapid weaning in most of SSA (18-20, 41-45) while other data illustrated reductions in postnatal MTCT with use of maternal and/or infant antiretrovirals during breastfeeding.(44, 46-48) WHO HIV and infant feeding guidelines were updated accordingly, recommending exclusive breastfeeding for 6 months with continuation until at least 12 months of age in settings with high infant and child mortality, and antiretroviral prophylaxis or lifelong maternal ART to reduce the risk of postnatal transmission through breastfeeding.(40)

Understanding the impact of universal maternal ART with extended breastfeeding on HEU child health is an important step towards improving child health and survival in SSA. Given their substantial benefits it is plausible that these strategies, increasingly implemented across SSA,(49) may ameliorate the adverse effects of maternal HIV infection on child health.(19, 43, 50-54) To investigate this possibility, we systematically reviewed the association between maternal HIV status and child health outcomes in SSA in the absence of vertical transmission, among children aged ≤ 10 years of age. In addition to mortality, we focused on conditions that contribute significantly to under-five mortality and burden of disease on the continent, and are strongly influenced by breastfeeding; specifically, we examined diarrhoea, pneumonia, undernutrition and developmental delay.(51-53, 55, 56) Our specific objectives were to (i) summarize and (ii) critically assess reports of child mortality/morbidity among HEU compared to HIV-unexposed (HU) children, and (iii) to examine variation by maternal ART status and breastfeeding practices.

METHODS

Criteria for study inclusion
The population of interest was pre-adolescent children (defined as age ≤10 years)(57) born to HIV-infected women, with known negative HIV status based on age-appropriate laboratory methods. Studies were excluded if child HIV status was not reported, was based only on survival and clinical criteria, or was based on antibody testing alone prior to 18 months of age. To reduce biases due to left censoring we limited our review to longitudinal study designs and excluded case-control and cross-sectional studies. We also excluded studies where participants were sampled on the basis of maternal morbidity (other than HIV infection) and/or child health status (other than HIV exposure). We included only studies where HEU children were compared to HU children from a similar setting or community.

The main exposure of interest was maternal HIV status during pregnancy and/or breastfeeding. We included studies where maternal HIV status was extrapolated from the presence of anti-HIV antibodies in children under the age of 12 months. Where data were available, we evaluated breast-
feeding practices and maternal HIV disease severity as additional exposures. Primary outcomes of interest for the review were (i) mortality; (ii) diarrhoea, pneumonia and health care utilization; (iii) growth parameters focusing on weight and length; and (iv) measures of early childhood development.

**Search methods for identification of studies**

We built database-specific search strategies using a combination of terms for “HIV” + “mother” + “uninfected” + “child”, without date limitations, and searched MEDLINE (via Pubmed), EMBASE, Cinahl Plus, PsycINFO, Academic Search Premier, Global Health and Psychosocial Instruments. For the PubMed search, we included both MeSH and free text terms, with no language restrictions, Appendix 1; the most recent search was conducted on October 28, 2015. We used the EBSCOhost research database to search for relevant conference abstracts, reviewed the reference lists of reports that met our inclusion criteria, and examined the bibliographies of editorials and review articles found in our searches. (3, 4, 58-69)

**Data collection and analysis**

We conducted the review in accordance with the PRISMA guidelines (Appendix 2; PROSPERO registration number CRD42015017639, Appendix 3). After iterative database searches, we screened all titles and abstracts to identify reports for full-text review, and developed the data abstraction form. Two authors (SLR and KN) independently reviewed all full-text reports; disagreements were resolved through discussion with the senior author (LM).

Given the substantive risk of bias (RoB) inherent to observational studies, we decided *a priori* to utilize the Newcastle-Ottawa Scale (NOS) (70) to assess RoB, in accordance to the Cochrane Handbook.(71) Domains of potential bias were external validity, confounding, selection bias and information bias; for each domain we used questions from either the NOS or, when application of the NOS did not yield sufficient information, the Cochrane Risk of Bias Assessment Tool for Non-Randomized Studies of Interventions (ACROBAT-NRSI).(72) Following the format of the latter, we utilized 11 “signalling” questions in total (Appendix 4), each of which could have a single answer: either yes (low RoB in the related domain), probably yes (moderate RoB), no (serious to critical RoB) or insufficient information to assess (unable to allocate RoB). Based on the answers within each domain, each study was allocated an overall RoB: “Low”, the study is comparable to a well-performed randomized trial; “Moderate”, the study is sound for a non-randomized study; “Serious”, the study has some serious problems; “Critical” the study is too problematic to provide useful evidence on the
effects of exposure; or “No information”, where insufficient information was provided on which to base a overall judgement. Finally, for data synthesis purposes, we excluded all studies with insufficient information and those judged to have more than a moderate overall RoB (Appendix 4).

In summarising study findings, we presented unadjusted mean differences, risk ratios, and incidence rate ratios, with 95% confidence intervals (CI). Adjusted measures of association were included when available. Measures of association were calculated in Stata 12.0 (StataCorp, College Station, TX, USA) using raw data presented in reports; where no raw data were presented we used the measure provided by the authors. As we anticipated substantial clinical and methodological heterogeneity we explored potential data synthesis only for mortality, limited to results from studies with low/moderate risk of bias, using a random effects meta-analytic model. We formally tested for statistical heterogeneity within sub-groups of perinatal/maternal use of ART, using the chi² test with 10% level of significance, presented as an I² statistic. (73-75) We tested for small study effects (such as potential publication bias) through the use of a funnel plot and Harbord’s modified test.(76)

RESULTS

Description of included studies
Thirty-seven reports, from 29 studies conducted in 12 African countries and involving 32,173 (11,164 HEU) children were included, as shown in Figure 1 (characteristics of and reasons for exclusion of studies are shown in Appendix 5; characteristics of included studies are shown in Appendix 6). Three studies contributed data on the same outcome at different time points (mortality,(77, 78) neurodevelopment(79, 80) and growth,(81, 82) respectively); while 5 studies contributed two reports each, on different outcomes.(5, 32, 33, 83-89) Overall, 28 prospective studies were included,(77, 80, 81, 84-87, 89-109) of which 11 were randomized controlled trials (13 reports),(5, 81, 82, 84, 90, 94-96, 98-101, 110) and 2 interventional cohort studies (4 reports) focusing on PMTCT.(33, 83, 85, 86) The majority of studies (72%, 21/29) enrolled participants during the antenatal period or soon after birth.(33, 77, 80, 84, 88, 89, 91-94, 96-99, 101-105, 107, 108) Most reports (31/37, 84%) focused on health outcomes among infants and/or children ≤36 months old; there were only 6 reports on health outcomes in HEU children older than 3 years.(78, 82, 87, 105, 109, 111).

Maternal treatment and health status
An overview of studies by maternal antiretroviral use is shown in Table 1. There were 2 reports on child outcomes under universal maternal ART; in both studies, HEU children were studied either during or immediately after participation in clinical trials.(100, 101) The majority of reports were on HEU children whose mothers received no perinatal antiretroviral drugs (19/37, 51%).(5, 77, 78, 80, 81,
Eighteen reports (49%) provided limited to no information on early infant feeding. (77, 78, 80, 83, 85, 87, 88, 91, 92, 94, 95, 98-100, 106, 107, 109, 111) Available infant feeding details were summarized by study according to WHO infant feeding (Appendix 7). (113) Most reports predated the 2001 WHO HIV and infant feeding guidelines (15 reports from 11 studies), (5, 77, 78, 80, 83-85, 91-93, 103, 105, 107, 108, 111) or were from the period between 2001 and 2009, when exclusive breastfeeding with rapid weaning was recommended if replacement feeding was not possible (20 reports from 17 studies). (32, 33, 81, 82, 86-90, 94, 95, 97-99, 101, 102, 104, 106, 109, 112) Two reports from post-2009 study populations provided limited infant feeding data. (96, 100) Both indicated breastfeeding durations for HEU children beyond 6 months, without reporting rapid weaning; however, the majority of HEU children discontinued breastfeeding before 12 months of age, while HU children were breastfed for longer. Overall, infant feeding reporting was suboptimal, and adjustment or stratification of results according to early infant feeding modality was presented in fewer than a third of reports (10/37, 27% of total). (32, 81, 86, 89, 90, 96, 98, 102, 107, 114)

Methodological quality of included studies
Study-level risk of bias varied widely (Appendix 4). Six reports were considered subject to serious overall risk of bias, predominantly selection bias resulting from participant sampling or differential follow-up. (87, 92, 95, 97, 103, 112) Four reports provided insufficient information on which to base a risk of bias assessment; (93, 99, 104, 108) one study was at critical risk of selection bias. (109) Two studies (four reports) were considered comparable to well-performed randomized trials (low risk of bias); (5, 33, 84, 86) the remaining studies were considered sound for non-randomized trials (moderate risk of bias). In 20 studies (26 reports), (32, 33, 77, 80-83, 85-90, 92, 94, 96-99, 101, 102, 104, 106-108, 111) post-partum HIV seroconversion was not excluded among initially HIV-negative breastfeeding women, potentially increasing the risk of exposure misclassification bias. (115)
Only 32% (12/37) of reports considered potential confounders in examining the association between HIV-exposure and child health outcomes. (5, 32, 33, 81, 82, 85, 86, 89, 96, 98, 100, 101) Confounder selection was commonly based on statistical significance testing and seldom defined a priori; varying between analyses, these included social/economic measures,(32, 81, 82, 86, 89, 96, 98, 100) early infant feeding,(32, 33, 81, 86, 89, 96) child age,(5, 32, 96-98, 100) low birth weight(32, 85, 100) and maternal health/survival.(5, 85, 89) The majority of studies reported group-averaged associations between maternal HIV status and child health outcomes; only four reports examined effect modification by other clinical or demographic factors.

**Association between HEU status and child health outcomes**

**Mortality**

There were 19 reports of unadjusted estimates of child mortality, 13 of which were considered to be at low/moderate risk of bias (Tables 1, 2). (77, 80, 84-86, 89, 94, 96, 102, 105-107, 116) Raw data were available for calculation of risk ratios in 11 reports; two reports are presented only with hazard ratios as provided in the original manuscript (Table 2 and Figure 2). (86, 105) There was substantial evidence of between-study heterogeneity overall ($I^2$, 75.1%, p<0.001) and within subgroups of maternal use of antiretroviral drugs (Figure 2). Furthermore, the funnel plot (Appendix 8) provided evidence of small study effect, most likely due to publication bias (p=0.04, Harbord’s modified test), with a paucity of small studies reporting no increased or very small decreased risk of mortality among HEU. Nonetheless, there was a clear trend towards increased mortality among HEU children versus HU comparators, particularly among non-breastfeeding HEU populations without universal maternal access to ART, although the majority of studies had small sample sizes and in turn, imprecise findings. (77, 78, 80, 84, 85, 96, 106) The two largest studies, both considered to be at relatively low risk of bias, provide particular insights relevant to current PMTCT strategies. In an early PMTCT trial, antiretroviral-naive Zimbabwean women and their newborns were randomly allocated to receive either vitamin A or placebo in a factorial (2X2) design. Although randomization was not stratified by HIV status, clear mortality differences were noted in these subgroups. Compared to HU infants, HEU infants experienced a significantly higher 12-month mortality risk: unadjusted risk ratio (RR), 3.71 (95% CI 0.73-13.86). (84) However, the effect of maternal HIV exposure on infant mortality was modified by maternal disease severity: HEU children born to mothers with CD4 cell counts <200 cells/μL were at substantially higher risk of death than those born to mothers with CD4 ≥ 400 cells/μL (aHR 2.62, 95% CI 1.8-3.8); maternal death predicted infant death among both HEU (aHR 2.68, 95% CI 1.86-3.87) and HU children. In a later South African study, predominantly breastfeeding
women received single-dose nevirapine (sdNVP) during pregnancy, with some access to triple ART for women with severe disease. After adjusting for breastfeeding practices and socio-economic factors, there was no significant difference in the hazard of child death comparing HEU to HU children (aHR 0.77, 95% CI 0.49-1.21). Exclusive breastfeeding (EBF) was achieved by a substantial number of both HIV-infected (81.4% by 6-8 weeks) and HIV-uninfected women (92.9% by 6-8 weeks). Compared to children who were EBF, both partially breastfed and never breastfed children were at substantially higher risk of death before 12 months, even after adjusting for child HIV status and socio-economic variables: aHR 2.6 (95% CI 1.9-3.8) and aHR 3.6 (95% CI 2.5-5.2), respectively. Of note, there were no published reports comparing mortality between HEU and HU children in the context of universal maternal ART (Tables 1, 2 and Appendix 6).

Hospitalization/sick child visits
Hospitalization and “sick-child” visits appeared to occur more commonly among HEU than HU children. There were 10 reports of unadjusted hospitalization and/or “sick-child” clinic visit risk estimates. Overall, four reports were from populations without access to antiretroviral drugs; 6 from populations using predominantly 1-2 antiretroviral drugs for PMTCT; and there were no reports on populations receiving ART during pregnancy and breast feeding (Table 1).

Estimates from the 6 reports with low/moderate risk of bias are shown in Appendix 9. Two reports identified factors modifying the relationship between maternal HIV status and childhood hospitalization/clinic visitation risk. In a Zimbabwean RCT (effect of vitamin A on MTCT), where access to ART was limited, the association between maternal HIV status and child risk differed significantly by levels of maternal HIV disease severity: compared to predominantly breastfed HU infants, predominantly breastfed HEU infants born to mothers with baseline CD4 counts of >800 cells/mm³ had no increased risk of sick clinic visits (IRR 1.02, 95% CI 0.89 – 1.16) whereas those born to mothers with lower CD4 cell counts showed incrementally increasing risks, with the highest risk occurring among infants born to mothers with CD4 <200 cells/mm³ (IRR 1.33, 95% CI 1.17 – 1.50). More recent data from an Ugandan anti-malarial RCT demonstrated effect modification by infant feeding modality: while non-breastfed HEU infants born to women receiving ART had a remarkably higher risk of hospitalization than their breastfed HU counterparts (adjusted IRR 19.6, 95% CI 4.95-77.3), breastfed HEU and breastfed HU children had approximately similar risks, although precision for this estimate was low (adjusted IRR 1.50, 95% CI 0.14 – 16.4).
Diarrhoeal disease

Ten reports provided unadjusted estimates of risk for diarrhoeal disease. \(5, 83, 86, 95-97, 102, 107, 108, 112\) Six reports were assessed to be at low/moderate risk of bias; \(5, 83, 86, 96, 102, 107\) 3 included stratified/adjusted estimates \(5, 86, 96\) (Table 1, Appendix 9). Between-study comparison of diarrhoea estimates was limited by varying definitions and measurement of diarrhoeal disease. 5 of the 10 reports were on populations without access to antiretroviral drugs. \(5, 83, 95, 107, 108\) Four of these populations were predominantly mixed fed, combining breastmilk with early introduction of complementary feeds; \(5, 83, 107, 108\) one report provided no details on infant feeding. \(95\) A single study with no use of antiretroviral drugs (moderate risk of bias) found an increased risk of persistent diarrhoea among HEU (IR 4.9 per 100 child-years) vs. HU infants (IR 2.7 per 100 child-years); those with earlier introduction to solids and/or formula milk were at highest risk irrespective of maternal HIV status. \(107\) No significant increases in diarrhoeal risk were observed in the other studies reporting on populations without access to antiretroviral drugs. There were 5 reports of diarrhoeal risk among infants born to mothers receiving predominantly 1-2 antiretroviral drugs for PMTCT with limited access to ART; three were assessed as having a low/moderate risk of bias. \(86, 96, 102\) Two of these reports indicated no significant association between maternal HIV and child diarrhoeal risk, while the third found an appreciable association which was explained entirely by the absence of breastfeeding among HEU infants. \(86, 96, 102\) There were no reports on childhood diarrhoeal risk among breastfed HEU children born to women receiving universal ART (Table 1, Appendix 9).

Pneumonia

There were 7 reports on pneumonia risk overall, \(5, 95-97, 102, 105\) with 3 reports from settings where women had no access to antiretroviral drugs, \(5, 95, 105\) and four from populations using predominantly 1-2 antiretroviral drugs for PMTCT with a limited number of women with advanced HIV disease receiving ART. \(96, 97, 102, 112\) there were no reports on populations with universal maternal use of ART (Table 1). Varying case definitions were used and the majority of estimates were unadjusted for potential confounders. Four reports were judged as having a low/moderate risk of bias. \(5, 96, 102, 105\) Only 1 study reported the use of co-trimoxazole preventive therapy among HEU infants. \(96\) Generally the reports yielded low precision estimates indicating a somewhat increased risk of pneumonia among HEU children (Appendix 9). \(5, 96, 102, 105\)

The effect of HIV exposure on childhood pneumonia was influenced by maternal use of ART and breastfeeding. In the Zimbabwean vitamin A trial, where predominantly breastfeeding women had no access to ART, the incidence of pneumonia-related hospitalization was almost three-fold.
higher (IRR 2.7, 95% CI 1.6 -4.7) among HEU than HU infants. (5) By comparison, in a Ugandan study where some HIV-infected women used ART, breastfed HEU and breastfed HU children experienced a similar risk of pneumonia (IRR 1.34, 95% CI 0.44-4.07), whereas non-breastfed HEU children had a 4-fold higher incidence than their breastfed HU counterparts (IRR 3.84, 95% CI 2.06-7.17) (Appendix 9).

Growth

Overall, 15 studies reported weight estimates adjusted for age. (32, 33, 81, 82, 90, 91, 95-98, 101-104, 111, 112) (109) Of the 7 reports providing weight-for-age Z-score (WAZ) estimates with low/moderate risk of bias,(81, 82, 90, 91, 98, 101, 111) 3 were from populations without access to antiretroviral drugs, (81, 91, 111) 4 from populations using predominantly 1-2 antiretroviral drugs for PMTCT(33, 82, 90, 98), and one from a study providing universal ART. (101) Two studies reported weight-velocity-for-age Z-scores (WVZ), one from a population using a single antiretroviral drug for PMTCT (32) and one from a population with universal maternal ART use(101) (Table 1, Appendix 10). Most point estimates were small with low precision and unclear clinical significance; HEU children tended to have similar long term weight gain compared to HU children after adjusting for low birth weight, feeding practices and socio-economic factors.(32, 90, 91) The largest study reported Ugandan HEU infants to have somewhat lower mean WAZ at 12 months than HU infants from the same setting (adjusted difference in mean WAZ -0.13, 95% CI -0.35 to 0.00). The estimate was not adjusted for maternal HIV disease stage or infant feeding; although the majority of children received some breastmilk, no information was provided regarding exclusivity or subsequent complementary feeding practices.(98) By contrast, a randomized trial investigating the effect of a fortified diet among 18-month old Zambian children reported significantly lower WAZ scores among the HEU children, even after adjusting for infant feeding and socio-economic markers (adjusted difference in mean WAZ, -0.14; 95% CI -0.37 to -0.09). HEU children had been significantly less likely to receive breastfeeding in early infancy than HU children. (81) However, in a later report on those children still in follow-up at school-going age, the observed differences between HEU and HU children were negated after adjusting for socio-economic confounders. (82)

Overall, there were 13 reports of linear growth. Of the 8 reports at low/moderate risk of bias, 3 were from populations without access to antiretroviral drugs,(81, 91, 111) and 4 from populations using predominantly 1-2 antiretroviral drugs for PMTCT.(32, 82, 90, 98) While most estimates were based on length- or height-for-age Z-scores (LAZ or HAZ), two studies reported length-velocity-for-age Z-scores (LVZ).(32, 101). Overall, sample sizes were small, with low precision estimates. Adjusted estimates of differences in mean LAZ/HAZ demonstrated no significant differences between HEU and HU children; (32, 81, 82, 98) maternal HIV disease severity predicted poor linear growth. (32,
Similarly, the risk of stunting (defined as % children with LAZ/HAZ-scores < -2SD from the expected mean) did not differ between HEU and HU children. A single report from a breastfeeding population with universal maternal ART also suggested no difference in risk of stunting between HEU and HU children at 17-18 months (RR 1.00, 95% CI 0.51-1.95), although there was some indication of effect modification by gender, with HEU girls demonstrating a slightly lower HVZ than HU girls of the same age (-0.82, 95% CI -1.52 to -0.12).

**Early childhood development**

Eight studies contributed 9 reports of early childhood development. Two studies utilized un-validated or unspecified measures of neurodevelopment, and 2 other studies were considered at substantial risk of selection bias. The remaining 4 studies contributed 5 reports (Appendix 11), 3 of which focused on children under 3 years of age. Different developmental measurement tools were used in each, limiting meaningful between-study comparisons. Two reports (from a single study at different time points) were for children born to mothers without access to antiretroviral drugs; and 2 reports were for children born to mothers who received 1-2 antiretroviral drugs for PMTCT and a single report was for preschool children born to women receiving universal ART (Table 1). No study reported significant differences in developmental outcomes between preschool HEU and HU children. A single report on 6-12 year old HEU and HU Congolese children found no significant differences in intelligence and scholastic achievement and skills as measured by standardised tests; the children had been mostly breastfed, and no antiretroviral drugs had been used by their mothers. By contrast, HEU Zambian school-age children had significantly lower maths grades than their HU counterparts; differences remained after adjusting for several potential confounders including socio-economic measures and parental education. The HIV-infected mothers had largely received sdNVP as prophylaxis, with some women accessing ART for advanced HIV disease; HEU children were breastfed for shorter durations than HU children. Of note, no reports stratified results by either early infant feeding modality or maternal health measures.

**DISCUSSION**

This review highlights a highly heterogeneous literature examining the impact of maternal HIV status on major child health outcomes among HEU children in SSA, with marked diversity across studies in maternal ART use, infant feeding and treatment of major confounding variables. Despite these limitations some general themes emerge. Reports from populations with limited or no access to maternal ART generally indicated increased risk of mortality and some morbidity among HEU compared to...
HU children. However, the observed associations appear to be strongly influenced by maternal HIV disease severity, ART use and infant feeding, with worse outcomes associated with advanced maternal disease, limited ART use, and restricted or no breastfeeding. We found no reports comparing mortality, diarrhoea, pneumonia or hospitalization between HU and breastfed HEU children born to women receiving universal ART, and very sparse data on child growth and neurodevelopment under these conditions. In this light, the available evidence regarding the effect of maternal HIV status on child health may have very limited application to the approaches of universal maternal ART and breastfeeding that are promoted across much of sub-Saharan Africa today.

Interpretation of the evidence on infectious morbidity (diarrhoea and pneumonia) was limited by varying case definitions and a reliance on maternal recall. Still there appeared to be a trend towards higher risk of pneumonia among HEU children, with some evidence of amelioration of risk by breastfeeding.(96, 102) Diarrhoeal risk among HEU and HU appeared similar when infant feeding practices were accounted for, with highest risk occurring among non-breastfed children and those with premature introduction to solid foods; these findings are wholly in keeping with evidence from HU populations.(96, 102, 107) In addition, across studies undernutrition and stunting were prevalent in both HEU and HU populations, with growth most strongly influenced by early infant feeding(32, 33, 81, 90) and maternal health.(32, 33) Among HEU children, optimal growth was observed in breastfed children whose mothers were at an earlier stage of HIV disease.

There were few data on associations between maternal HIV status and early childhood development, and notable methodological limitations. Although there was a reassuring lack of substantial differences between HEU and HU children with regards to early neurodevelopmental outcomes in preschool populations, there was some indication of lower educational achievement among older HEU children born to women with limited access to ART.(82) The diverse tools used to assess neurodevelopment make cross-study comparisons challenging, however.

Overall, insights from the currently available literature are sharply limited around two factors that are likely to modify the putative associations between maternal HIV infection and child health outcomes. First, advanced maternal HIV disease during pregnancy and postpartum has been consistently associated with a range of adverse child health outcomes. Improving maternal health through ART use has the potential to mitigate many – but perhaps not all - of these effects. Second, poor infant nutrition, and limited breastfeeding in particular, is a well understood cause of morbidity and mortality across resource-limited settings.(35-37) The most commonly promoted intervention to address this, exclusive breastfeeding with adequate early complementary feeding strategies (including continuation of breastfeeding rather than rapid weaning), has been shown to improve each of the child health outcomes considered here regardless of HIV exposure.(52, 117-119)
Thus, the existing literature provides little insight into contexts where universal maternal ART is being implemented with high levels of breastfeeding, per current policies and programmes in sub-Saharan Africa.\(^{(49)}\) In fact, while studies providing direct comparisons are few, the currently available literature suggests that major child health outcomes in optimally-breastfed children born to mothers using ART may be the same as those of HU children from similar settings.

**Methodological concerns**

We included only longitudinal studies where HEU and HU children were sampled independent of child health status (other than HIV exposure) and were from the same communities. This approach was used to help reduce the potential for selection biases and/or confounding that may influence our assessment of the impact of maternal HIV status on child health. First, although case-based sampling approaches can play an important role in describing HEU child health status and understanding disease features, they are prone to selection bias. While there exists a larger body of evidence employing case-control methods (where children are sampled based on both their HIV exposure and a potentially-related child health outcome) the potential for differential sampling of cases is profound. In addition, we limited studies to those including an HU comparator group drawn from the same community as the HEU group. While international standards for measuring a variety of child health outcomes are widely used (eg., WHO tables for anthropometry), local ‘normal’ child health standards may vary substantially across communities due to socio-economic and environmental parameters. In turn, any attempt to isolate the association between maternal HIV status and subsequent child health must clearly include comparators selected from the local populations that gave rise to the HEU children.\(^{(71, 72)}\)

While use of longitudinal study design and local comparators may help to address some forms of bias, the putative associations between maternal HIV exposure and child health is still likely to be confounded by the adverse economic and psychosocial circumstances associated with maternal HIV across many parts of sub-Saharan Africa. Of the 25 reports considered to be at moderate risk of bias, only 11 presented estimates adjusted for confounding; moreover, only two of the studies in this review were considered to be well conducted with complete reporting, contributing four of the 37 reports – approximately 10% of the evidence base on HEU child health – highlighting a substantial shortcoming in the existing literature on this issue.
**Limitations**

This review is subject to several limitations. First, generalizability of the available data may be limited. Only 1 of the included studies was designed specifically to evaluate the outcomes of HEU compared to HU children; (104) the majority of studies included in this review contributed secondary data analyses, often based on cohorts of participants drawn from intervention trials. (5, 33, 81-86, 90, 94-96, 98, 99, 101, 102) Trial participants are not necessarily representative of the general population due to specific eligibility criteria, and may receive improved medical care through trial participation. (120) For example the estimated mortality risk among HU children enrolled in the Zimbabwean Vitamin A trial was substantially lower than the expected for the general population during the time of the study. (121) This may colour the relative increase in mortality risk observed in comparing HEU children to HU counterparts in this analysis. (84) We excluded evidence on HEU children from outside SSA as the nature of the HIV epidemics, and the distribution of major child health outcomes, varies dramatically across continents. (1) Yet reports from Europe, Asia, South America and the United States have reported findings similar to those included in this review. (65, 122-126) Second, while we attempted to minimize the risk of publication bias by including conference abstracts and searching several databases, the possibility of studies meeting our eligibility criteria appearing outside these sources remains unclear. In addition, we cannot account for changes in the sensitivity and specificity of laboratory methods to exclude infant HIV infection over the past 25 years; some misclassification of HIV-infected children as being HEU may have biased earlier reports, although it is unclear whether such misclassification could substantively alter the findings of studies.

**Implications for research and policy**

It is clear that the combination of universal, early maternal ART with optimal breastfeeding has the potential to substantially improve child health across SSA, and in turn, there is an urgent need for additional research on HEU versus HU child outcomes in this context. Critical questions that remain unanswered include (a) whether increased mortality and/or morbidity exists even among optimally breastfed HEU children born to mothers receiving ART; (b) if yes, what additional factors exist that may be amenable to intervention; and (c) which operational interventions are most effective at optimizing breastfeeding practices and maximizing ART use among HIV-infected women. Meeting these needs will require robust data from methodologically sound, prospective studies from a variety of settings, with the inclusion of otherwise similar HU comparison groups.
CONCLUSION

In summary, HEU children comprise a large and growing proportion of the African continent’s population yet there are relatively few rigorous studies investigating their health outcomes as compared to those of HU children. Limited evidence suggests an increased risk of mortality and infectious morbidity among HEU children born to mothers without access to ART, with the highest risks occurring in the absence of breastfeeding. Insufficient comparative data exist on HEU health outcomes under the practices of universal maternal ART with extended breastfeeding, with some indication that HEU child health outcomes may be comparable to those of HU children under these conditions. Providing HIV-infected mothers with ART and supporting them to optimally feed their infants has the potential to substantially improve the health of HEU children across SSA. At the same time, it is critical to keep in mind that in many parts of SSA high rates of infant mortality and morbidity are the norm among HU populations and much work remains to be done to improve health outcomes for all children on the continent.

ACKNOWLEDGEMENTS

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REFERENCES


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26. WHO. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. 2015.


28. Spaulding A, Rutherford GW, Siegfried N. Tenofovir or zidovudine in three-drug combination therapy with one nucleoside reverse transcriptase inhibitor and one non-nucleoside reverse


49. The Interagency Task Team on the Prevention and Treatment of HIV Infection in Pregnant Women, Mothers and Children (IATT). Option B+ countries and PMTCT regimen 2015 [16


74. Ioannidis JP. Interpretation of tests of heterogeneity and bias in meta-analysis. Journal of


86. Rollins NC, Ndirangu J, Bland RM, Coutsoudis A, Coovadia HM, Newell ML. Exclusive breastfeeding, diarrhoeal morbidity and all-cause mortality in infants of HIV-infected and HIV


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FIGURE 1. Flowchart of study selection process
Table 1. Overview of included studies by reported outcomes, maternal antiretroviral use and methodological quality

<table>
<thead>
<tr>
<th>MATERIAL USE OF ANTIRETROVIRALS IN HIV-INFECTED STUDY POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL REPORTS (n=37)</td>
</tr>
<tr>
<td>REPORTED OUTCOMES (Total number of reports)</td>
</tr>
<tr>
<td>Mortality (10)</td>
</tr>
<tr>
<td>Hospital admissions/health care visits (10)</td>
</tr>
<tr>
<td>Diarrhea (10)</td>
</tr>
<tr>
<td>Pneumonia (7)</td>
</tr>
<tr>
<td>Growth (16)</td>
</tr>
<tr>
<td>RCD (9)</td>
</tr>
</tbody>
</table>

Abbreviations: PMTCT: prevention of mother to child transmission of HIV; ART: antiretroviral therapy; ECD: early childhood development.

Table 2. Effect of maternal HIV status on mortality among HIV-uninfected children in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Study</th>
<th>Original study design &amp; primary aim</th>
<th>Predominant infant feeding method among HUE</th>
<th>Outcome, age, sample size</th>
<th>Incidence among HUE children</th>
<th>Incidence among HUE children</th>
<th>Crude association (95% CI)</th>
<th>Adjusted association (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tha et al. (1999)</td>
<td>Prospective observational cohort: incidence, clinical characteristics &amp; mortality of acute, recurrent and persistent diarrhea among HIV-children</td>
<td>At least some breastfeeding, with early mixed feeding</td>
<td>Mortality, 0-24 months (n=330)</td>
<td>1.4% (2/139)</td>
<td>1.9% (15/191)</td>
<td>RR = 0.78 (0.04-0.99)</td>
<td>-</td>
</tr>
<tr>
<td>Drotar (2007)</td>
<td>Prospective observational cohort: neonate developmental outcomes of HIV-children</td>
<td>Not stated, likely predominately breastfed</td>
<td>Mortality, 0-24 months (n=317)</td>
<td>5.8% (14/241)</td>
<td>4.9% (11/115)</td>
<td>RR = 0.74 (0.50-0.93)</td>
<td>1.95 (0.95-3.83)</td>
</tr>
<tr>
<td>Tallah (1999)</td>
<td>Prospective interventional cohort: PMTCT and birth canal cleaning</td>
<td>Not stated, likely predominately breastfed</td>
<td>Mortality rates, 12-36 months of age (n=618)</td>
<td>RR = 0.74 (0.1-1.7)</td>
<td>RR = 1.28 (0.99-3.22)</td>
<td>RR = 1.20 (0.99-3.25)</td>
<td>1.67 (1.0-3.3)</td>
</tr>
<tr>
<td>Spira (1999)</td>
<td>Prospective observational cohort: natural history of maternal and child HIV, MTCT, predictors and impact on health services</td>
<td>Predominantly breastfed</td>
<td>Under-5 mortality (n=347)</td>
<td>Not stated</td>
<td>Not stated</td>
<td>RR = 0.6 (1.1-1.6)</td>
<td>-</td>
</tr>
<tr>
<td>Ora et al. (2000) &amp; Schin van der Beek (2003)</td>
<td>Prospective observational cohort of HIV-1 and HIV-2 infected women: MTCT, disease progression and child survival</td>
<td>Not stated</td>
<td>Mortality, 0-18 months (n=512)</td>
<td>11.7% (62/528)</td>
<td>6% (27/449)</td>
<td>RR = 0.8 (0.0-0.1)</td>
<td>1.84 (0.02-0.37)</td>
</tr>
<tr>
<td>Marquido (2007)</td>
<td>Randomized controlled trial of maternal and neonatal vitamin A: impact on MTCT and health outcomes</td>
<td>Prolonged breastfeeding, limited EEF</td>
<td>Time points: 6th, 7th, 8th, 9th</td>
<td>Time points: 10th, 11th, 12th, 13th</td>
<td>Time points: 12th, 13th</td>
<td>1.9 (1.0-3.5)</td>
<td>2.0 (1.0-3.5)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Study</th>
<th>Original study design &amp; primary aim</th>
<th>Predominant infant feeding method among HEU</th>
<th>Outcome, age, sample size</th>
<th>Incidence among HEU children</th>
<th>Incidence among HIV children</th>
<th>Crude association (95% CI)</th>
<th>HEU vs HIV</th>
<th>Adjusted association (95% CI)</th>
<th>HEU vs HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicide</td>
<td>Prospective observational cohort: immunogenicity of measles vaccine by HIV status</td>
<td>Not stated</td>
<td>Mortality, 3-16 months (n=187)</td>
<td>5% (13/266)</td>
<td>1.6% (2/127)</td>
<td>HR = 3.2 (0.7-14.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chilenguasi</td>
<td>Randomized controlled trial of intermittent preventive treatment for malaria</td>
<td>Not stated</td>
<td>Mortality at 12 months (n=190)</td>
<td>7.2% (13/159)</td>
<td>4.8% (7/145)</td>
<td>RR = 1.48 (0.89-2.47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheppa</td>
<td>Prospective observational cohort: evaluation of South African PMTCT program</td>
<td>Predominantly mixed or exclusive formula feeding (mostly inappropriate)</td>
<td>Mortality, 6-24 months (n=680)</td>
<td>3.7% (16/452)</td>
<td>4.1% (8/198)</td>
<td>RR = 0.94 (0.41-2.17)</td>
<td>alpha = 0.7</td>
<td>(0.3-1.5)</td>
<td></td>
</tr>
<tr>
<td>Shapero</td>
<td>Randomized controlled trial evaluating ARV-based PMTCT strategies</td>
<td>Breastfeeding (n=604)</td>
<td>Mortality at 6 and at 24 months (n=672)</td>
<td>3.6% (23)</td>
<td>6.7% (20)</td>
<td>RR = 0.57 (0.35-0.93)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rollins</td>
<td>Non-randomized intervention cohort: the effect of infant feeding on HIV transmission and child survival</td>
<td>Exclusive breastfeeding for first 6 months followed by weaning</td>
<td>Mortality, 0-12 months (n=209)</td>
<td>2.6% (5/203)</td>
<td>2.6% (2/78)</td>
<td>RR = 1.39 (0.91-2.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marques</td>
<td>Randomized controlled trial of drug regimens for malaria prevention</td>
<td>Breastfeeding</td>
<td>Mortality, 6-24 months (n=973)</td>
<td>3.76% (7/186)</td>
<td>0.29% (1/348)</td>
<td>RR = 13.10 (1.62-107.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PMTCT** (prophylaxis maternal to infant transmission of HIV-1): maternal prophylaxis initiated as a part of PMTCT irrespective of disease staging

No reports.

1 Measures of association calculated based on data presented in manuscript.

PMTCT: prevention of mother-to-child transmission of HIV-1; HEU, human immunodeficiency virus-exposed uninfected; HIV, HIV-uninfected; HIV+, HIV-infected; CI, confidence interval; RR, risk ratio; IR, incidence rate (IR); incidence rate ratio; HR, hazard ratio; aHR, adjusted hazard ratio; py, person-years.
Figure 3. Flowchart of study selection process

MEDLINE database search
n=996

EMBASE database search
n=626

EBSCOHOST database search
n=2472

Reference searches
n=4

Abstracts and Titles screened after duplicates removed, n=1349

Records excluded
n=1248

Full-text articles screened
n=101

Full-text articles excluded, n=64
Reasons for ineligibility:
- HIV infection not definitively excluded among HEU infants < 18 months of age, n=20
- Results not disaggregated by both maternal and infant HIV status, n=18
- No comparative data on HIV-unexposed infants, n=4
- Study design, n=7
- Maternal status unknown, or only HIV-negative women included, n=2
- No clinical outcomes of interest, n=8
- Sampling based on maternal morbidity other than HIV infection, n=1
- Conference abstract or interim report subsequently fully published, n=3
- Setting outside of sub-Saharan Africa, n=1

Articles included in review
n=37
(29 studies)

Articles with serious/critical risk of bias
n=11

Articles with low/moderate risk of bias
n=26
Figure 2. Forest plot of mortality among HIV-exposed uninfected children compared to HIV-unexposed children, by maternal antiretroviral use

<table>
<thead>
<tr>
<th>Study (publication year) by maternal use of antiretroviral therapy:</th>
<th>Events, Events, %</th>
<th>Odds Ratio (CI)</th>
<th>Treatment</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No maternal use of antiretrovirals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trees (1989)</td>
<td>0.16 (0.04, 0.78)</td>
<td>2130</td>
<td>15191</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Drake (1987)</td>
<td>0.74 (0.06, 13.03)</td>
<td>14341</td>
<td>15116</td>
<td>4.32</td>
<td></td>
</tr>
<tr>
<td>Tara (1989)</td>
<td>1.30 (0.23, 7.4)</td>
<td>30456</td>
<td>31065</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Otis (2003)</td>
<td>1.94 (1.90, 3.77)</td>
<td>9011</td>
<td>27869</td>
<td>11.69</td>
<td></td>
</tr>
<tr>
<td>Mwangi (2007)</td>
<td>0.11 (0.02, 0.53)</td>
<td>1036</td>
<td>94865</td>
<td>17.19</td>
<td></td>
</tr>
<tr>
<td>Tariyokh (2008)</td>
<td>3.91 (1.17, 12.01)</td>
<td>19335</td>
<td>31177</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Chinagong (2008)</td>
<td>6.09 (0.6, 64.7)</td>
<td>16287</td>
<td>16051</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Spira (2009)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Subtotal (1st 11 studies)</td>
<td>1.76 (1.03, 3.01)</td>
<td>396532</td>
<td>2749305</td>
<td>78.51</td>
<td></td>
</tr>
</tbody>
</table>

**Maternal use of 1-2 antiretrovirals for PMTCT**

<table>
<thead>
<tr>
<th>Study (publication year)</th>
<th>Events, Events, %</th>
<th>Odds Ratio (CI)</th>
<th>Treatment</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiave (2010)</td>
<td>0.94 (0.41, 2.17)</td>
<td>16442</td>
<td>98718</td>
<td>19.59</td>
<td></td>
</tr>
<tr>
<td>Rosado (2013)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Subtotal (1st 2 studies)</td>
<td>0.94 (0.41, 2.17)</td>
<td>16442</td>
<td>98718</td>
<td>19.59</td>
<td></td>
</tr>
</tbody>
</table>

**Maternal use of 1-2 antiretrovirals for PMTCT with triple antiretroviral therapy for those with advanced HIV disease stages**

<table>
<thead>
<tr>
<th>Study (publication year)</th>
<th>Events, Events, %</th>
<th>Odds Ratio (CI)</th>
<th>Treatment</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigu (2007)</td>
<td>4.02 (1.19, 13.94)</td>
<td>35564</td>
<td>21257</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Mwangi (2007)</td>
<td>10.15 (1.62, 65.54)</td>
<td>7160</td>
<td>5946</td>
<td>4.11</td>
<td></td>
</tr>
<tr>
<td>Subtotal (1st 2 studies)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

**Overall**

<table>
<thead>
<tr>
<th>Events, Events, %</th>
<th>Odds Ratio (CI)</th>
<th>Treatment</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.95 (1.17, 3.23)</td>
<td>4697114</td>
<td>28571862</td>
<td>190.00</td>
<td></td>
</tr>
</tbody>
</table>

*Results for studies excluded due to missing raw data:
Spira (2009), Mwangi (2007), Shigu (2007), Mwangi (2007)*