# Effect of co-trimoxazole on mortality in HIV-exposed but uninfected children in Botswana (the Mpepu Study): a double-blind, randomised, placebo-controlled trial

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## Summary

**Background** Co-trimoxazole prophylaxis reduces mortality among HIV-infected children, but efficacy in HIV-exposed but uninfected (HEU) children in a non-malarial, low-breastfeeding setting with a low risk of mother-to-child transmission of HIV is unclear.

**Methods** HEU children in Botswana were randomly assigned to receive co-trimoxazole (100 mg/20 mg once daily until age 6 months and 200 mg/40 mg once daily thereafter) or placebo from age 14–34 days to age 15 months. Mothers chose whether to breastfeed or formula feed their children. Breastfed children were randomly assigned to breastfeeding for 6 months (Botswana guidelines) or 12 months (WHO guidelines). The primary outcome, analysed by a modified intention-to-treat approach, was cumulative child mortality from treatment assignment to age 18 months. We also assessed HIV-free survival by duration of breastfeeding. This trial is registered with ClinicalTrials.gov, number NCT01229761.

**Findings** From June 7, 2011, to April 2, 2015, 2848 HEU children were randomly assigned to receive co-trimoxazole (n=1423) or placebo (n=1425). The data and safety monitoring board stopped the study early because of a low likelihood of benefit with co-trimoxazole. Only 153 (5%) children were lost to follow-up (76 in the co-trimoxazole group and 77 in the placebo group), and 2053 (72%) received treatment continuously to age 15 months, death, or study closure. Mortality after the start of study treatment was similar in the two study groups: 30 children died in the co-trimoxazole group, compared with 34 in the placebo group (estimated mortality at 18 months 2·4% *vs* 2·6%; difference –0·2%, 95% CI –1·5 to 1·0, p=0·70). We saw no difference in hospital admissions between groups (12·5% in the co-trimoxazole group *vs* 17·4% in the placebo group, p=0·19) or grade 3–4 clinical adverse events (16·5% *vs* 18·4%, p=0·18). Grade 3–4 anaemia did not differ between groups (8·1% *vs* 8·3%, p=0·93), but grade 3–4 neutropenia was more frequent in the co-trimoxazole group than in the placebo group (8·1% *vs* 5·8%, p=0·03). More co-trimoxazole resistance in commensal *Escherichia coli* isolated from stool samples was seen in children aged 3 or 6 months in the co-trimoxazole group than in the placebo group (p=0·001 and p=0·01, respectively). 572 (20%) children were breastfed. HIV infection and mortality did not differ significantly by duration of breastfeeding (3·9% for 6 months *vs* 1·9% for 12 months, p=0·21).

**Interpretation** Prophylactic co-trimoxazole seems to offer no survival benefit among HEU children in non-malarial, low-breastfeeding areas with a low risk of mother-to-child transmission of HIV.

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## Introduction

Co-trimoxazole prophylaxis reduces mortality among HIV-infected children by preventing opportunistic infections, diarrhoeal and respiratory illnesses, and malaria, and use is recommended up to at least age 5 years.[1–5] WHO recommends that all HIV-exposed children receive co-trimoxazole until HIV infection has been ruled out and HIV exposure from breastmilk has ended.[6,7] With improvements in availability of antiretroviral treatment (ART), however, mother-to-child HIV transmission has decreased, and in some areas the vast majority of HIV-exposed children now remain uninfected. The risks and benefits of co-trimoxazole prophylaxis in these children are unknown. A small randomised trial involving HIV-exposed but uninfected (HEU) infants in Uganda showed lower incidence of malaria among children who continued prophylactic co-trimoxazole to age 4 years than among those who stopped after 2 years of treatment.[8] Reductions in the incidence of malaria associated with co-trimoxazole prophylaxis have been reported in several other studies.[8–13] Observational data are conflicting for non-malarial health benefits of co-trimoxazole in HEU children, with some studies showing no association between co-trimoxazole and common childhood illnesses, such as lower respiratory tract infections or diarrhoea,[14] and other studies suggesting a

## Research in context

### Evidence before this study

Co-trimoxazole prophylaxis reduces mortality among HIV-infected children, and is recommended by WHO for all children born to HIV-infected mothers until the child is confirmed not to be infected with HIV and the period of HIV infection risk has ended. With declines in mother-to-child transmission of HIV due to improved antiretroviral availability, most children born to HIV-infected mothers now remain uninfected. However, the risk of mortality is higher among HIV-exposed but uninfected (HEU) children than in HIV-unexposed children, and long-term co-trimoxazole prophylaxis in children has been considered as a potential intervention to mitigate this excess risk. The effects of co-trimoxazole prophylaxis on health outcomes of HEU children in non-malarial areas are unknown.

### Added value of this study

We did a randomised trial to assess the use of prophylactic co-trimoxazole among HIV-exposed uninfected children in a non-malarial setting. We randomly assigned 2848 babies free from HIV infection born to HIV-infected women to receive co-trimoxazole or placebo from age 14–34 days up to age 15 months. Only 20% of children were breastfed. Child mortality occurring between randomisation and age 18 months did not differ between the co-trimoxazole and placebo groups.

### Implications of all the available evidence

Previous evidence showed that co-trimoxazole prophylaxis reduces morbidity and mortality in HIV-infected children, and lowered the incidence of malaria among HEU children residing in malarial settings. However, our study, which was done in a non-malarial region of Botswana with a well established programme for prevention of mother-to-child transmission, showed no clinical benefit in HEU children of HIV-infected mothers. These findings collectively suggest that in non-malarial settings with low HIV transmission rates and relatively low child mortality, prophylaxis with co-trimoxazole might not improve child health outcomes.

protective effect of co-trimoxazole.[15] Although mortality among HEU children is higher than that among children not exposed to HIV,[16–19] whether they would benefit from prophylactic co-trimoxazole needs to be assessed in randomised clinical trials in non-malarial settings.

We did a double-blinded, randomised, placebo-controlled trial to investigate whether co-trimoxazole would offer a survival benefit for HEU children in a non-malarial region of Botswana. This cohort also offered an opportunity to assess a potential relation between breastfeeding duration and child mortality.

## Methods

### Study design and participants

In this trial, known as the Mpepu Study (Mpepu means "to carry a baby on your back" in Setswana), we enrolled HIV-infected women and their children in southern Botswana, which is an area without malaria transmission. Participants were identified at either public antenatal clinics or maternity wards in Gaborone (a city), Molepolole (a village), and Lobatse (a town, included up to August, 2012). Women who were in at least week 26 of pregnancy through to 34 days post partum were eligible. Other maternal inclusion criteria were documented HIV-1-positive status, age 18 years or older, ability to provide written informed consent and attend the study clinic for follow-up, and Botswana citizenship. Exclusion criteria for randomisation of children included a previous positive HIV result on a qualitative DNA PCR test, any medical condition making survival to 18 months unlikely, clinical jaundice, known allergy to sulphonamide drugs or other contraindication to starting co-trimoxazole, and grade 4 asymptomatic or grade 3 symptomatic anaemia or neutropenia. Children with negative HIV DNA PCR results at birth who had positive results in blood samples taken at the time of randomisation were excluded from analyses.

Throughout the study period, Botswana feeding guidelines supported the provision of free formula but allowed for breastfeeding by mothers receiving ART. These guidelines differ from most other countries in Africa, which support breastfeeding in the setting of maternal ART or infant prophylaxis.[20] Women who had not chosen a feeding method before enrolment in the study were provided with education on the risks and benefits of formula versus breastfeeding by study staff, from government-approved counselling information. Study staff also provided further information on the method selected (eg, safe formula preparation or exclusive breastfeeding followed by weaning over a 4-week period). Free maternal three-drug ART was available to all participating mothers during pregnancy and breastfeeding through the Botswana Government's health system. All neonates received antiretroviral prophylaxis in the first 4 weeks of life, regardless of feeding method. If a breastfeeding mother was not taking ART for any reason, prophylactic nevirapine was provided to her child, dosed per WHO guidelines.[21] From May, 2011 (maternal enrolment), to January, 2013, we followed the Botswana guidelines for prevention of mother-to-child transmission of HIV, which recommended single-dose nevirapine plus 4 weeks of zidovudine for antiretroviral prophylaxis in neonates. After January, 2013, we amended the study protocol to allow earlier use of co-trimoxazole, and, therefore, neonatal prophylaxis was revised to 4 weeks of nevirapine alone to avoid potential haematological toxic effects associated with concurrent use of zidovudine and co-trimoxazole. Most children received zidovudine for a brief period even after January, 2013.

The Botswana Government Health Research Development Committee and the Office for Human Research Administration, Harvard T H Chan School of Public Health, Boston, MA, USA, approved the study protocol and amendments. An independent data and safety monitoring board (DSMB) reviewed the safety and efficacy data approximately every 6 months and assessed futility. Participants signed written consent forms approved by the ethics review boards.

### Randomisation and masking

The study data manager in Botswana electronically generated random allocation sequences. HEU children were randomly assigned 1:1 to receive either 100 mg/20 mg co-trimoxazole once daily until age 6 months and 200 mg/40 mg co-trimoxazole once daily thereafter (standard WHO dosing at the time[22]) or placebo. Children were randomly assigned treatment in blocks of eight, stratified by study site. Before Jan 21, 2013, children were randomly assigned treatment when they were aged 28–34 days. From this date onwards, in an effort to address early mortality, we extended the age full-term babies weighing more than 2500 g could be allocated treatment to 14–34 days; age of allocation remained as 28–34 days in babies born before 37 weeks' gestation or who weighed less than 2500 g at a visit between age 14 and 27 days. Children of breastfeeding mothers who gave consent were also randomly assigned to breastfeeding for 6 months (the recommended duration in Botswana) or 12 months (the duration recommended by WHO during the study period). We allocated these children by factorial randomisation to co-trimoxazole versus placebo and to 6 versus 12 months of breastfeeding (ie, into one of four possible groups).

Apart from the data manager, all study staff, investigators, and participants were unaware of treatment allocation. To mask treatment, co-trimoxazole and placebo were dispensed as syrup with similar colour, density, and taste, in brown opaque plastic bottles.

### Procedures

Before treatment allocation, we did full blood and CD4 cell counts in enrolled women. HIV-1 RNA was quantified only in women enrolled up to Nov 6, 2012, due to cost constraints, with the automated COBAS Amplicor/AmpliPrep HIV-1 Monitor Test (version 1.5, Roche Molecular Systems, Branchburg, NJ, USA). Children were clinically assessed at birth, randomisation, ages 2 months and 3 months, and every 3 months thereafter until age 18 months. Peripheral blood was taken from all babies at the birth and random treatment assignment visits, and at ages 3, 6, 9, 12, and 15 months from breastfeeding children for HIV testing by qualitative PCR DNA assay with Amplicor HIV-1 testing (Roche Diagnostic Systems, Branchburg, NJ, USA). Full blood counts for children were done at the time of treatment assignment and ages 3, 6, and 15 months, and HIV ELISA was done at age 18 months. Children who had positive HIV tests at or after random treatment assignment were given active co-trimoxazole and ART provided by the Botswana Government, and were retained in the study follow-up and primary analysis.

### Definitions

Maternal HIV infection status was established from documentation in health records of a positive HIV test or by in-study testing with ELISA, dual rapid enzyme immunoassay, or detectable HIV-1 RNA assay. HIV-infected children were defined as having positive HIV DNA PCR results on two occasions (or one occasion followed by death before repeat testing). Preterm was defined as birth earlier than 37 weeks' gestation, and low birthweight as less than 2500 g. Adherence to study drug was measured as the number of drug refills dispensed at study visits and by maternal or caregiver report at each clinic visit. For children who went off study treatment early due to non-attendance of study visits, we used the earlier of reported discontinuation or the first of consecutive missed visits, including the month 12 visit, as the imputed date of treatment discontinuation. Clinical adverse events and laboratory results were graded with the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0.[23]

### Outcomes

The primary outcome was cumulative mortality up to age 18 months in children without HIV infection at treatment assignment. We had originally planned a second primary outcome of child HIV-free survival after 6 months versus 12 months of breastfeeding, which had been based on the assumption that approximately 80% of infants would be breastfed. Owing to the much lower proportion (20%) of children being breastfed, however, we amended the protocol in January, 2013, and recategorised this outcome to be a secondary outcome. As a secondary composite clinical endpoint we assessed the occurrence of death, admission to hospital, or a grade 3 or 4 clinical adverse event. Other secondary clinical endpoints, analysed separately, were the cumulative incidence of admission to hospital, any grade 3–4 adverse events or grade 2 rash, grade 3–4 anaemia, grade 3–4 neutropenia, and a composite of HIV infection or death at 18 months.

### Co-trimoxazole resistance testing

From May, 2014, stool samples were collected from children, when possible, at the time of treatment assignment or at age 3 or 6 months. Samples were stored at –70°C before culture on MacConkey agar. Antibiotic susceptibility testing was done with the Kirby Bauer method on Mueller Hinton agar for 25 µg co-trimoxazole in samples that grew *Escherichia coli*. We used Clinical and Laboratory Standards Institute performance standards for disc diffusion to classify resistant isolates.[24]

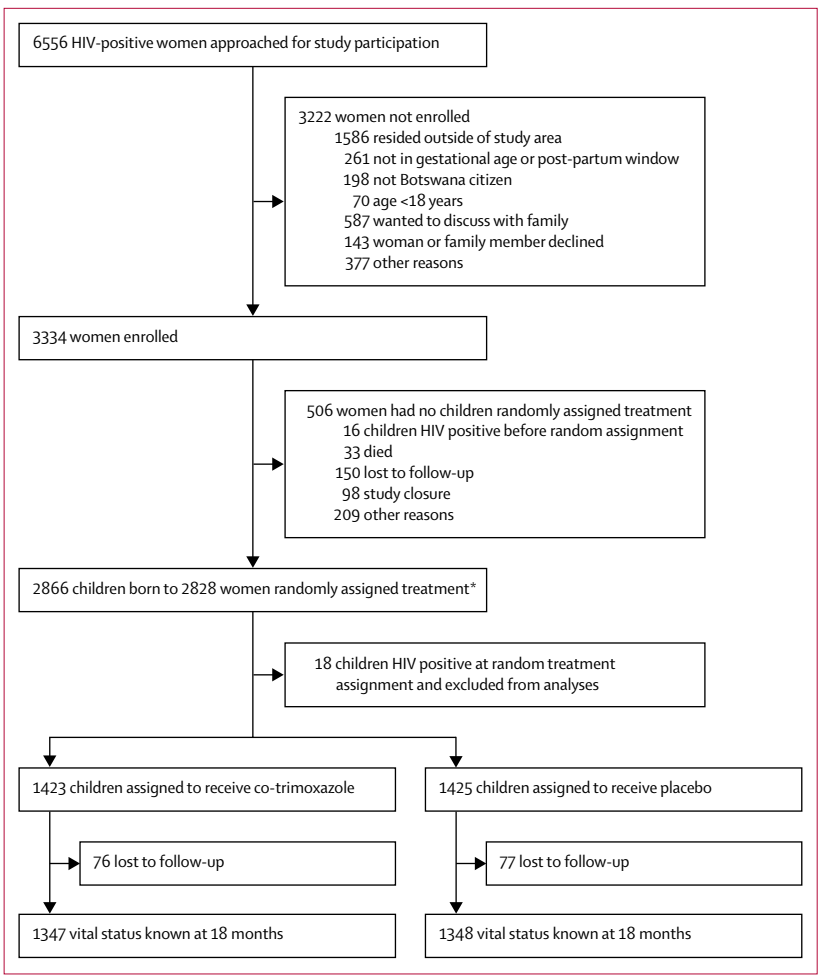


***Figure 1*: Trial profile**
*36 women had twins and one woman had triplets.

### Statistical analysis

We calculated that 3016 children randomly assigned treatment would need to be retained in follow-up to provide 80% power to detect a 40% decrease in mortality from treatment assignment to age 18 months, from 5·2% (based on mortality estimates from previous clinical trial data[25]) to 3·1%, with a two-sided type I error rate of 0·05. For children not known to have died, follow-up was calculated from treatment assignment to the latest date during the study the child was known to be alive or exactly 18 months from the date of birth if they were known to be alive beyond that age. We used Kaplan-Meier survival estimates to compare proportions of children alive at age 18 months in the co-trimoxazole and placebo groups, and used Greenwood's formula to calculate the SE of the difference. We did analyses in a modified intention-to-treat population, from which we excluded infants randomly assigned treatment after having negative DNA PCR results for HIV at birth and who later had positive DNA PCR results in blood samples taken at the time of treatment assignment.

For the secondary endpoints we analysed the differences (and SEs) in estimated proportions between groups at age 18 months, derived from Kaplan-Meier analysis. We used interval-censored survival methods[26] to assess all endpoints for clinical adverse events, admission to hospital, and diarrhoea, because dates for some events were not collected (eg, diarrhoea between study visits).

We did additional exploratory analyses of mortality in children who did not miss any medication refills, with follow-up censored at the first missed visit or discontinuation of study treatment, whichever was earliest. We also assessed whether mortality was affected by different degrees of adherence (≥1 or ≥2 missed days of treatment). We compared bacterial resistance in the two study groups by Fisher's exact test. We used Cox proportional hazards models separately adjusted for maternal and child baseline covariates to assess the effect of treatment on the primary endpoint, after checking that the proportional hazards assumption was satisfied. This trial is registered with ClinicalTrials.gov, number NCT01229761.

### Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

Between May 11, 2011, and April 2, 2015 we enrolled mothers, and between June 7, 2011, and April 2, 2015, babies were randomly assigned to treatment groups. Approximately 45 311 women gave birth at the maternity sites actively recruiting for the Mpepu Study, of whom 10 752 (24%) had confirmed HIV infection. 6556 were approached for the study, and 3334 (51%) were enrolled (figure 1). Among the enrolled women, 506 had babies who were not randomly assigned treatment, of whom 16 (<1%) tested positive for HIV before allocation (figure 1). 18 (<1%) babies had positive HIV results from blood samples drawn at the time of random assignment and were excluded from analyses per protocol. 2848 of the target 3016 children without HIV infection were randomly assigned to receive co-trimoxazole (n=1423) or placebo (n=1425). On April 2, 2015, accrual was stopped early because the DSMB judged that a benefit with co-trimoxazole would be unlikely. Characteristics of mothers and children were well balanced across the two study groups at random assignment (table 1).

Loss-to-follow-up was low. The vital status of 2695 (95%) of 2848 children randomly assigned treatment was known at age 18 months or at study closure (figure 1). Data were censored for 1106 children (553 [39%] of 1423 in the co-trimoxazole group and 553 [39%] of 1425 in the placebo group) before the 18-month visit, mainly because of study closure (953 [86%]). 2053 (72%) children

| | Co-trimoxazole group (n=1423) | Placebo group (n=1425) |
|---|---|---|
| **Mothers*** | | |
| Study site | | |
| Gaborone (city) | 847 (60%) | 842 (59%) |
| Molepolole (village) | 470 (33%) | 476 (33%) |
| Lobatse (town)† | 106 (7%) | 107 (8%) |
| Age at enrolment (years) | 31 (26–35) | 30 (26–34) |
| Education | | |
| None or primary | 232 (16%) | 229 (16%) |
| Secondary | 1087 (76%) | 1081 (76%) |
| Tertiary | 104 (7%) | 115 (8%) |
| Personal monthly income (US$)‡ | | |
| Unsure | 5 (<1%) | 7 (<1%) |
| None | 932 (65%) | 932 (65%) |
| <70 | 54 (4%) | 71 (5%) |
| 71–140 | 141 (10%) | 121 (8%) |
| 141–710 | 265 (19%) | 265 (19%) |
| >710 | 26 (2%) | 29 (2%) |
| Electricity in the home | 754 (53%) | 776 (54%) |
| Enrolled antepartum | 259 (18%) | 273 (19%) |
| CD4 cell count at enrolment (cells per μL) | 504 (367–674) | 508 (353–687) |
| HIV-1 RNA concentration at enrolment (copies per mL)§ | ≤40 (≤40–1293) | ≤40 (≤40–1038) |
| Receiving three-drug antiretroviral treatment before delivery | | |
| No | 174 (12%) | 181 (13%) |
| Yes | 1173 (82%) | 1173 (82%) |
| No antiretrovirals during pregnancy | 76 (5%) | 71 (5%) |
| (Continued from previous column) | | |
| **Children** | | |
| Gestational age (weeks) | 39 (37–40) | 39 (37–40) |
| Premature (>32 but ≤37 weeks' gestation) | 426 (30%) | 403 (28%) |
| Very premature (≤32 weeks' gestation) | 36 (3%) | 52 (4%) |
| Birthweight (kg) | | |
| Median | 2·97 (2·64–3·28) | 2·90 (2·60–3·23) |
| Low (>1·5 but ≤2·5 kg) | 254 (18%) | 273 (19%) |
| Very low (≤1·5 kg) | 6 (0%) | 9 (1%) |
| Received single-dose nevirapine at delivery | 1375 (97%) | 1374 (96%) |
| Received zidovudine at delivery | 1403 (99%) | 1402 (98%) |
| Feeding method at random assignment | | |
| Formula | 1136 (80%) | 1140 (80%) |
| Breastfeeding | 287 (20%) | 285 (20%) |
| Duration not randomly assigned¶ | 1 (<1%) | 2 (<1%) |
| Randomly assigned to 6 months' duration | 142 (10%) | 138 (10%) |
| Randomly assigned to 12 months' duration | 144 (10%) | 145 (10%) |
| Congenital abnormality | 20 (1%) | 22 (2%) |
| Age at random assignment (days) | | |
| Median (IQR) | 28 (16–30) | 28 (16–30) |
| 14–27 | 587 (41%) | 570 (40%) |
| 28–34‖ | 836 (59%) | 851 (60%) |

Data are number (%) or median (IQR). *2811 women contributed data for 2848 children (1412 in the co-trimoxazole group and 1399 in the placebo group). †Included in recruitment until August, 2012. ‡Converted from Botswana Pula with the average conversion rate for the study period. §In the first 1514 women enrolled in the study. ¶Three breastfed babies were mistakenly recorded as being formula fed, and were not randomly assigned a breastfeeding duration. ‖Includes two babies assigned treatment at age 35–37 days.

***Table 1*: Characteristics of mothers of and children at random assignment**

attended all study visits, and thus received all medication refills, up to age 15 months, death, or study closure. 272 (10%) children missed one or more visits but were taking the study treatment at the time of the 15-month visit, death, or study closure. The time to discontinuation of study treatment did not differ significantly between groups (p=0·35). Nine children were taken off study treatment before age 15 months or study closure because of adverse events (four in the co-trimoxazole group: neutropenia n=2 and anaemia n=2; five in the placebo group: rash n=1, Stevens-Johnson syndrome n=1, hepatitis n=2, and anaemia n=1). Nine children discontinued treatment due to participant choice (five in the co-trimoxazole group and four in the placebo group) or a positive HIV test while taking the study treatment (one in the placebo group).

Adherence to the study treatment was similar in the two groups at all visits. Caregivers reported complete adherence in 1344 (65%) of the 2053 children who attended all study visits up to the earlier of age 15 months, death, or study closure (664 [64%] in the co-trimoxazole group and 680 [67%] in the placebo group). Thus, total adherence by both methods of assessment (no missed refill visits and no missed doses in caregiver reports) was 47% among all children randomly assigned study treatment. 608 (43%) children in the co-trimoxazole group and 597 (42%) in the placebo group had either missed at least one refill visit or had a caregiver report of missing at least one dose, and 523 (37%) and 521 (37%), respectively, had missed at least one refill visit or had caregiver report of missing at least two doses.

64 children died between random assignment of treatment and age 18 months: 30 in the co-trimoxazole group (diarrhoeal disease n=17, pneumonia n=7, sepsis n=1, malnutrition n=1, other n=1, and unknown n=3) and 34 in the placebo group (diarrheal disease n=17, pneumonia n=8, sepsis n=5, other n=2, and unknown n=2). No deaths were judged to be probably or definitely related to study treatment. Three deaths were thought to be possibly related to study treatment, of which one was in the co-trimoxazole group and was due to confirmed acute

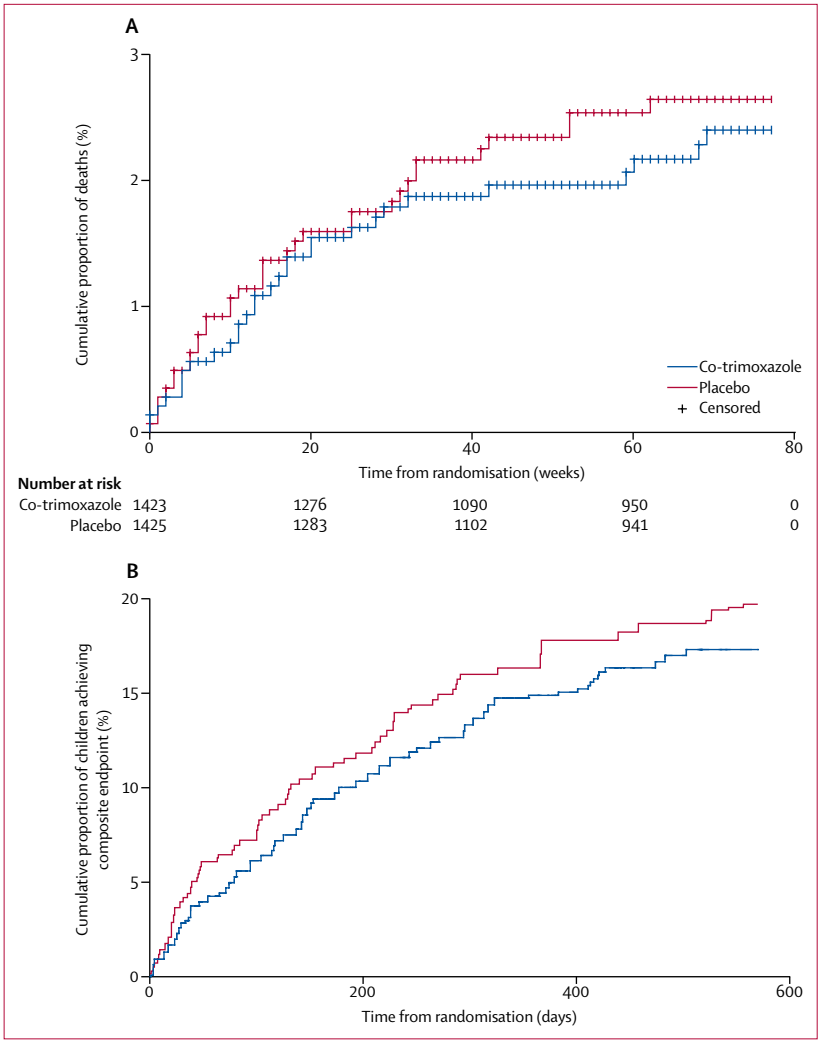


***Figure 2*: Cumulative proportions of deaths and of the composite endpoint of death, admission to hospital, and grade 3–4 clinical adverse events in children from baseline to the end of the study**
(A) Deaths. (B) Composite clinical endpoint.

| | Co-trimoxazole group (n=1423)* | Placebo group (n=1425)* | Difference (95% CI) | p value |
|---|---|---|---|---|
| **Primary outcome** | | | | |
| Deaths | 30 (2·4%) | 34 (2·6%) | –0·2% (–1·5 to 1·0) | 0·70 |
| **Secondary outcomes** | | | | |
| ≥1 admissions to hospital | 125 (12·5%) | 143 (17·4%) | –4·9% (–12·2 to 2·4) | 0·19 |
| ≥1 grade 3–4 clinical adverse event and grade 2 rash | 176 (16·5%) | 192 (18·4%) | –1·9% (–4·7 to 0·9) | 0·18 |
| Death, admission to hospital, or grade 3–4 clinical adverse events | 212 (17·3%) | 237 (19·6%) | –2·2% (–5·2 to 0·7) | 0·13 |
| Grade 3–4 laboratory events | | | | |
| Anaemia | 83 (8·1%) | 81 (8·3%) | –0·1% (–2·8 to 2·6) | 0·93 |
| Neutropenia | 95 (8·1%) | 68 (5·8%) | –2·3% (–0·2 to 4·3) | 0·03 |

Adverse events are graded with the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0.[23] *Percentages expressed as the estimated proportions at age 18 months.

***Table 2*: Outcomes**

gastroenteritis. The estimated cumulative proportions of deaths after treatment assignment and by age 18 months were 2·4% for co-trimoxazole and 2·6% for placebo (difference –0·2%, 95% CI –1·5 to 1·0, p=0·70; figure 2A). Similar results were found in the exploratory analyses of children with no missed medication refills, with estimated cumulative proportions of those dying up to and including age 18 months of 2·0% in the co-trimoxazole group and 2·5% in the placebo group (difference –0·5% 95% CI –1·7 to 0·7, p=0·43). Mortality was not affected by missing any days of treatment (≥1 or ≥2 days; data not shown).

Among 2848 children randomly assigned treatment, 362 had grade 3 or worse clinical adverse events and six children (three in each group) had grade 2 rash (table 2). The proportions of children who had at least one grade 3–4 adverse event, were admitted to hospital at least once, and who met the composite clinical endpoint of death, admission to hospital, or a grade 3–4 clinical adverse event did not differ between groups (table 2, figure 2B). Frequency was similar in the co-trimoxazole and placebo groups for pneumonia (2·5% *vs* 3·6%, p=0·33) and diarrhoea (10·3% *vs* 9·0%, p=0·26). No kernicterus was seen among children who started treatment at the earliest age (14–27 days). Laboratory data were available for 2510 (88%) of 2848 children randomly assigned treatment. 319 (13%) had at least one grade 3 or worse haematological abnormality. The frequency of grade 3–4 anaemia did not differ between groups, but that of grade 3–4 neutropenia was significantly higher in the co-trimoxazole group (table 2).

Of the 572 women who were breastfeeding at the time of treatment assignment, 569 (99%) were randomly assigned to breastfeed for 6 months (n=280) or 12 months (n=289). In the 6-month breastfeeding group, the median duration of breastfeeding was 6 months (IQR 6–6). 234 (84%) children were weaned before they completed study follow-up, of whom 168 (72%) were breastfed for the assigned duration. In the 12-month breastfeeding group, the median duration of breastfeeding was 8 months (IQR 5–12). 221 (76%) children were weaned before they completed study follow-up, of whom 73 (33%) were breastfed for the assigned duration. Among the babies randomly assigned to 12 months of breastfeeding, 144 (65%) were weaned before 12 months and four (2%) after 12 months, and among those assigned to 6 months of breastfeeding, 57 (24%) were weaned before and nine (4%) after 6 months.

ELISA at age 18 months was available for 259 (45%) of 572 breastfed children, and of the 313 without ELISA, 299 (96%) had had an HIV PCR test done either after weaning or within 3 months of his or her last visit. Tests for the remaining 14 children were missing due to early study closure, loss to follow-up, or death. HIV infection was detected in five children after randomisation, two who were breastfed (one randomised to 6 months and one to 12 months of breastfeeding, but who were both breastfed for 12 months before their first positive HIV test) and

three children not reported to have been breastfed, who we took to have had either false-negative results at the time of random assignment or unreported breastfeeding. One of the children first tested positive at the 3-month visit, two at the 12-month visit, and two at the 18-month visit. All five children were alive at the final study visit.

The frequency of death and HIV infection did not differ by feeding method or breastfeeding duration (table 3). Additionally, we found no difference between the co-trimoxazole and placebo groups for mortality alone among children who were formula fed or breastfed or by feeding duration (data not shown), although the study was not powered to detect these interactions.

Resistance to co-trimoxazole was based on 206 *E coli* isolates grown from stool specimens. For children with samples collected at the time of treatment assignment, the proportions with co-trimoxazole-resistant *E coli* were similar in the co-trimoxazole and placebo groups (11 [65%] of 17 *vs* 17 [61%] of 28) at randomisation. For children with samples collected at 3 months or 6 months, however, the proportions with resistance were higher in the co-trimoxazole group (3 months, 37 [95%] of 39 *vs* 19 [51%] of 37, p=0·001; 6 months, 32 [84%] of 38 *vs* 27 [58%] of 47, p=0·01).

| | HIV infection | Death or HIV infection* | Estimated proportions at age 18 months for death and HIV infection (95% CI) |
|---|---|---|---|
| Formula fed only | 3 of 2276 | 56 of 2276 | 3·0% (2·1–4·0) |
| Breastfed only† | | | |
| 6 months | 1 of 280 | 9 of 280 | 3·9% (1·3–6·4) |
| 12 months | 1 of 289 | 4 of 289 | 1·9% (0·01–3·7) |

*No deaths were in children with HIV infection. †Excludes three children who were breastfed but not randomly assigned to a feeding duration.

***Table 3*: Frequency of death and HIV infection, by feeding method**

## Discussion

Prophylactic co-trimoxazole was not associated with any survival benefit in HEU children born to HIV-infected women in southern Botswana. Although mortality was low overall, we also found no difference by study group in the prespecified composite clinical endpoint of death, admission to hospital, and grade 3–4 clinical adverse events. Co-trimoxazole was generally well tolerated and was not associated with either anaemia or adverse clinical outcomes, but was associated with increased numbers of cases of grade 3–4 neutropenia. Bacterial co-trimoxazole resistance in commensal stool flora was greater in the co-trimoxazole group than in the placebo group.

Our findings contrast with those from several other studies assessing HEU children. An observational study in Malawi reported a hazard ratio of 0·48 (p=0·03) for death among children who received prophylactic co-trimoxazole,[15] and various studies, mainly from malarial regions and all in Africa, have suggested a benefit with co-trimoxazole prophylaxis among HIV-affected and unaffected populations.[5,8–13] All these studies were done in areas with high childhood mortality.

Several factors might have accounted for the lack of benefit we saw with prophylactic co-trimoxazole. Although previous trials have reported benefits with co-trimoxazole in the prevention of routine respiratory and diarrhoeal bacterial illnesses,[1,2] including in settings with high prevalence of resistant pathogens,[3,4] the main contribution of co-trimoxazole might be in preventing opportunistic infections (its intended purpose) in HIV-positive children. We were unable to determine the number of deaths attributable to standard bacterial pathogens as compared with opportunistic infections. The contribution of malaria to mortality in previous studies might have been underestimated, and the benefits from co-trimoxazole in those studies have been suggested to be due largely to reductions in malaria. For example, in a household study in Uganda, co-trimoxazole prophylaxis in an individual infected with HIV significantly reduced the frequency of malaria and deaths in the entire household.[3] Other studies have shown reduced incidence of clinical malaria[9,10,27,28] and parasite burden[11,12] in people who have received co-trimoxazole prophylaxis. Bacterial resistance to co-trimoxazole is common throughout Africa,[29–31] which might have further diminished the effect of co-trimoxazole on preventing death from standard diarrhoeal and respiratory pathogens. Little is known about the effect of co-trimoxazole resistance when this drug is used for prophylaxis rather than treatment, but might be of concern if it is ineffective against standard bacterial pathogens.[32] Giving co-trimoxazole at the recommended doses is thought to result in low concentrations in up to a third of children,[33] which might have altered efficacy in our study. Lastly, adherence might have affected the efficacy of co-trimoxazole, although we found no significant difference between groups in mortality in the exploratory as-treated or the intention-to treat analyses, which suggests that adherence was not the primary explanation for our findings.

Given the low mortality in our placebo group, the 95% CI for the absolute difference in mortality between groups was wide. In previous studies in Africa[16–19] including in Botswana,[34] mortality was higher among HEU children than among those not exposed to HIV, particularly among those fed with formula.[25,35] We estimated that mortality would be 5·2% between random assignment of treatment and age 18 months in the placebo group, based on a previous clinical trial at the same study sites,[25] but this value was double that in our findings. The lower mortality we found might have been due to improvements in clinical care and in vaccination coverage over time, improved maternal health owing to widespread ART availability, or improved safety of feeding and preparation of formula. The Prevnar and rotavirus vaccines were introduced in Botswana during the study period, in mid-2012, and might have lowered mortality from respiratory and diarrhoeal diseases.[36–39]

Mortality might also have been lower than anticipated because we excluded children who were thought unlikely to survive to age 18 months and those who died before age 34 days. Of note, 33 (1%) enrolled neonates died before random treatment assignment. Finally, mortality could be lower among children participating in a clinical trial than among those in a programmatic setting. Despite low mortality, our better-powered comparison of the composite outcome of death, admission to hospital, and grade 3–4 clinical adverse events also did not reveal a difference between treatment groups.

This study yielded new safety data for co-trimoxazole used in early life. No increase was seen in the frequency of grade 3–4 anaemia compared with placebo even with use from as early as age 14 days. WHO and other guidelines suggest starting co-trimoxazole prophylaxis at age 4–6 weeks, partly because of the risk of kernicterus with sulfafurazole use in preterm neonates.[40] We did not see this complication among the babies treated from age 14 days in this study, all of whom were born at full term and weighed 2500 g or greater when co-trimoxazole was started. Significantly more children had grade 3–4 neutropenia in the co-trimoxazole than in the placebo group, although the absolute difference was small and we saw no clinical consequences. Few data exist regarding the implications of medication-induced neutropenia for child health outcomes in Africa, and the normal ranges for neutrophil counts in African children might be lower than in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events.[41–44] Lastly, we detected an increase in co-trimoxazole resistance among commensal *E coli* stool isolates in the co-trimoxazole group; the clinical consequences of this finding are unknown.

Our study had several limitations. First, mortality was lower than expected. The study was originally powered to detect a difference between groups of 40% or more. Despite the low mortality, we had power to detect a 50% or greater difference in mortality (which amounted to a small but clinically meaningful absolute difference in mortality between co-trimoxazole *vs* placebo: 95% CI –1·5% to 1·0%). The absence of a difference in the composite outcome of death, hospital admission, and grade 3–4 clinical adverse events (for which the study was well powered) supports the absence of a clinically meaningful effect of co-trimoxazole. Second, following changes to WHO guidelines in 2010,[45] and the reporting of data on the safety of breastfeeding in the setting of ART prophylaxis in Botswana,[46] we anticipated that a higher percentage of women in our study would opt to breastfeed. However, with only 20% choosing breastfeeding instead of the estimated 80%, secondary random assignment to a 6-month or 12-month breastfeeding duration was underpowered to show any benefit from longer-term breastfeeding. We did, however, note some challenges in maintaining longer breastfeeding in Botswana. 44% of all women approached were enrolled in the study. The reasons for refusal (figure 1) suggest little risk of bias regarding study entry, with few refusals being due to neonatal illness. Nevertheless, we cannot rule out the possibility that mothers of the sickest infants were not available to be invited to participate. Lastly, the DSMB stopped the study early based on the predefined stopping guidelines, which led to 39% of children per group having data censored before age 18 months.

Extended co-trimoxazole prophylaxis provided no survival benefit in HEU children in southern Botswana up to age 18 months. Despite being generally well tolerated, long-term use of co-trimoxazole in HEU children at low risk of late mother-to-child transmission of HIV added cost and complexity to care, increased antimicrobial resistance, and was associated with slightly increased risk of neutropenia. Thus, in non-malarial regions with low risk of HIV infection through mother-to-child transmission, long-term co-trimoxazole prophylaxis for HEU children might not be needed.

#### Contributors

SL MH, KM, LM, JM, ME, and RS designed the study. SL, KP, GA, KM, LM, JM, ME, and RS were responsible for the conduct of the trial. EvW directed data collection. EvW and JL managed the data. MH led the data analyses, to which KP, KB, and JL contributed, and KP participated in data quality management. SM led the laboratory activities. All authors participated in writing the paper.

#### Data safety monitoring board

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#### Declaration of interests

We declare no competing interests.

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