**Estimating the External Validity of Randomized Controlled Trials: A Comparison of Morbidity and Mortality Between an RCT and an Observational Study in Botswana**

Neil Thivalapilla, Shahin Lockmana,b,c,d, Kathleen Powisa,b,e, Rebecca Zashb,f, Jean Leidnerg, Gbolahan Ajibolab, Mompati Mmalaneb, Joseph Makhemab, Roger L Shapiro a,b,c,f

**Affiliations:**

a Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

b Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

c Harvard Medical School, Boston, Massachusetts, USA

d Brigham and Women’s Hospital, Boston, Massachusetts, USA

e Massachusetts General Hospital, Boston, Massachusetts, USA

f Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

g Goodtables Data Consulting, Norman, Oklahoma, USA

**Corresponding Author:**

Neil Thivalapill, MS

677 Huntington Ave.

Department of Epidemiology

Kresge Building 9th Fl. – Box 95

Harvard T.H. Chan School of Public Health

e. [thivalapill@hsph.harvard.edu](mailto:thivalapill@hsph.harvard.edu)

p. (+1) 516-395-0204

**The Mpepu Trial:**

**Study Design:** The Mpepu Study was a double-blind, randomized, placebo-controlled trial in which 2,848 HIV-Exposed Uninfected (HEU) infants enrolled within the first 34 days of life between June 2011 and April 2015 and randomized to receive co-trimoxazole or placebo.[[1]](#endnote-1) Women with documented HIV-1-infection were recruited from public antenatal clinics or maternity wards in southern Botswana between the 26th week of pregnancy up to 34 days postpartum, The study was conducted in an area of Botswana without malaria transmission. Recruitment took place in Gaborone (urban setting), Molepolole (large village), and Lobatse (town). Mothers who elected to breastfeeding their infants gave consent to be randomly assigned to breastfeeding for 6 months (the recommended duration in Botswana) or 12 months (the duration recommended by WHO). Breastfed children were allocated by factorial randomization to CTX vs. Placebo and to 6 vs. 12 months of breastfeeding. The trial was stopped for futility as the data and safety monitoring board concluded a low likelihood of benefit with CTX.

**Exclusion Criteria for Randomization of Children**: Children were excluded from randomization if they had a previous positive HIV result on a qualitative DNA PCR test, a medical condition that made survival to 18 months unlikely, jaundice, known allergy to co-trimoxazole, or anemia or neutropenia that met the Division of AIDS criteria for either a grade 4 event even if the child was asymptomatic or, for symptomatic children, a grade 3 event.[[2]](#endnote-2)

**Setting**: The government of Botswana provided free formula feeding but also allowed mothers receiving antiretroviral treatment (ART) to breastfeed. The government also provided free maternal three-drug ART for all maternal participants during pregnancy and breastfeeding. All infants received antiretroviral (ARV) prophylaxis in the first four weeks of life, regardless of feeding method. Breastfed infants of mothers who were not taking ART were provided prophylactic nevirapine that was dosed according to WHO guidelines.

**Care**: All mothers were counseled on infant ARV prophylaxis and on administration of CTX versus placebo. Mothers were counseled on weaning at the randomization visit if they chose to breastfeed and those who chose to formula feed were counseled on safe feeding practices and the safe use of formula. Children were clinically assessed at birth, randomization (occurring between 14-34 days of life), 2 months, 3 months, and every 3 months thereafter until age 18 months by a nurse at all visits and by a physician at most visits. Adherence to study drug or placebo was measured as the number of drug refills dispensed at study visits and by maternal or caregiver report at each clinic visit. If a scheduled visit was missed, a study nurse or counselor would locate the mother and child by phone or home visit, if permission was granted. Routine care was provided to study participants through the Botswana government and national health system guidelines provided free maternal ART and PMTCT to mothers living with HIV and their children. Botswana offered most standard childhood vaccines including BCG, DTAP, HBV, HIB, MMR and polio. During the study, the pneumococcal conjugate vaccine and rotavirus vaccine became available. In keeping with national standards of care for children under the age of 5 in Botswana, if a child was found to have an acute or chronic illness, missed a schedule vaccination, or showed evidence of poor growth or developmental delay at a study visit, the child was referred by the study staff to a government health facility for evaluation.

**Guideline Changes for PMTCT:** Between May 2011 to January 2013, the trial adhered to Botswana’s guidelines for the prevention of mother to child transmission (PMTCT) of HIV which was a single dose of nevirapine and four weeks of prophylactic zidovudine (ZDV) for newborns. After 2013, however, the trial protocol was changed to allow earlier use of CTX and newborn ARV prophylaxis was modified to 4 weeks of nevirapine to avert hematological toxicity from the combination of ZDV and CTX. Therefore, before January 21st, 2013, children were randomized to CTX versus placebo between the ages of 28-34 days. After this date, full-term infants who weighed more than 2500 grams were randomized between 14-34 days. Infants born before 37 weeks or those weighing less than 2500 grams prior to 28 days were randomized to CTX or placebo between 28-34 days.

**The Maikaelelo Study:**

**Study Setting**: The Maikaelelo Study was an observational cohort study that enrolled mother-infant pairs from five public hospital maternity wards in Botswana, including Francistown (urban setting), Mochudi (large village), Ramotswa (village), Maun (town), and Kanye (large village). Between January 2012 and March 2013, the Maikaelelo study enrolled 1,499 HIV-infected and 1,501 HIV-negative mothers and their 3,033 infants, of which 1,515 were HEU.[[3]](#endnote-3) In the primary analysis of Maikaelelo, HIV-exposed children with unknown infection were considered HIV-uninfected.

**Exclusion of Mothers**: Mothers were excluded if they lived more than 100 kilometers away, were unwilling to be contacted by telephone, or could not provide a contact number.

**Assessment:** Follow-up visits were conducted via telephone only, without any in-person contact. Intervals of assessment occurred at one month, three months, and then every three months until 24 months postpartum. Maternal characteristics including medical history, demographics, and HIV testing and treatment results were assessed at baseline. The caregiver was asked to report on vital status of the mother and infant, as well as any hospitalizations, medications, infant vaccinations, infant feeding method, and any interim infant HIV testing during follow-up telephone calls. Mothers continued to receive follow-up telephone calls until death, death of the infant, or until the end of the study. Infants were followed until death or the end of the study. Child deaths, maternal deaths, and the date of death were collected from the primary caregiver reached by phone at follow-up. Verbal autopsy was performed for children who died using structured interviews, with cause of death being categorized by a consensus of study physicians who were blinded to all information except the data in the verbal autopsy.

**Care**: Routine care was provided to study participants through the Botswana government and national health system guidelines provided free maternal ART and PMTCT to mothers living with HIV and their children. Botswana offered most standard childhood vaccines including BCG, DTAP, HBV, HIB, MMR and polio. During the study, the pneumococcal conjugate vaccine and rotavirus vaccine became available.

**Infant Feeding:** Prior to June 2012, the government of Botswana recommended that women living with HIV to exclusively formula feed and provided free formula to eligible mothers. In June of 2012, however, the guidelines were updated to allow mothers receiving ART who were virally suppressed at delivery to choose to breastfeed.

**Botswana MTCT and ART guidelines (both studies):** Between February 2012 to May 2012, pregnant HIV-positive women with CD4 count </=350 cells/mm3 or WHO Clinical HIV Stage III or IV started combination ART and those with CD4 count >350 cells/mm3 started ZDV alone during pregnancy. However, beginning in June of 2012, all pregnant women, regardless of CD4 cell count, were eligible to initiate and indefinitely continue combination ART (Efavirenz/ZDV/3TC).

1. Lockman, S., Hughes, M., Powis, K., Ajibola, G., Bennett, K., Moyo, S., van Widenfelt, E., Leidner, J., McIntosh, K., Mazhani, L., Makhema, J., Essex, M., & Shapiro, R. (2017). Effect of co-trimoxazole on mortality in HIV-exposed but uninfected children in Botswana (the Mpepu Study): A double-blind, randomised, placebo-controlled trial. *The Lancet Global Health*, *5*(5), e491–e500. <https://doi.org/10.1016/S2214-109X(17)30143-2> [↑](#endnote-ref-1)
2. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017]. Available from: <https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf> [↑](#endnote-ref-2)
3. Zash, R., Souda, S., Leidner, J., Ribaudo, H., Binda, K., Moyo, S., Powis, K. M., Petlo, C., Mmalane, M., Makhema, J., Essex, M., Lockman, S., & Shapiro, R. (2016). HIV-exposed children account for more than half of 24-month mortality in Botswana. *BMC Pediatrics*, *16*. <https://doi.org/10.1186/s12887-016-0635-5> [↑](#endnote-ref-3)