Incident HIV among pregnant and breast-feeding women in sub-Saharan Africa: a systematic review and meta-analysis

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Objectives: A previous meta-analysis reported high HIV incidence among pregnant and breast-feeding women in sub-Saharan Africa (SSA), but limited evidence of elevated risk of HIV acquisition during pregnancy or breast-feeding when compared with non-pregnant periods. The rapidly evolving HIV prevention and treatment landscape since publication of this review may have important implications for maternal HIV incidence.

Design: Systematic review and meta-analysis.

Methods: We searched four databases and abstracts from relevant conferences through 1 December 2018, for literature on maternal HIV incidence in SSA. We used random-effects meta-analysis to summarize incidence rates and ratios, and to estimate 95% prediction intervals. We evaluated potential sources of heterogeneity with random-effects meta-regression.

Results: Thirty-seven publications contributed 100 758 person-years of follow-up. The estimated average HIV incidence rate among pregnant and breast-feeding women was 3.6 per 100 person-years (95% prediction interval: 1.2–11.1), while the estimated average associations between pregnancy and risk of HIV acquisition, and breast-feeding and risk of HIV acquisition, were close to the null. Wide 95% prediction intervals around summary estimates highlighted the variability of HIV incidence across populations of pregnant and breast-feeding women in SSA. Average HIV incidence appeared associated with age, partner HIV status, and calendar time. Average incidence was highest among studies conducted pre-2010 (4.1/100 person-years, 95% prediction interval: 1.1–12.2) and lowest among studies conducted post-2014 (2.1/100 person-years, 95% prediction interval: 0.7–6.5).

Conclusion: Substantial HIV incidence among pregnant and breast-feeding women in SSA, even in the current era of combination HIV prevention and treatment, underscores the need for prevention tailored to high-risk pregnant and breast-feeding women.

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Introduction

HIV acquisition among pregnant and breast-feeding women increases risk of maternal morbidity and mortality, and accounts for a significant, and growing, proportion of pediatric HIV infections globally [1]. A meta-analysis of 19 studies conducted between 1980 and 2012 estimated an average HIV incidence rate of 3.8/100 person-years [95% confidence interval (CI): 3.0–4.6] among pregnant and breast-feeding women in sub-Saharan Africa (SSA) [2]. Although this estimate is above the World Health Organization's (WHO) threshold for substantial risk of HIV acquisition [3], the rapidly evolving HIV prevention and treatment landscape since publication of this review may have important implications for maternal HIV incidence.

In 2013, the WHO updated HIV treatment guidelines, expanding antiretroviral therapy (ART) eligibility to $CD4^+ \leq 500$ cells/μl [4], and in 2015, it recommended universal treatment for HIV [5]. These changes, together with increased uptake of HIV testing and counseling and medical male circumcision [6–8], coincided with a 30% decline in the estimated number of new adult HIV infections in SSA between 2010 and 2017 [9]. Similar temporal trends in HIV incidence have been observed in three population-based cohort studies in SSA [10–12], with more gradual declines observed among women than among men [11,12]. Although combination HIV prevention and treatment interventions may not directly target pregnant and breast-feeding women, these populations may experience downstream benefits in HIV prevention. In at least one study [13], maternal HIV incidence was considerably lower in a cohort of pregnant and breast-feeding women participating in a community-based HIV prevention program than estimates of maternal incidence from the previous review [2].

Although the previous review observed evidence of heterogeneity among study-specific estimates of the incidence rate and the association between pregnancy and risk of HIV acquisition, their investigation into the underlying factors contributing to this variability was limited [2]. A better understanding of features contributing to variation in estimates is critical for guiding future research and policy, and for developing efficient strategies to reduce horizontal and vertical HIV transmission during pregnancy and breast-feeding.

In this updated review of literature from SSA between 1980 and 2018, we sought to summarize estimates of HIV incidence among pregnant and breast-feeding women; summarize estimates of the associations between pregnancy and risk of maternal HIV acquisition and between breast-feeding and risk of HIV acquisition; and identify population and methodological characteristics contributing to variation in study-specific estimates of incidence and association.

Methods

This review is registered with PROSPERO (CRD42017079577) and follows the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Guidelines [14].

Study selection and data abstraction

We searched PubMed, Embase, PsycInfo, and the Cochrane Library for relevant literature published between 1 January 1980 and 1 December 2018 (Table S1, http://links.lww.com/QAD/B657). We also searched online abstract archives from HIV Research for Prevention Conference (2014–2018), Conference of Retroviruses and Opportunistic Infections (2014–2018), and International AIDS Society Conferences (2001–2018) using the terms ('pregnant', 'pregnancy', or 'postpartum') and ('incident', 'incidence', or 'seroconvert').

We screened resulting titles and abstracts to identify publications that referred to HIV incidence among women or to pregnancy/breast-feeding and HIV. We conducted a full text review of included publications to identify primary research reports with estimates of (or sufficient information to derive) the incidence rate of HIV among pregnant and breast-feeding women, the incidence rate ratio (IRR) or hazard ratio contrasting HIV incidence between pregnant and nonpregnant periods, and/or the IRR or hazard ratio contrasting HIV incidence between breast-feeding and non-breast-feeding periods. Included studies were restricted to those published in English and conducted in SSA. We requested additional information from authors when publications contained relevant but insufficient information, and reviewed the bibliographies of included publications for relevant references.

Two investigators reviewed each publication at screening and full-text review; disagreements were resolved by consensus. Data on outcomes and exposures of interest and key population and methodological features of each study were abstracted into standardized tables by one reviewer and checked by two others. When more than one publication reported the same outcome from the same study population over the same period, we included the report considered most complete.

Outcome and exposure definitions

HIV incidence, the primary outcome, was defined as the number of new HIV infections per 100 person-years. Pregnancy and breast-feeding represented periods of interest in studies contributing incidence rate estimates, and represented exposures of interest in studies estimating the IRR or hazard ratio. We accepted all definitions in our primary analyses. In a sensitivity analysis, we excluded studies where the breast-feeding period exceeded 24 months postpartum [15].

Statistical approach

We used inverse-variance-weighted random-effects meta-analysis to estimate natural log-transformed measures of the average HIV incidence rate among pregnant and breast-feeding women, the average association between pregnancy and risk of HIV acquisition, the average association between breast-feeding and risk of HIV acquisition, and 95% prediction intervals around summary estimates. The 95% prediction intervals convey the estimated spread of the random-effects distribution, and can be informally interpreted as 95% CI for the true rate or association to be estimated in a randomly selected study population [16–18]. When zero seroconversions were reported, we applied a half-integer continuity correction to prevent the estimate from being omitted. As IRRs roughly approximate hazard ratios [19], we pooled these estimates for meta-analysis and assumed approximate collapsibility since HIV acquisition is rare [20]. Summary estimates and 95% prediction intervals were exponentiated for interpretability.

Because of the potential for publication bias, we drew funnel plots and analyzed them with the symmetry test of Egger *et al.* and with Duval and Tweedie's trim-and-fill imputation method [21,22]. We analyzed overall heterogeneity using 95% prediction intervals and the P value for Cochrane's Q statistic. We used stratified analyses and univariate random-effects meta-regression to analyze heterogeneity further by comparing average rates and associations by population characteristics of included studies. Meta-regression was also used to explore associations between estimates and methodological aspects related to study quality [23,24]. When a single study contributed information to more than one stratum of a variable, we used robust variances to account for correlation [25]. Given the large number of studies contributing estimates of the incidence rate, we also constructed separate multivariable models for each potential source of heterogeneity of the incidence rate. Each model adjusted for region, years of study implementation, and calendar time to account for differences in HIV prevalence and ART coverage. All analyses were conducted using the Metafor package in R, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) [26].

Sources of heterogeneity

Characteristics related to underlying HIV risk – region, calendar time, age, membership of a high-risk population, and participant enrollment in an HIV-prevention clinical trial – may be associated with estimates of incidence and association. As studies contributing estimates of the association had limited variability in calendar time, and did not provide age-stratified results, these features were only evaluated as sources of heterogeneity of the incidence rate.

We defined region using the World Bank's classifications, and calendar time based on mid-year of study implementation. We examined calendar time continuously, as a quadratic function, and categorically with three periods: precombination HIV prevention (before 2010), early adoption (2010–2014), and program expansion (after 2014). These periods correspond to important updates to WHO HIV treatment and prevention recommendations [4,5,27,28], and their implementation across SSA [6–8]. We defined age groups based on the most commonly reported categorization in the literature: less than 20, 20–24, 25–29, and at least 30. Other age group categorizations were considered in sensitivity analyses. We used a binary variable to distinguish between studies that enrolled a 'high-risk' cohort (e.g. HIV-discordant couples or female sex workers) and those that did not. We stratified by type of 'high-risk' group in sensitivity analyses. Studies were also classified according to whether participants were enrolled in a clinical trial evaluating an HIV prevention intervention.

The following features related to the measurement of incident infections and person-time may also be associated with estimates of incidence and association: study design, use of results from repeat HIV testing to identify seroconversions, reproductive periods observed over follow-up, use of HIV DNA/RNA PCR in the HIV-testing algorithm, and method for estimating date of HIV infection. As all studies contributing estimates of the association used repeat HIV testing and observed all reproductive periods over follow-up, these features were only evaluated as sources of heterogeneity of the incidence rate.

Finally, estimates of the IRR or hazard ratio may be related to the inclusion of breast-feeding-exposed periods in the reference group, adjustment for confounders, and adjustment for time-varying measures of condom use and intercourse frequency.

Results

Our search yielded 5186 nonduplicate abstracts (Fig. 1). Screening resulted in 202 publications for full-text review, of which 57 met inclusion criteria. After excluding 20 publications because of overlapping cohorts and outcomes, 37 publications remained (Table 1). Thirty-four contributed estimates of the HIV incidence rate [13,29–61], and 10 contributed estimates of either the IRR or hazard ratio [55–64]. Follow-up ranged from 45 person-years to 57 240 person-years. Most studies were conducted in southern Africa ($n = 20$) [13,29,30,32,34,35,39–44,48,51,52,54,55,60,61,64]. The mid-point of follow-up occurred before 2010 in 26 studies [29,32,34–36,38–44,48,50,52–64], between 2010 and 2014 in eight [30,31,33,37,46,47,49,55], and after 2014 in three [13,45,51]. Two studies reported results stratified by calendar time [55,56]. In seven studies, participants
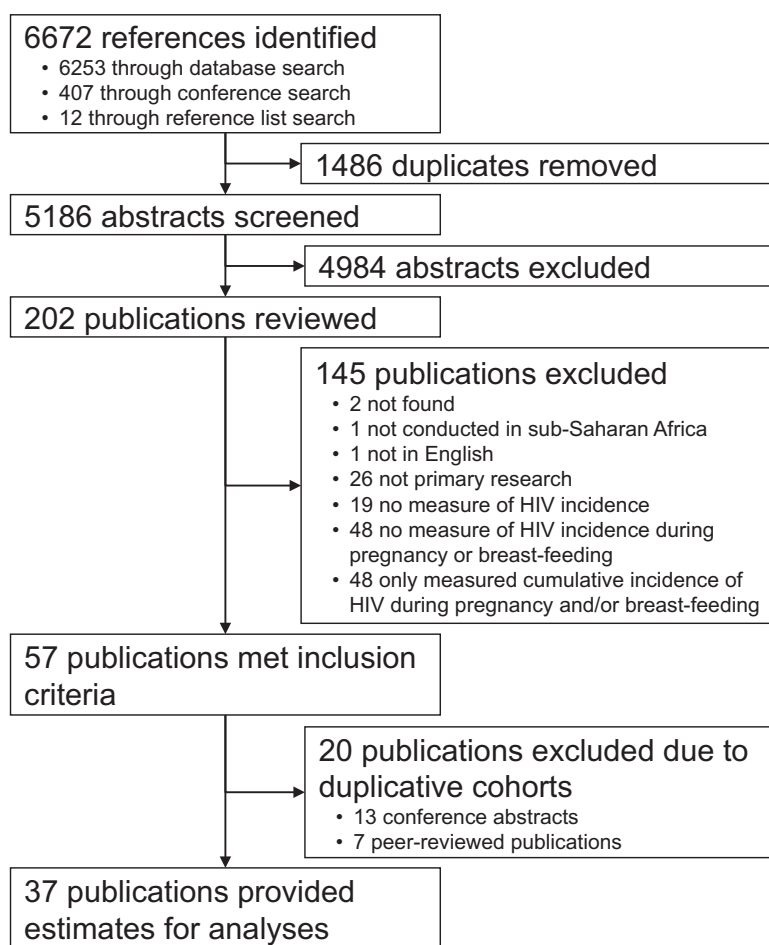
Fig. 1. Study selection flowchart.

were enrolled in an HIV prevention trial [32,43,54,60–62,64]. Four studies enrolled high-risk study populations [54,57,62,63], and two studies reported results stratified by risk-group [58,59]. Eight studies reported estimates of incidence stratified by age [13,30,38,39,44,48,55,58].

There was limited variability in how studies measured incidence after accounting for study design (Table S2, http://links.lww.com/QAD/B658). Prospective cohort studies ($n = 24$) enrolled HIV-seronegative women and retested them over follow-up to identify changes in HIV serostatus. Twenty-one prospective cohort studies contributed estimates of the incidence rate among pregnant and breast-feeding women [13,29,30,32,37–40,42–44,48–50,52,54,58–61], and eight contributed estimates of the IRR or hazard ratio [57–64]. Eleven cross-sectional studies contributed estimates of the incidence rate among pregnant and breast-feeding women [31,33–36,41,45–47,51,53]. In these studies, HIV status at the time of the first antenatal visit was retrospectively assessed at the time of enrollment, which occurred in the third trimester [31,41,46,47], at delivery [33–35,51], or in the postpartum period [36,45,53]. Women classified as HIV-negative in pregnancy were enrolled and current HIV

serostatus was assessed to identify new HIV infections. Finally, two studies nested within large population-based surveillance studies contributed estimates of both the incidence rate and the hazard ratio [55,56]. These studies used prospectively collected data from HIV surveillance assessments to assess changes in serostatus over time.

HIV incidence during pregnancy and breast-feeding

Studies contributing estimates of incidence during pregnancy typically captured the period between the first antenatal visit and delivery, while studies contributing estimates of incidence during breast-feeding captured the period from delivery up to 24 months postpartum depending on length of follow-up (Table 1).

Thirty-four studies contributed 100 758 person-years of follow-up and generated 44 estimates of HIV incidence among pregnant and/or breast-feeding women. Ten studies reported stratified estimates of incidence during pregnancy and during breast-feeding [13,37,39,44,54–59]. Using all available estimates, we observed little difference in the average HIV incidence rate during pregnancy only ($n = 22$, 3.4/100 person-years, 95%

Table 1. Description of studies meeting inclusion criteria.

Publication	Region	Study design	Study years	Incident cases of HIV	Person-years	Definition of pregnancy	Definition of breast-feeding	Exposed group	vs.	Unexposed group	High-risk participant recruitment	Parent study was a clinical trial of an HIV prevention intervention
Studies that only contributed estimates of the incidence rate during pregnancy												
De Schacht et al. [29]	Southern Africa	Prospective cohort	2008–2011	14	328	Date of enrollment to date of delivery. All participants enrolled during the index pregnancy. Median gestational age at enrollment was 24 weeks	-	-		-	No	No
Egbe et al. [31]	Western Africa	Cross-sectional	2011	9	147	Date of first ANC visit to date of delivery. Women were 16–20 weeks pregnant at their first ANC visit	-	-		-	No	No
Imade et al. [33]	Western Africa	Cross-sectional	2010–2012	4	235	Date of first ANC visit to date of delivery. Information on gestational age at first ANC is not provided	-	-		-	No	No
Keating et al. [34]	Southern Africa	Cross-sectional	2009	11	275	Date of first ANC visit to date of delivery. Average gestational age at first ANC was 25 weeks	-	-		-	No	No
Kieffer et al. [35]	Southern Africa	Cross-sectional	2008–2008	58	388	Date of first ANC visit to date of delivery. Information on gestational age at first ANC is not provided	-	-		-	No	No
Moodley et al. [41]	Southern Africa	Cross-sectional	2006–2007	72	679	Date of first ANC visit to date of subsequent ANC visit. Average of 24 weeks between first ANC visit and subsequent ANC visit	-	-		-	No	No
Phiri et al. [51]	Southern Africa	Cross-sectional	2015	83	4888	Date of first ANC visit to date of delivery. First ANC was assumed to have occurred approximately 4.5 months before delivery	-	-		-	No	No
Rogers et al. [46]	Eastern Africa	Cross-sectional	2011–2014	2	45	Date of first ANC visit to date of subsequent ANC visit. Average period between ANC visits is not provided	-	-		-	No	No
Tabu et al. [47]	Eastern Africa	Cross-sectional	2012	5	311	Not clearly defined, but the entire period between the first HIV test and the final HIV test occurred in pregnancy	-	-		-	No	No
Traore [49]	Western Africa	Prospective cohort	2010–2011	0	126	First ANC visit to the end of pregnancy. Average gestational age at first ANC was 14 weeks. Average length of follow-up was 22.8 weeks	-	-		-	No	No
Studies that only contributed estimates of the incidence rate during breast-feeding												
De Schacht et al. [30]	Southern Africa	Prospective cohort	2008–2012	41	1278	-	Date of enrollment to 18 months postpartum. Enrollment occurred 0–8 weeks postpartum, with the majority of women enrolling within 4 days of delivery. Median length of follow-up was 18.2 months	-		-	No	No

Table 1 (*continued*)

Publication	Region	Study design	Study years	Incident cases of HIV	Person-years	Definition of pregnancy	Definition of breast-feeding	Exposed group	vs.	Unexposed group	High-risk participant recruitment	Parent study was a clinical trial of an HIV prevention intervention
Humphrey et al. [32]	Southern Africa	Prospective cohort	1997–2001	269	7763	-	Date of enrollment to 18 months postpartum. Enrollment occurred between 0 and 96h after delivery. Women were analytically censored 12 months postpartum	-		-	No	NCT00198718: Evaluated the effect of maternal vitamin A supplementation soon after delivery on HIV incidence.
Leroy et al. [38]	Eastern Africa	Prospective cohort	1988–1992	17	390	-	Date of delivery to 24 months postpartum. Average length of follow-up was 32 months, but stratified analyses enabled the restriction of estimates to 24 months postpartum	-		-	No	No
Miotti et al. [40]	Southern Africa	Prospective cohort	1989–1993	43	988	-	Date of delivery to 24 months postpartum. Average length of follow-up was not provided	-		-	No	No
Van de Perre et al. [50]	Eastern Africa	Prospective cohort	1998–1990	18	474	-	Date of delivery to 24 months postpartum. Follow-up continued to 36 months postpartum, but stratified estimates enabled ascertainment of incidence between delivery and 24 months only	-		-	No	No

Studies that contributed estimates of the incidence rate during pregnancy and breast-feeding

Publication	Region	Study design	Study years	Incident cases of HIV	Person-years	Definition of pregnancy	Definition of breast-feeding	Exposed group	vs.	Unexposed group	High-risk participant recruitment	Parent study was a clinical trial of an HIV prevention intervention
Fatti et al. [13]	Southern Africa	Prospective cohort	2013–2016	11	828	Date of first ANC visit to date of delivery. Median gestational age at first ANC was 16 weeks	Date of delivery to 18 months postpartum. Average length of follow-up was not provided. Eleven percent of women were lost to follow-up by 12 months postpartum	-		-	No	No
John et al. [53]	Eastern Africa	Cross-sectional	-	118	2565	First ANC to approximately 9 months postpartum. Information on gestational age at first ANC is not provided. All women in this study had an HIV test at approximately 9 months postpartum	-	-		-	No	No
Kinuthia et al. [36]	Eastern Africa	Cross-sectional	-	53	779	First ANC to approximately 6 weeks postpartum. Information on gestational age at first ANC is not provided. All women in this study had an HIV test approximately 6 weeks postpartum	-	-		-	No	No
Kinuthia et al. [37]	Eastern Africa	Prospective cohort	2011–2013	25	1278	Date of first ANC visit to date of delivery. Median gestational at first ANC was 27 weeks	Date of delivery to 9 months postpartum. Average length of follow-up was not provided. Ninety-eight percent of participants were retained through 9 months postpartum	-		-	No	No

Table 1 (*continued*)

Publication	Region	Study design	Study years	Incident cases of HIV	Person-years	Definition of pregnancy	Definition of breast-feeding	Exposed group	vs.	Unexposed group	High-risk participant recruitment	Parent study was a clinical trial of an HIV prevention intervention
								Contrast estimated by the IRR or hazard ratio				
Mbizvo et al. [39]	Southern Africa	Prospective cohort	1991–1995	66	1375	Date of first ANC visit to date of delivery. Information on gestational age at first ANC is not provided	Date of delivery to 24 months postpartum. Average length of follow-up was not provided	-		-	No	No
Mepham et al. [52]	Southern Africa	Prospective cohort	2001–2005	38	717	First ANC to approximately 6 months postpartum. Median gestational age at first ANC was 25 weeks. All women in this study had an HIV test conducted at approximately 6 months postpartum	-	-		-	No	No
Moodley et al. [42]	Southern Africa	Prospective cohort	2005–2007	48	1946	First ANC to approximately 12 months postpartum. Median gestational age at first ANC was 25 weeks. Average length of follow-up was not provided. Eighty-eight percent completed study follow-up	-	-		-	No	No
Moodley et al. [43]	Southern Africa	Prospective cohort	2008–2010	6	109	First ANC to approximately 14 weeks postpartum. Median gestational age at first ANC was 24 weeks. Average length of follow-up was not provided	-	-		-	No	NCT01683461: Evaluated the efficacy of enhanced HIV counseling during antenatal and postnatal periods on incidence of STIs and risk-taking behavior
Munjoma et al. [44]	Southern Africa	Prospective cohort	2002–2008	17	298	First ANC to approximately 9 months postpartum. Information on gestational age at first ANC is not provided. Average length of follow-up was 38.2 months; stratified estimates enabled the ascertainment of incidence between first ANC and 9 months only	-	-		-	No	No
Nikuze et al. [45]	Eastern Africa	Cross-sectional	2016	33	805	Not clearly defined, but the last HIV test occurred approximately 9 months postpartum	-	-		-	No	No
Taha et al. [48]	Southern Africa	Prospective cohort	1990–1995	97	2302	First ANC visit to 6 years postpartum. Average length of follow-up was 30.2 months. Stratified results are not provided	-	-		-	No	No
Thomson et al. [54][b]	Southern Africa	Prospective cohort	2004–2013	24	447	LMP to end of pregnancy	Variable based on pregnancy outcome: • Pregnancy loss at <6 weeks gestation: N/A. • Pregnancy loss between 6 and 20 weeks gestation: first 28 days following pregnancy loss • Pregnancy loss ≥20 weeks gestation or infant death before 6 months of age: first 42 days following pregnancy/infant loss • Live birth and no infant death before 6 months of age: delivery to 6 months postpartum	-		-	Recruited HIV sero-discordant couples only	NCT00194519: Evaluated the efficacy of twice daily acyclovir on HIV incidence NCT00557245: Evaluating the efficacy of PrEP for HIV prevention

Table 1 (*continued*)

Studies that contributed estimates of the incidence rate during pregnancy and/or breast-feeding, and estimates of the IRR or hazard ratio

Publication	Region	Study design	Study years	Incident cases of HIV	Person-years	Definition of pregnancy	Definition of breast-feeding	Exposed group	vs.	Unexposed group	High-risk participant recruitment	Parent study was a clinical trial of an HIV prevention intervention
Braunstein et al. [57]	Eastern Africa	Prospective cohort	2006–2008	17	625	Not clearly defined.	Not measured over follow-up. Study ascertained breast-feeding status at baseline, but did not appear to measure changes to this status over follow-up	Incidence during pregnant Period	vs.	Incidence during nonpregnant periods	Recruited FSWs only	No
Chetty et al. [55]	Southern Africa	Nested surveillance study	2010–2015	66	1857	Variable based on pregnancy outcome: • Pregnancies ending in a still or live birth: the period between LMP and date of delivery • Pregnancies ending in a miscarriage or termination: the period between LPM to date of expected delivery	Delivery to 2 months postpartum	Incidence during pregnant period. Incidence during breast-feeding period	vs. vs.	Incidence during nonpregnant, non-breast-feeding periods. Incidence during nonpregnant, non-breast-feeding periods	No	No
Gray et al. [58] [a]	Eastern Africa	Prospective cohort	1994–1999	63	4040	The period between pregnancy identification and the immediate postpartum period. Average time between pregnancy identification and delivery was 4.6 months	Date of first HIV test in the postpartum period to date of repeat HIV test. Average time between HIV tests during breast-feeding was 1.05 years	Incidence among pregnant periods. Incidence among breast-feeding periods	vs. vs.	Incidence among nonpregnant, non-breast-feeding periods. Incidence among nonpregnant, non-breast-feeding periods	This study ascertained information on male partner HIV status for some women and provided estimates of the incidence rate among women in HIV sero-discordant relationships	No
Marston et al. [56] [a]	Southern and Eastern Africa	Nested surveillance study	1994–2011	767	57240	LMP to date of delivery	Date of delivery to 12 months postpartum	Incidence during pregnant period. Incidence during breast-feeding period	vs. vs.	Incidence during nonpregnant, non-breast-feeding periods. Incidence during nonpregnant, non-breast-feeding periods	No	No
Morrison et al. [59]	Southern and Eastern Africa	Prospective cohort	1999–2004	63	3056	Pregnancy status was ascertained at each study visit. It is not clear if the pregnancy status at a current visit defined the exposure status of person-time in the segment before the current visit or the segment after the current visit, or both	Lactation status was ascertained at each study visit. It is not clear if lactation at a current visit defined the exposure status of person-time in the segment before the current visit or the segment after the current visit, or both	Incidence during pregnant period. Incidence during breast-feeding period	vs. vs.	Incidence during nonpregnant, non-breast-feeding periods (without use of hormonal contraception). Incidence during nonpregnant, non-breast-feeding periods (without use of hormonal contraception)	This study recruited a small sub-cohort of high-risk women from STI clinics, sex worker networks, and military bases, and reported incidence rates stratified by risk-group	No
Reid et al. [60]	Southern Africa	Prospective cohort	2003-2007	72	1758	Date of first positive pregnancy test to 6 weeks after first negative pregnancy test. In analytical models, 'being pregnant' was turned on in the quarter of the first positive pregnancy test and remained on through the quarter of the last positive test	Not measured	Incidence during pregnant period	vs.	Incidence during nonpregnant periods	No	NCT00076232: Evaluated the efficacy of twice daily acyclovir on HIV incidence

Contrast estimated by the IRR or hazard ratio

Table 1 (*continued*)

Publication	Region	Study design	Study years	Incident cases of HIV	Person-years	Definition of pregnancy	Definition of breast-feeding	Exposed group	vs.	Unexposed group	High-risk participant recruitment	Parent study was a clinical trial of an HIV prevention intervention
Teasdale et al. [61]c	Southern Africa	Prospective cohort	2003–2006	16	417	Variable based on pregnancy outcome. • When a live birth was reported less than 6 weeks from next study visit: date of first positive pregnancy test to date of first negative test • When a live birth was reported at least 6 weeks from next study visit: date of first positive pregnancy test to date of last positive test	Not measured	Incidence during pregnant period	vs.	Incidence during nonpregnant periods (without use of hormonal contraception)	No	NCT00121459: Evaluated the effectiveness of the vaginal diaphragm for preventing HIV infections
Studies that only contributed estimates of the IRR or hazard ratio												
Mugo et al. [62]b	Southern and Eastern Africa	Prospective cohort	2004–2007	17	231	The period between LMP to 6 weeks postpartum	Not measured	Incidence during pregnant period	vs.	Incidence during nonpregnant period	Recruited only HIV sero-discordant couples	NCT00194519: Description in Thomson et al. [54]
Vandepitte et al. [63]	Eastern Africa	Prospective cohort	2008–2011	-	-	Pregnancy status was ascertained at each study visit. It is not clear if the pregnancy status at a current visit defined the exposure status of person-time in the segment before the current visit or the segment after the current visit, or both	Not measured	Incidence during pregnant period	vs.	Incidence during nonpregnant period	95% of cohort reported sex work at enrollment	No
Wand and Ramjee [64]c	Southern Africa	Prospective cohort	2002-2005	-	-	The period between the last negative pregnancy test and the last positive pregnancy test	Not measured	Incidence during pregnant period	vs.	Incidence during nonpregnant period	No	A portion of the study population was enrolled in NCT00121459: Description in Teasdale et al. [61]

ANC, antenatal care; FSW, female sex worker; HTC, HIV testing and counseling; IRR, incidence rate ratio; LMP, last menstrual period; PrEP, preexposure prophylaxis; STI, sexually transmitted infection.
aThe Rakai Community Cohort Study was a study site for both Gray et al. [58] and Marston et al. [56]. Periods of follow-up are minimally overlapping, enabling both cohorts to contribute estimates to this meta-analysis.
bThe Partners in Prevention HSV/HIV Transmission study contributed data from the same time period to both Mugo et al. [62] and Thomson et al. [54]. To prevent double counting this cohort, we excluded the incidence rate estimated by Mugo et al. in favor of the incidence rate estimated by Thomson et al. as this estimate included additional follow-up time from the Partners PrEP study. As our inclusion criteria restricted estimates of effect to the IRR or hazard ratio, we excluded the risk ratio estimated by Thomson et al. in favor of the hazard ratio estimated by Mugo et al.
cThe Methods for Improving Reproductive Health in Africa (MIRA) study contributed data to both Wand and Ramjee [64] and Teasdale et al. [61]; however, the data included in each publication did not completely overlap. Teasdale et al. included results from both the South African and Zimbabwean MIRA site from 2003 to 2006, whereas Wand et al. included results from the South African MIRA site from 2002 to 2005. We included both studies in our main analysis but conducted sensitivity analyses where we included one of these studies at a time.

prediction interval: 1.1–10.4), breast-feeding only ($n = 17$, 3.1/100 person-years, 95% prediction interval: 1.0–9.5), and pregnancy and breast-feeding combined ($n = 5$, 4.6/100 person-years, 95% prediction interval: 1.4–15.4). We, therefore, combined estimates into a single HIV incidence rate during 'pregnancy and breast-feeding' for subsequent analyses. The estimated average of the HIV incidence rates during pregnancy and breast-feeding was 3.6 per 100 person-years (95% prediction interval: 1.2–11.1; Figure S1, http://links.lww.com/QAD/B653). Our results were unchanged after excluding one study with follow-up exceeding 24 months postpartum [48]. There was no visual or statistical evidence of funnel plot asymmetry ($P = 0.3$). Cochrane's Q statistic indicated evidence of heterogeneity ($P < 0.001$), which was consistent with the wide 95% prediction interval.

The average HIV incidence rate among pregnant and breast-feeding women was associated with age, calendar time, study design, and method of estimating the timing of HIV infection (Table 2). Average HIV incidence rates were lower among women at least 30 years old than among women less than 20 years old (ratio of average incidence rates: 0.5, 95% CI: 0.3–0.7), and this inverse relationship was robust to different categorizations of age (Table S3, http://links.lww.com/QAD/B659). HIV incidence appeared to have an inverted u-shaped association with calendar time (Figure S2, http://links.lww.com/QAD/B654). After adjusting for region and length of study, the average incidence rate for studies conducted after 2014 was 0.4 times the average rate for studies conducted prior to 2010 (95% CI: 0.2–0.7). Incidence was also associated with study design. Average rates were the highest among cross-sectional studies (4.7/100 person-years, 95% prediction interval: 1.6–13.5), followed by prospective cohort studies (3.4/100 person-years, 95% prediction interval: 1.2–9.4) and surveillance studies (2.2/100 person-years, 95% prediction interval: 0.6–7.4). Studies that defined the date of seroconversion as the date of the first positive HIV test observed higher incidence rates than studies that used a date between the last negative and first positive HIV test (ratio of average incidence rates: 4.3, 95% CI: 1.4–13.2).

After stratifying by type of high-risk population, we observed higher estimated incidence rates among pregnant and breast-feeding women with known HIV-positive partners than rates estimated in a more general study population (ratio of average incidence rates: 4.7, 95% CI: 2.2–10.2; Table S4, http://links.lww.com/QAD/B660).

Pregnancy and HIV acquisition
Ten studies contributed estimates of the association between pregnancy and HIV acquisition. In four, nonpregnant, non-breast-feeding periods served as the referent [55,56,58,59]; in six, nonpregnant periods (which included breast-feeding) were defined as the referent [57,60–64]. There were variability definitions of 'nonpregnant' and 'nonpregnant/non-breast-feeding' because of heterogeneous definitions of pregnancy and breast-feeding (Table 1). All studies used methods that allowed women to contribute person-time to both exposed and unexposed periods.

The average hazard ratio estimating the association between pregnancy and risk of HIV acquisition was 0.9 (95% prediction interval: 0.2–3.8; Figure S3, http://links.lww.com/QAD/B655). Although we observed statistical evidence of funnel plot asymmetry ($P = 0.05$), results were largely unchanged after using a trim-and-fill analysis to impute one possibly missing result (average hazard ratio: 1.0, 95% prediction interval: 0.3–3.3). We also observed evidence of heterogeneity among study-specific estimates of the association ($P < 0.001$), which was consistent with the wide 95% prediction interval spanning the null. Stratified analyses and meta-regression revealed limited evidence of associations between the average hazard ratios and the measured characteristics of contributing studies (Table 3). Two estimates were generated by studies with partially overlapping cohorts [61,64]; exclusion of either did not change these results substantially (Tables S5, http://links.lww.com/QAD/B661 and S6, http://links.lww.com/QAD/B662).

Breast-feeding and HIV acquisition
Four studies compared the risk of HIV acquisition during breast-feeding to risk during nonpregnant and non-breast-feeding periods. The average hazard ratio estimating the association between breast-feeding and risk of HIV acquisition was 1.0 (95% prediction interval: 0.6–1.6; Figure S4, http://links.lww.com/QAD/B656). We did not observe statistical evidence of funnel plot asymmetry ($P = 0.2$). Compared with estimates of the association between pregnancy and risk of HIV acquisition, estimates of the association between breast-feeding and risk of HIV acquisition were more tightly clustered around the null. We observed little evidence of heterogeneity between the study-specific hazard ratio estimates ($P = 0.6$), and our analyses revealed limited evidence of associations between the average hazard ratios and the measured characteristics of contributing studies (Table 4).

Discussion

In this meta-analysis update − which included 15 new studies and over 77 000 additional person-years of follow-up − the estimated average HIV incidence rate among pregnant and breast-feeding women was above the 'substantial risk' threshold described by the WHO [3], whereas the estimated average associations between pregnancy and risk of HIV acquisition, and breast-feeding and risk of HIV acquisition, were close to the null.

Table 2. Stratified analysis and meta-regression of the incidence rate of HIV during pregnancy and breast-feeding.

Variable	Stratum-specific estimates			Univariate meta-regression				Multivariate meta-regression[a]			
	Number of estimates	Pooled incidence rate	(95% PI)	Ratio of the pooled incidence rate	(95% CI)	P value	Omnibus P value	Ratio of the pooled incidence rate	(95% CI)	P value	Omnibus P value
Region											
Southern Africa	18	4.2	(1.4–12.3)	1.0	–	–	–	1.0	–	–	–
Eastern Africa	10	3.3	(1.1–9.9)	0.8	(0.5–1.2)	0.3	–	0.7	(0.4–1.1)	0.1	–
Southeastern Africa	3	2.4	(0.7–8.1)	0.6	(0.3–1.1)	0.1	–	0.9	(0.3–2.3)	0.8	–
Western Africa	3	2.9	(0.7–11.5)	0.7	(0.3–1.8)	0.4	0.3	0.7	(0.3–1.8)	0.4	0.4
Calendar time[b]											
Pre-2010	23	4.1	(1.4–12.2)	1.0	–	–	–	1.0	–	–	–
2010–2014	8	2.9	(0.9–8.8)	0.7	(0.4–1.2)	0.2	–	0.7	(0.4–1.2)	0.2	–
Post-2014	4	2.1	(0.7–6.5)	0.5	(0.3–0.9)	0.02	0.04	0.4	(0.2–0.7)	0.002	0.007
High-risk cohort											
No	32	3.6	(1.2–11.3)	1.0	–	–	–	1.0	–	–	–
Yes	4	4.3	(1.2–15.9)	1.2	(0.5–1.8)	0.8	0.8	1.2	(0.6–2.5)	0.3	0.3
Participants enrolled in an HIV-prevention clinical trial											
No	29	3.5	(1.2–10.5)	1.0	–	–	–	1.0	–	–	–
Yes	5	4.3	(1.3–14.2)	1.2	(0.6–2.4)	0.6	0.6	1.9	(0.9–4.1)	0.2	0.2
Age group											
<20 years	7	3.8	(1.5–10.1)	1.0	–	–	–	1.0	–	–	–
20–24 years	6	2.8	(1.1–7.4)	0.7	(0.6–1.0)	0.05	–	0.7	(0.5–1.0)	0.05	–
25–29 years	5	2.5	(0.9–6.6)	0.6	(0.5–0.9)	0.01	–	0.6	(0.4–0.9)	0.01	–
30+ years	6	1.8	(0.7–4.8)	0.5	(0.3–0.7)	<0.001	<0.001	0.5	(0.3–0.7)	<0.001	<0.001
Undefined	16	–	–	–	–	–	–	–	–	–	–
Study design											
Prospective cohort study	21	3.4	(1.2–9.4)	1.0	–	–	–	1.0	–	–	–
Cross-sectional retesting study	11	4.7	(1.6–13.5)	1.4	(0.9–2.1)	0.1	–	1.8	(1.0–3.4)	0.06	–
Nested surveillance study	2	2.2	(0.6–7.4)	0.6	(0.3–1.4)	0.2	0.1	0.9	(0.3–2.8)	0.8	0.1
Use of results from repeat HIV testing to identify HIV seroconversions											
Yes	30	3.7	(1.3–11.1)	1.0	–	–	–	1.0	–	–	–
No	4	2.8	(0.8–9.4)	0.8	(0.4–1.4)	0.4	0.4	0.6	(0.2–1.6)	0.3	0.3
Reproductive periods observed during study follow-up											
Pregnancy only	11	4.3	(1.4–13.0)	1.0	–	–	–	1.0	–	–	–
Breast-feeding only	5	3.8	(1.2–11.9)	0.9	(0.5–1.6)	0.7	–	0.9	(0.4–1.7)	0.6	–
Pregnancy and breast-feeding	10	3.8	(1.2–11.6)	0.9	(0.5–1.5)	0.7	–	0.7	(0.4–1.3)	0.2	–
Pregnancy, breast-feeding, nonpregnant/non-breast-feeding	8	2.7	(0.9–8.3)	0.6	(0.4–1.1)	0.1	0.4	0.6	(0.3–1.3)	0.2	0.6
Estimated timing of HIV seroconversion											
Date between last negative and first positive HIV test	11	2.6	(0.9–7.3)	1.0	–	–	–	1.0	–	–	–
Date of the first positive HIV test	4	5.3	(1.8–16.1)	2.0	(1.1–3.7)	0.02	0.02	4.3	(1.4–13.2)	0.01	0.01
Undefined	19	–	–	–	–	–	–	–	–	–	–
HIV RNA/DNA PCR used in HIV testing algorithm											
No	21	4.2	(1.5–12.0)	1.0	–	–	–	1.0	–	–	–
Yes	5	3.1	(1.0–9.5)	0.7	(0.4–1.3)	0.3	0.3	0.9	(0.4–2.1)	0.8	0.8
Undefined	8	–	–	–	–	–	–	–	–	–	–

CI, confidence interval; HR, hazard ratio; PI, prediction interval.
[a]Unless otherwise indicated, models were adjusted for region, length of study implementation, and calendar time.
[b]Multivariate meta-regression adjusted for region and length of study implementation only.

Table 3. Stratified analysis and meta-regression of the association between pregnancy and risk of HIV acquisition.

Variable	Stratum-specific estimates			Univariate meta-regression results			
	Number of estimates	Pooled HR	(95% PI)	Ratio of the pooled HR	(95% CI)	P value	Omnibus P value
Region of Africa							
Southern Africa	4	0.8	(0.2–3.9)	1.0	–	–	–
Eastern Africa	3	0.9	(0.2–4.8)	1.1	(0.3–3.7)	0.9	–
Southeastern Africa	3	0.9	(0.2–4.5)	1.1	(0.4–3.3)	0.9	1.0
High-risk cohort							
No	7	0.9	(0.2–3.5)	1.0	–	–	–
Yes	3	0.8	(0.2–3.8)	0.9	(0.3–2.6)	0.8	0.8
Participants enrolled in an HIV-prevention clinical trial							
No	6	0.7	(0.2–2.5)	1.0	–	–	–
Yes	4	1.2	(0.3–4.4)	1.7	(0.7–3.9)	0.2	0.2
Study design							
Prospective cohort study	8	1.1	(0.3–3.4)	1.0	–	–	–
Nested surveillance study	2	0.6	(0.2–2.3)	0.6	(0.2–1.5)	0.2	0.2
Use of HIV RNA/DNA PCR in HIV testing algorithm							
No	5	1.1	(0.3–4.4)	1.0	–	–	–
Yes	3	0.9	(0.2–3.8)	0.8	(0.3–2.3)	0.7	0.7
Undefined	2	–	–	–	–	–	–
Estimated timing of HIV seroconversion							
Date between last negative and first positive HIV test	5	1.0	(0.3–3.3)	1.0	–	–	–
Date of the first positive HIV test	3	1.0	(0.3–3.7)	1.1	(0.4–2.7)	0.9	0.9
Undefined	2	–	–	–	–	–	–
Referent group							
Not pregnant	6	0.8	(0.2–3.3)	1.0	–	–	–
Not pregnant or breast-feeding	4	1.0	(0.2–4.4)	1.2	(0.5–3.1)	0.7	0.7
Adjustment for confounders							
Yes	8	0.8	(0.2–2.8)	1.0	–	–	–
No	2	1.3	(0.3–5.8)	1.6	(0.6–4.6)	0.4	0.4
Adjusted for time-varying measures of condom use and intercourse frequency[a]							
No	5	0.7	(0.2–2.8)	1.0	-	-	-
Yes	3	1.0	(0.2–4.1)	1.3	(0.5–3.7)	0.6	0.6

CI, confidence interval; HR, hazard ratio; PI, prediction interval.
[a]Only among studies that used adjusted models.

Prediction intervals around each of our summary estimates were wide, highlighting the variability of HIV incidence across populations of pregnant and breast-feeding women in SSA.

Our results were consistent with findings from a previous meta-analysis that reported high average HIV incidence during pregnancy and breast-feeding [2]. Hormonal changes during pregnancy may increase susceptibility to HIV through changes in the vaginal epithelial thickness, microbiome, and CCR5 coreceptor expression [65,66]. Pregnancy activates the innate immune system, increasing inflammation and concentration of dendritic cells in the female genital tract, while suppressing the adaptive immune response [67,68]. Such immunologic changes may increase risk of HIV acquisition [69–71], and can last for several months postpartum [72,73]. Behavioral changes occurring during pregnancy may also influence risk of HIV acquisition. Couples may be more likely to engage in unprotected sex during pregnancy [34,58,74], and male partners may be more likely to seek extra-partnership sexual liaisons during extended periods of pregnancy-related or breast-feeding-related abstinence [34,75–77].

Substantial heterogeneity of the incidence rates, however, cautions us from interpreting the average HIV incidence rate estimated in this study as the incidence rate among pregnant and breast-feeding women in SSA. Our results suggest maternal HIV incidence rates may lower among older compared with younger pregnant and breast-feeding women, and higher among women in HIV serodiscordant relationships. Additionally, we observed changes in average HIV incidence over calendar time that follow temporal trends observed in the region since the 1980s: a steady rise in HIV incidence until the early 2000s [78], largely driven by increasing HIV prevalence without viral suppression [79], followed by a slow decline that may be attributed to expanded HIV testing and counseling, medical male circumcision, and ART services. Inverted u-shaped trends in HIV incidence over time have been observed in large population-based cohorts in SSA [10–12], with reported associations between HIV incidence and community-level coverage of ART and medical male circumcision. Models predict that integrated behavioral and biomedical interventions will reduce HIV incidence generally [80,81], and among pregnant women specifically [82], and two cluster randomized trials of combination HIV prevention with universal ART

Table 4. Stratified analysis and meta-regression of the association between breast-feeding and risk of HIV acquisition.

Variable[a]	Stratum-specific estimates[b]			Univariate meta-regression results[c]			
	Number of estimates	Pooled HR	(95% PI)	Ratio of the pooled HR	(95% CI)	P value	Omnibus P value
Region of Africa							
Southeastern Africa	2	0.9	(0.8–1.0)	–	–	–	–
Eastern Africa	1	1.1	NA	NA	NA	NA	NA
Southern Africa	1	1.2	NA	NA	NA	NA	NA
Study design							
Prospective cohort	2	1.1	(0.9–1.5)	–	–	–	–
Surveillance study	2	0.9	(0.8–1.0)	0.8	(0.6–1.1)	0.2	0.2
Estimated timing of HIV seroconversion							
Date between last negative and first positive HIV test	3	1.0	(0.9–1.1)	–	–	–	–
Date of the first positive HIV test	1	1.1	NA	NA	NA	NA	NA

CI, confidence interval; HR, hazard ratio; PI, prediction interval.
[a]There was limited variability in the population and methodological features of included studies. All estimates were adjusted for confounders and generated by studies that used 'not pregnant, not breast-feeding' as the referent group. No estimates were generated among a high-risk population or among a population enrolled in an HIV-prevention clinical trial. Only prospective cohort studies adjusted for time-varying sexual behaviors.
[b]95% PI computed only when the number of estimates in the strata exceeds 1.
[c]Meta-regression computed only when the number of estimates exceeded 1 in both the comparison group and the referent stratum.

demonstrated some reductions in community-wide HIV incidence [83,84]. Although we expect that HIV-negative pregnant and breast-feeding women may serve as beneficiaries of expanded combination HIV prevention, impact will likely vary across sub-groups.

Prediction intervals around estimates of the average association between pregnancy and risk of HIV acquisition and between breast-feeding and risk of HIV acquisition, were wide with lower and upper bounds on either side of the null. This variability is not unexpected; pregnancy and breast-feeding are periods marked by significant biological and behavioral changes that may have different effects on risk of HIV. For example, the potential increased risk of HIV arising from the pregnancy-induced physiological changes described earlier may be offset by a reduction in sexual intercourse that frequently occurs during late pregnancy and early breast-feeding [34,54,58,74]. The direction of the observed association between pregnancy or breast-feeding and risk of HIV acquisition may, therefore, depend on both study context and analytical decisions regarding covariate measurement and adjustment [85]. Furthermore, as the physiological and behavioral changes that accompany pregnancy and breast-feeding are dynamic, decisions regarding how to define pregnancy, breast-feeding, and the referent state may influence the direction of the observed association. For example, the inclusion of breast-feeding in the referent group may produce estimates closer to the null as incidence rates during breast-feeding appear similar to those during pregnancy, whereas single categories for pregnancy and breast-feeding may obscure periods during pregnancy or breast-feeding when risk is truly elevated or suppressed. Work by Thomson et al. [54] suggests that physiological changes during pregnancy increase susceptibility to HIV, particularly in late pregnancy and early breast-feeding. However, additional work is needed to better understand the interaction between biological susceptibility and behavioral changes on risk of HIV acquisition among pregnant and breast-feeding women in different SSA contexts.

Our results should be interpreted in light of possible limitations. It is unclear if contributing studies enrolled representative cohorts of women, so the extent to which our estimates generalize to all pregnant and breast-feeding women in SSA is unknown. It is possible that investigators targeted clinics in areas of elevated HIV incidence, which may bias estimates of incidence upwards. Few estimates of the incidence rate captured the first trimester of pregnancy, and given the variability of risk over the course of pregnancy [54,58,62], this may bias estimates of incidence. The directionality of this bias is unclear; two studies report higher incidence during early compared with late pregnancy [58,62], whereas one reports the reverse [54]. For this reason, misclassification of early or late pregnancy-exposed periods as nonpregnant person-time may also bias estimates of the association in unknown directions. Finally, our analyses were restricted by the number of studies and the information provided by each study. The small number of estimates may have limited our power to detect associations between estimates and underlying sources of heterogeneity. Differences in populations and methodological features of contributing studies may not have been adequately captured by variables used in meta-regression models, and several important population features were unmeasured by contributing studies.

Although many countries in SSA have placed considerable focus on identifying and treating HIV-infected pregnant and breast-feeding women, HIV-uninfected women have received considerably less attention in antenatal and postnatal settings. Our results support the expansion of bio-behavioral HIV prevention interventions and repeat testing throughout pregnancy and breast-

feeding to women at high risk of HIV acquisition. Further work is needed to identify risk factors for HIV acquisition during pregnancy and breast-feeding to facilitate targeted prevention interventions in antenatal and postnatal settings. Offering female-controlled strategies, such as tenofovir-based oral preexposure prophylaxis, and promoting couple-based prevention approaches in these settings, are important next steps that may reduce the risk of HIV-related maternal morbidity and mortality, and ensure continued progress towards the elimination of mother-to-child transmission of HIV.

Conflicts of interest
There are no conflicts of interest.

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