Postnatal HIV Transmission rates at age six and 12 months in infants of HIV-infected women on ART initiating breastfeeding: a systematic review of the literature

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BACKGROUND

The 2010 WHO infant feeding guidelines in the context of HIV infection recommend exclusive breastfeeding (EBF) for six months followed by complementary feeding and continued breastfeeding (CBF) for up to one year, under the cover of antiretroviral treatment (ART) to either the mother or the infant. However, when these recommendations were drawn up there was limited information about the risk of HIV transmission postnatally in women who were on ART during pregnancy and continued after delivery, or where the infant received ART prophylaxis to prevent mother-to-child transmission (PMTCT). In the past five years further evidence has become available from studies and programmes where PMTCT postnatally was achieved through maternal ART or infant ARV prophylaxis. A remaining question in this context relates to a possible increased risk of transmission when infants receive other feeds in addition to breastmilk in the first six months of life, which in the pre-ART and pre-PMTCT era has been shown to be associated with a substantially increased risk.

In October 2015 the 2010 WHO guidelines on HIV and Infant Feeding will be reviewed in light of the expanding use of ART in pregnant and breastfeeding women. In addition to the question on HIV-free survival by duration of maternal ART and duration of breastfeeding, addressed in a separate review, the other question that this meeting will address is: Is maternal ART effective at reducing the risk of postnatal HIV transmission even if the mothers mix breastfeed their infants?

We present the results from a systematic review and GRADE Evidence summary tables in preparation for this WHO guideline meeting, addressing the question of overall and postnatal HIV transmission rates at six, nine and 12 and 18 months in infants born to women who were on ART, by infant feeding modality in the first six months of life. We also present additional information, provided for each study included here, on infant feeding recommendations given to women in the studies.

METHODS

INCLUSION CRITERIA

Types of studies

The review considered both experimental and observational studies e.g. randomised control studies, cohort studies and longitudinal studies, and included HIV positive mothers receiving antiretroviral therapy and their breastfed children. Infants may also have received prophylactic ARVs as per WHO 2010 guidelines.

Types of participants

HIV positive mothers receiving antiretroviral therapy (ART) from before or early pregnancy until at least 6 months postpartum and their breastfed children.

Types of exposures

The exposures were HIV antiretroviral therapy and feeding modality during breastfeeding (exclusive breastfeeding, mixed feeding).

Outcome Measures

The outcome measures were overall HIV transmission rate between birth and 6 months of age, and postnatal transmission rate between 4-6 weeks and 6 months, and overall and postnatal transmission rates at 12, 18 or 24 months, as provided by the study.

SEARCH STRATEGY

SB searched the English literature from multiple electronic databases including PubMed, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, and CINAHL for articles with a time limit of 2005 to 2015 (Table 1). The search words are shown in Table 2 and also provided in Appendix 1.

SB undertook the initial search of literature in discussion with LC and MLN. The search terms were adapted for other databases. Reference lists from relevant studies, grey literature as well as conference abstracts available online from the following conferences were also searched: the International IAS AIDS Conference in Melbourne 2014 and the 2013-2015 Conferences on Retroviruses and Opportunistic Infections (CROI).

Table 1. Electronic databases and conferences searched for Systematic Review 2.

Source	A: Identified	B: Selected by abstract[b]	C: Selected for full screening[c]	D: Included	D / B (%)
MEDLINE	506	50	25	9	18%
Web of Science	933	22[a]	10	2	9%
Conferences	-	-	-	-	-
TOTAL	**1439**	**72**	**35**	**11**	

a Excluded duplicates
b Abstracts were rejected if mothers were not on ART or transmission rates or number of infected children were not provided at 6 months; where there was any detail regarding population and outcome, the paper was selected for full screening
c Full texts were rejected if outcome was not provided or when it was not possible to identify outcome by ART or feeding status.

Table 2. Search Terms

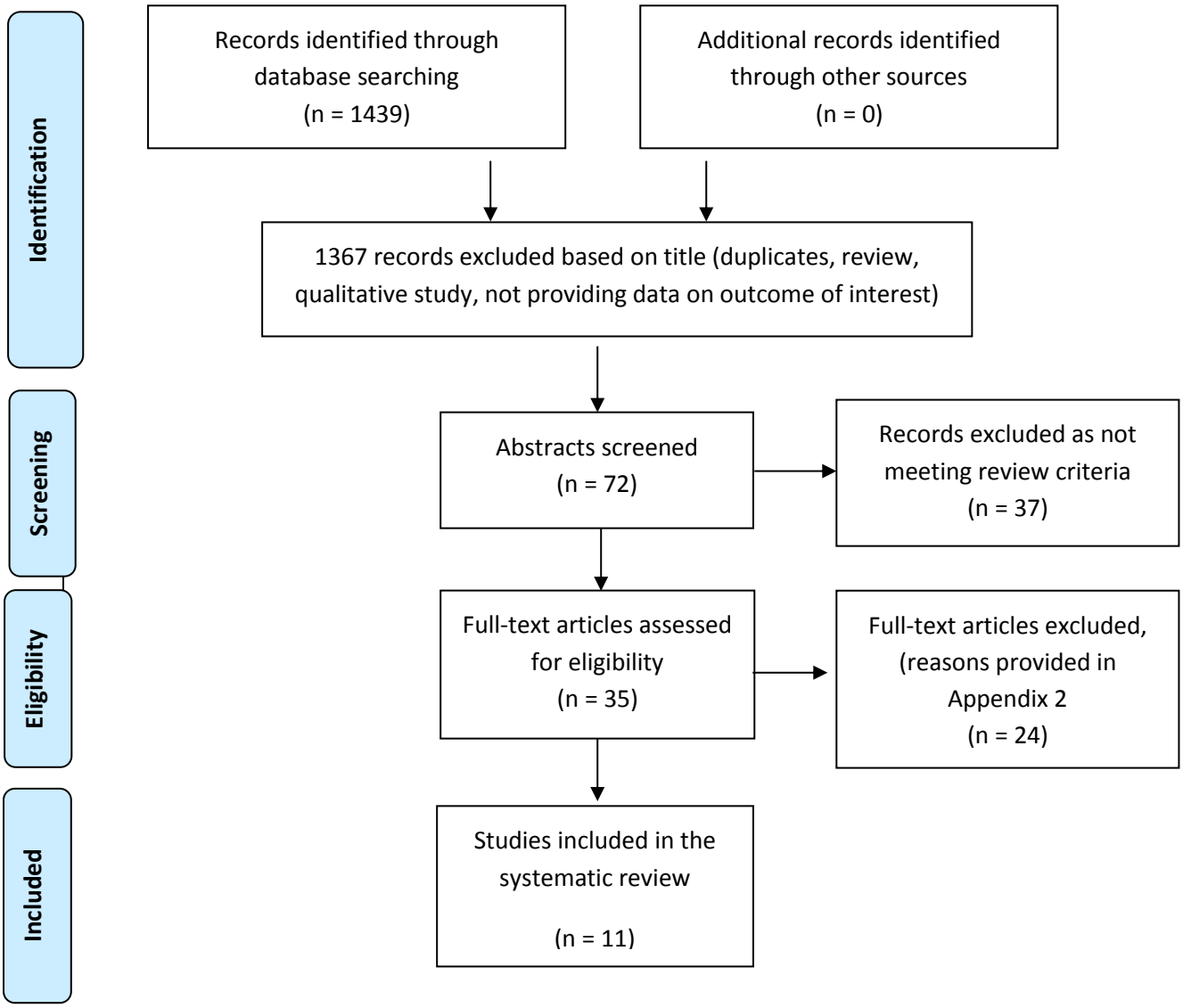
Domain	Description	Search Terms
Antiretroviral	*Maternal*	Maternal, mother*
	Antiretroviral	Antiretroviral, antiretroviral therapy, ART, ARV, HAART
HIV transmission	*HIV transmission*	HIV, Transm*, Transmission, infec*
Feeding	*Feeding type*	Breastfeeding, breast*, postnatal, feed*, mixed feed*, mixed
Publication Dates	*January 2005 to September 2015*	

Search terms were linked by OR or AND
** Used to include all the terms starting with the letters before the star*

The search process identified 1,439 citations, of which 1,367 were excluded on the basis of being a duplicate, review, qualitative study or not evaluating transmission at six months according to feeding modality (Figure 1). The abstracts of 72 studies were evaluated by SB and LC, and 35 studies were selected for full screening (Appendix 2 and 3). SB, LC and MLN went through the full screening, and 11 studies were selected. No studies were identified in addition to the first systematic review on HIV-free survival (reported separately) for this second systematic review; 11 studies included in the first review were also analysed in this review.

Agreement was 100% between the three reviewers (SB, LC, MLN). Studies were excluded mainly for not reporting transmission at six months of age, for not having mothers on ART and for describing feeding modality in detail without reporting transmission rates or number of infected children. The flow chart of the screening process is shown in Figure 1.

Figure 1. Flowchart of screening process



In addition, to obtain further information for this and the first report, we contacted ten first authors of papers identified in the first systematic review, to solicit additional information regarding infant feeding modality in the first six months of life. Seven questions were asked: 1) What feeding practice did the study team recommend to HIV-infected mothers? 2) If

breastfeeding, for how long were HIV-infected mothers recommended to breastfeed? 3) 3. What type of support was provided to mothers to assist them in their feeding practice (e.g. frequency of counselling before and after delivery, facility or community-based, home visits, skill of counsellors, support provided if mother encountered difficulties, availability of free formula milk)? 4) How was data regarding infant feeding practices collected e.g. self-reporting, use of feeding diaries, frequency of interviews, independent field team? 5) Were feeding practices disaggregated in the study database i.e. mixed feeding, exclusive breastfeeding? Was feeding type (exclusive vs. mixed feeding) included within a model examining postnatal transmission risk in the context of ARVs? 6) If feeding practices were characterised in the database, how were exclusive breastfeeding rates estimated i.e. a cross-sectional practice at e.g. 4 or 5 months, or estimated as cumulative practices determined by considering all data from birth? 7) What proportion of HIV-infected women in the study were truly exclusively breastfeeding at 4-6 months – is this from actual data or is this more of an impression from staff in the field?

ASSESSMENT OF QUALITY OF STUDIES

Newcastle-Ottawa Scale

A modified Newcastle-Ottawa Scale (NOS) was developed by authors to assess the quality of all studies included in the analysis; the criteria for assessment of the quality of studies are provided in Appendix 4. All studies were cohorts; four studies were nested in RCTs in which the randomisation was not based on the intervention of interest (feeding modality). Stars were awarded for each study based on selection of study participants and assessment of outcomes; assessment did not use all aspects of the NOS because the papers included in this research only assessed HIV transmission in breastfed children whose mothers were on ART, and did not include a comparison group [1]. Each study could score a maximum of five stars on Selection and four on Outcome. The factors considered included the representativeness of the study population in terms of the underlying population of HIV-positive pregnant women accessing PMTCT programme, ascertainment of exposure to ART and breastfeeding, report of maternal adherence to ART and feeding modality. Ascertainment of outcome (HIV transmission at six months of age and later) included type of outcome provided (rate including denominator at six months and later or only number of infected children) and whether the outcome was stratified by feeding, length of follow up and loss to follow up (Appendix 4).

GRADE

The information obtained from the NOS was used to comment on the quality of the included studies in GRADE. The quality assessment also considered study limitations, consistency of results, directness, imprecision and reporting bias. We further took into account the fact that quality of evidence could increase based on the direction of plausible bias. The GRADE Evidence Profiles are presented at the end of the results.

Synthesis of evidence

The studies covered different types of interventions and varied with regard to the outcomes of interest of indication, timing of initiation and duration of maternal ART, breastfeeding recommendations and practice. A detailed description of information provided by each study is given in Appendix 3. We present the evidence using a narrative synthesis, in addition to providing pooled estimates of transmission rates, with heterogeneity scores based on a random effects meta-analysis in Stata. Random effects regression is recommended for use in analysis of studies that were conducted differently [2]. The pooled estimate therefore represents the average estimate of HIV-free survival across the studies included in the analysis. We summarised the information in graphs depicting HIV transmission rates at six and 12 months of age. For most cases confidence intervals for estimates of HIV transmission were given, but where no confidence interval was available from the paper, a confidence interval was calculated based on the number of events and those at risk using the formula described by Eayres and shown in Appendix 6 [3].

RESULTS

Eleven studies were selected;, four of which were cohorts nested within randomised clinical trials [4-7]. In all studies mothers started ART before or during pregnancy, and continued until at least six months postnatally, according to the WHO recommendations at the time which recommended that mothers should initiate ART for PMTCT during pregnancy and stop at cessation of breastfeeding around six months postnatally. Eight studies followed this recommendation [4-11] with six-month ART, two provide lifelong ART for all women [12, 13] while Giuliano et al., 2013 [14] provided lifelong ART only for treatment-eligible mothers with very low CD4 count.

Quality of included studies

Table 3 presents the assessment of the quality of the studies based on the modified Newcastle-Ottawa Scale (Appendix 4). Ngoma et *al.* (2015) had the highest quality in terms of selection (5 stars) followed by Sagay et al (2015), Jamieson et al (2012) and Thomas et al (2011), all with three stars. For outcome, Alvarez-Uria et al (2012) had the highest quality (4 stars) since the outcome was stratified by feeding modality, however, no denominators were provided according to feeding modality and thus rates of transmission could not be calculated by infant feeding. Detailed rating of the studies is shown in Appendix 5.

Table 3. Modified Newcastle-Ottawa for assessment of HIV transmission in breastfed infants whose mothers were on ART

Author	Country	Selection	Outcome
Ngoma et al, 2015	Zambia	*****	**
Sagay et al, 2015	Nigeria	***	**
Thakwalakwa et al, 2014	Malawi	*	**
Giuliano et al, 2013	Malawi	**	**
Coovadia et al, 2012	South Africa, Tanzania, Uganda and Zimbabwe	**	**
Jamieson et al, 2012	Malawi	****	***
Alvarez-Uria et al, 2012	India	**	****
Thomas et al, 2011	Kenya	****	***
Marazzi et al, 2009	Mozambique	**	**
Kilewo et al, 2009	Tanzania	***	***
Peltier et al, 2009	Rwanda	****	***

HIV Transmission

Of the 11 studies identified, six reported the transmission rate at age six months [4, 7-10, 12], and the remaining five reported HIV transmissions after age six months [5, 6, 11, 13, 14]. For the estimates of Ngoma et al. (2015) and Marazzi et al. (2009), a confidence interval was not provided, but instead was calculated using the formula in Appendix 6. Two studies reported the number of infections at six months and the number of children at risk: Sagay et al (2015), which was a retrospective cohort and provided only one number as a denominator (N=856); and Jamieson et al (2012), for which the number of children at risk was obtained from Kaplan-Meier. A further two studies reported the number of transmissions but not the number of children at risk (Thakwalakwa et al, 2014 and Giuliano et al, 2012), and one study reported transmission only at age nine months, but noted that all transmissions occurred during breastfeeding (Peltier et al, 2009).

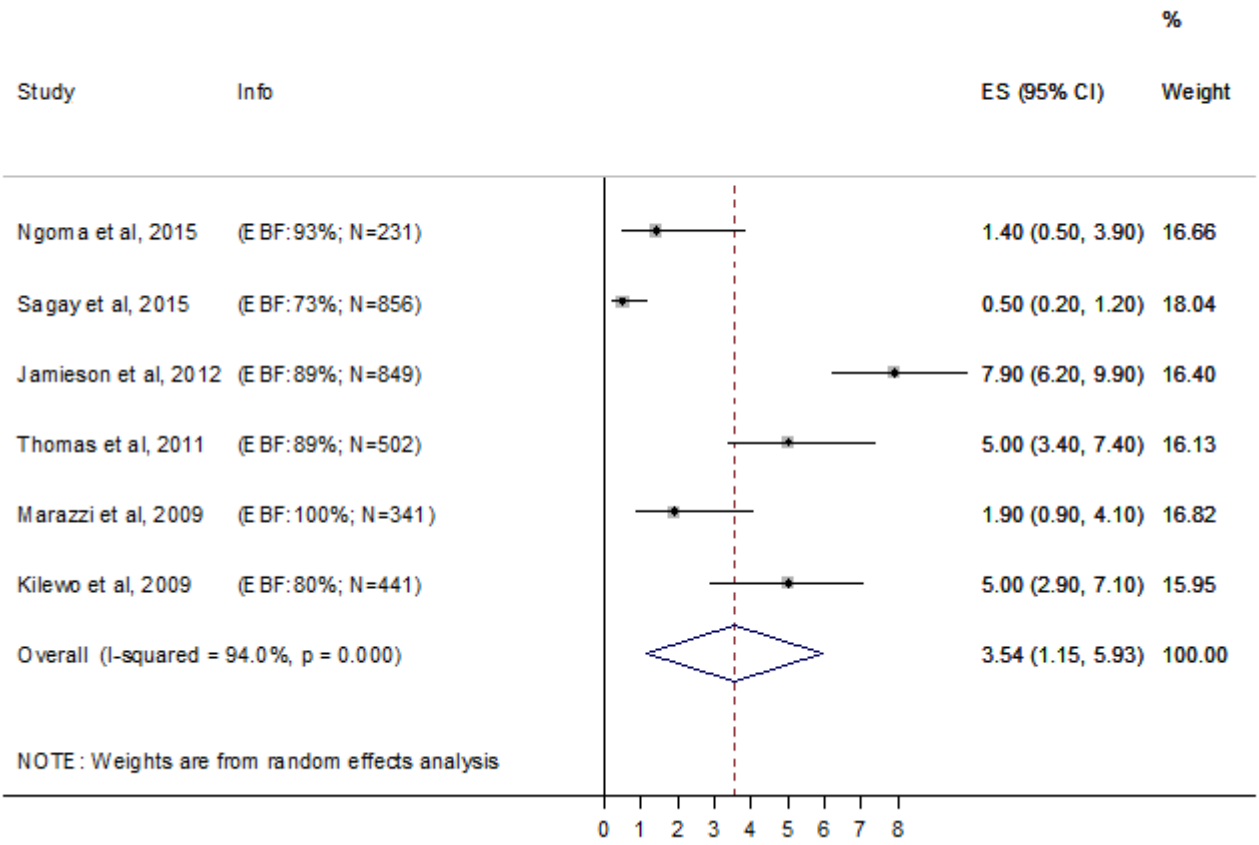
Overall transmission at age six months

Six studies provided overall transmission rates at age six months, which included peripartum transmissions [6,7, 9, 10, 12, 13]. Ngoma et al (2015) reported three peripartum transmissions (before six weeks, number of children at risk=219), and no postnatal transmissions between six weeks and six months, with an overall transmission rate at six months of 1.4% (95% CI 0.5% to 3.9%). Sagay et al (2015) reported a total of four infections at six months (N=856 was the only denominator provided; rate of transmission and CI calculated as 0.5%; 95% CI 0.2% to 1.2%). Thomas et al (2011) reported 24 transmissions, of which 20 occurred before six weeks, for an overall transmission rate of 5.0%; 95% CI 3.4%-7.4% (N=487). Kilewo et al (2009) reported 22 infections at six months, 18 before six weeks (N=423; overall transmission rate 5.0%; 95% CI 2.9%-7.1%). Marazzi et al (2009) reported six transmissions at six months (N=313, overall transmission rate 1.9%; 95% CI 0.9%-4.1%). Jamieson et al (2012) reported 67 infections; 21 infections after six weeks (N=849; overall transmission rate at age six months was 7.9%; 95% CI 6.2%-9.9%). Figure 2 shows the analyses of these six studies with overall transmission rates. The pooled estimate of overall transmission at six months was 3.54% (95% CI 1.15% to 5.93%), with considerable heterogeneity between studies (I^2 = 94%).

Jamieson et al. (2012) further provided HIV transmission rates up to 28 weeks (7 months) in three arms: infants whose mothers were on ART, infants who were on nevirapine but mothers

not on ART and a placebo group. The estimates for HIV transmissions in the three arms were: 7.9% (95% CI 6.2%-9.9%), 6.0% (95% CI 4.3%-7.4%) and 10.0% (95% CI 8.0%-12.7%) in the mother ART, infants on nevirapine and a placebo group respectively.

Figure 2. Overall transmission rate (including peripartum) at age **six** months, with 95% confidence intervals, in children who were breastfed and whose mothers were on ART.



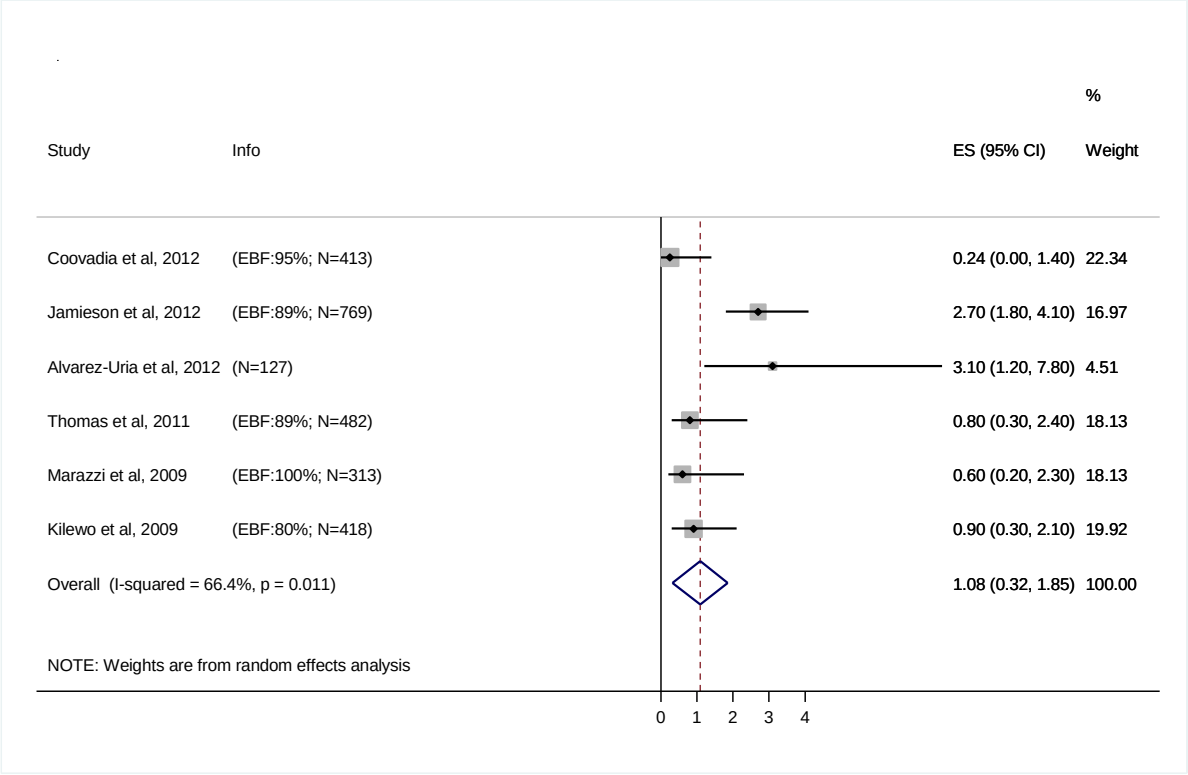
Note: Sagay et al (2015) and Jamieson et al (2012) did not provide a rate of transmission, but the rate was calculated on the basis of the number of children at risk provided in the paper. For Jamieson et al (2012) transmission rate was provided at 28 weeks. For Ngoma et al (2015) and Marazzi et al (2009), a confidence interval was not provided in the paper but calculated using the formula in Appendix 6.

Postnatal transmission between 4/6 weeks and 6 months

Six studies provided estimates of postnatal transmission rates, excluding peripartum infections diagnosed before six weeks of age; Marazzi et al (2009) provided the rate of transmission after four weeks of age; Coovadia et al (2012) reported one infection (N=413, rate 0.2%; 95%CI 0%-1.4%); Alvarez-Uria et al (2012) reported four (out of N=127) infections for a transmission rate of 3.1% (95% CI 1.2%-7.8%), and noted that one of the infected infants received mixed feeding. Marazzi et al (2009) reported two transmissions

(2/313, rate 0.6%; 95%CI 0.2%-2.3%). Thomas et al (2011) and Kilewo et al (2009) did not provide a rate of postnatal transmission at six months, but the number of transmissions and number of children at risk were reported. Both studies noted four transmissions between six weeks and six months of age (N= 482 for Thomas et al (2011), rate of postnatal transmission 0.80%; 95% CI 0.3%-2.4%; and N=418 for Kilewo et al (2009), rate of postnatal transmission was 0.90% (95% CI 0.3%-2.1%). Figure 3 shows the pooled analysis for the studies, with a pooled transmission rate of 1.08% (95%CI 0.32% to 1.85%). Heterogeneity was high (I^2 = 66.4%).

Figure 3. Postnatal transmission rate between 4-6 weeks of age and age **six** months, with 95% confidence intervals, in children who were breastfed and whose mothers were on ART.

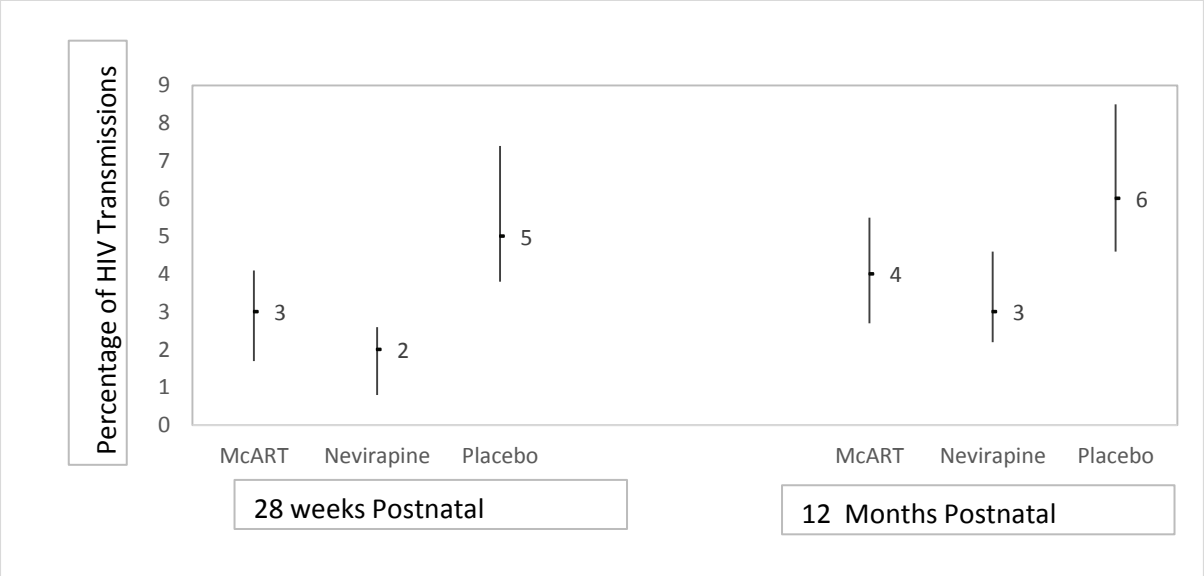


Note: Coovadia et al (2012), Alvarez-Uria et al (2012), Thomas et al (2011) and Kilewo et al (2009) excluded positive children at 6 weeks. Jamieson et al (2012) excluded transmission at 2 weeks and Marazzi et al (2009) excluded 4 weeks. Jamieson et al (2012), Thomas et al (2011) and Kilewo et al (2009) did not provide rate of transmission, but rate was calculated considering number of children at risk provided in the paper. Marazzi et al (2009), confidence interval was not provided and it was calculated using formula.

Jamieson et al. 2012 provided estimates of postnatal HIV transmissions at 28 weeks (seven months) and 12 months in three arms (Figure 4). The estimates at 28 weeks were 3% (95% CI 1.7%-4.1%), 2.0% (95% CI 0.8%-2.6%) and 5% (95% CI 3.8%-7.4%) in the mother on

ART, infants on nevirapine prophylaxis and placebo group respectively and at 12 months were 4% (95% CI 2.7%-5.5%), 3.0% (95% CI 2.2%-4.6%) and 6% (95% CI 4.6%-8.5%) in the mother on ART, infants on nevirapine prophylaxis and placebo group respectively.

Figure 4. Postnatal transmission rate at 28 weeks and 12 months of age, with 95% confidence intervals, in children who were breastfed in three different arms from Jamieson et al. 2012



Two studies reported the number of infections at six months but not the number of children at risk. Thakwalakwa et al. (2014) reported three infections before six months (280 children were born but no information provided on loss to follow up and deaths); Giuliano et al. (2013) reported four transmissions before six months, 288 children were included in the study, but the number of children at risk of transmission at six months was unclear.

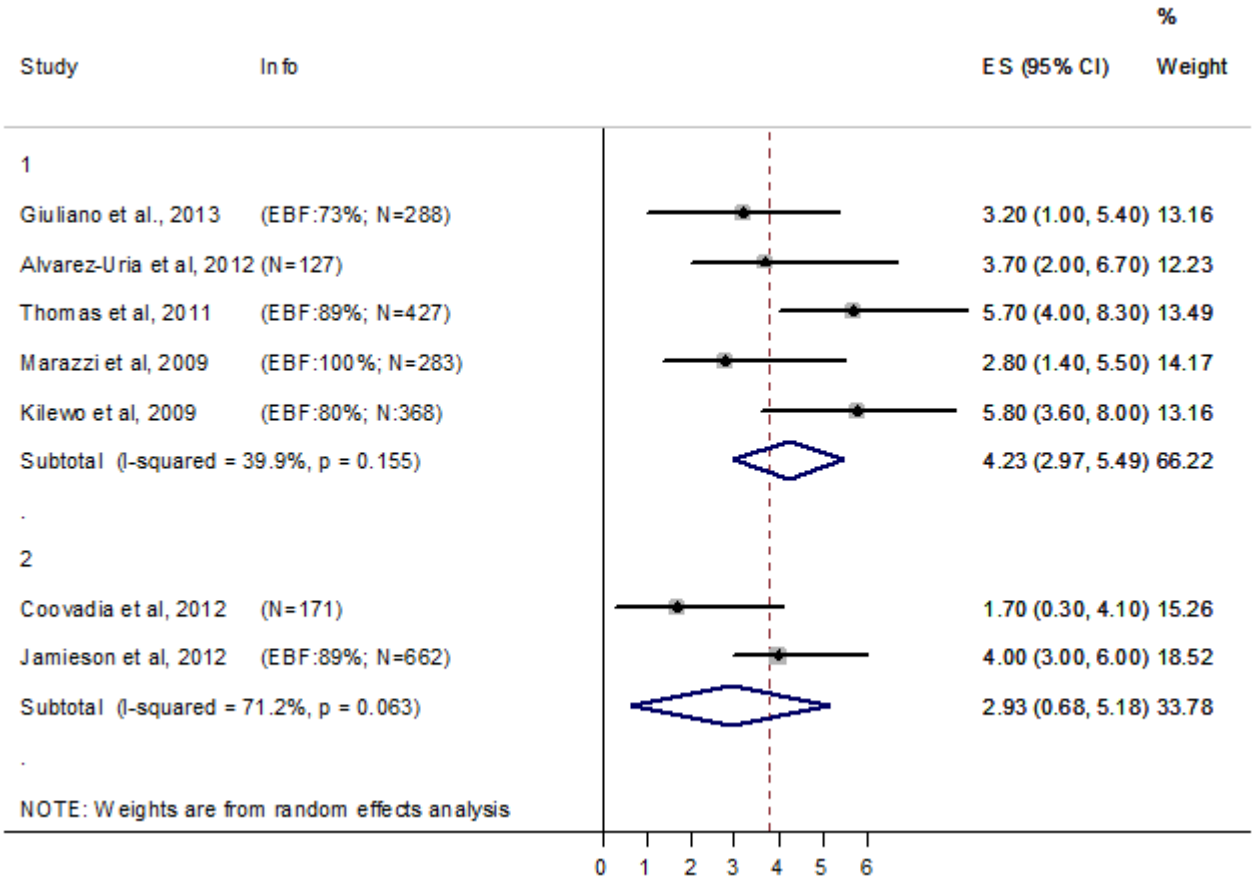
Overall rate of transmission after six months of age

Of the seven studies providing information on transmission rates at age 12 months, five reported overall HIV transmission rates (including peripartum) [7-10, 14] and two reported postnatal transmission rates [4, 6]. Coovadia et al. (2012) excluded transmission before six weeks of age, and Jamieson et al. (2012) before two weeks. The pooled estimates showed an overall rate of transmission at 12 months of 4.23% (95% CI 2.97%-5.49%); and a postnatal transmission rate of 2.93% (95% CI 0.68%-5.18) (Figure 5). Heterogeneity was higher in the

postnatal transmission group (I^2 =71.2%) than in the overall HIV transmission group (I^2 =39.9%) (Figure 5).

Ngoma et al. was the only study which reported estimated overall (including peripartum) rate of transmission at 18 months, with lifelong ART provided for all mothers. Nine transmissions were reported, with an overall transmission rate of 4.1% (95% CI 2.2%-7.6%; N=219).

Figure 5. Transmission rates at **12** months of age, with 95% confidence intervals, in children who were breastfed and whose mothers were on ART.
Group 1: Overall transmission.
Group 2: Postnatal transmission between 4/6 weeks and 12 months of age



Peltier et al (2009) provided an overall transmission rate at nine months of age, including perinatal transmission, with no transmission after cessation of breastfeeding. The total number of children at risk was 227, with four infections (only 1 after 6 weeks). The overall estimated rate of transmission at nine months was 1.8% (95% CI 0.7% to 4.8%). Only 15 mothers were reported to have mixed fed their children, but none of these were infected.

Additional information regarding feeding

No study provided a transmission rate according to infant feeding modality (exclusive breastfeeding or mixed feeding); in all studies mothers were recommended to (and assumed to have) exclusively breastfeed their infants for six months. Alvarez-Uria et al. 2012 noted that one of the infected children was mix-fed, but did not provide the rate of transmission by feeding modality. Additional information was asked from each study regarding how feeding type was assessed and supported during the study. Only two studies selected for this review answered the questions, and the remaining eight studies have only data extracted from papers (Tables 4-6).

Table 4. Responses to questions 1 and 2 regarding recommended feeding practice by study and duration of breastfeeding

	Studies	Recommendation	Duration of BF
Additional information	Ngoma et al, 2015	EBF for 6 months and complementary feeding between 6-12 months	12 months
	Giuliano et al, 2013	BF	4.5 months and wean over a 1.5 months
Information from published papers	Sagay et al, 2015	EBF for 6 months and complementary feeding between 6-12 months	1 year
	Jamieson et al, 2012	BF	6 months
	Alvarez-Uria et al, 2012	BF and FF	6 months
	Coovadia et al, 2012	BF	6 months
	Thomas et al, 2011	BF	6 months
	Marazzi et al, 2009	BF	6 months
	Kilewo et al, 2009	BF	6 months

Table 5. Responses regarding support for exclusive breastfeeding and type of data collection (questions 3 and 4)

	Studies	Frequency counselling	Local of counselling	Type of support	Person giving support	Type of data collection
Additional Information	Ngoma et al, 2015	4 wks post enrolment, 36 weeks gestation, at birth, 2 weeks, 6 weeks, 3,6,9,12,15, 18 and 24 months	2 week was home visit, others facility based.	Text message reminders, foo and transportation stipends	Registered nurse	interview
	Giuliano et al, 2013	Every 2 wks in first 2 months pregnancy and every month until BF cessation	Facility-based	counselling	Skilled personnel in nutrition practices	Self-report
Information from published papers	Jamieson et al, 2012	1, 2, 4, 6, 8, 12, 18, 21, 24, 28 weeks post partum	-	Counselling and breast-milk replacement food in case of BF cessation		Interview by standard questionnaire
	Alvarez-Uria et al, 2012	Not said	Not said	Not said	Not said	Not said
	Coovadia et al, 2012	7d, 2,5,6,8wks, 3,6,9,12,18 mo	Not said	Not said	Not said	Self-report
	Thomas et al, 2011	Weekly before delivery, and after delivery: 2,6,10, 14 weeks and 6, 9, 12, 15, 18, 24 months	Not said	Not said	Not said	Not said
	Marazzi et al, 2009	Not said	Not said	Counselling and nutritional supplement for pregnant and lactating mothers	nutritionists	Clinical data
	Kilewo et al, 2009	1, 3, 6 weeks and 3, 4, 5, 6 months	Facility based	counselling	Not said	Not said

Table 6. Responses regarding disaggregation of data on feeding practices (questions 5 to 7).

	Study	Disaggregation	Postnatal transmission disaggregated by feeding practice	BF rates
Additional Information	Ngoma et al, 2015	EBF and complementary feeding	No	95.5% of women were BF at 6 months of age.
	Giuliano et al, 2013	BF and MF	No	Month 1 : 96.7 % Month 2 : 92.6 % Month 3 : 86.6 % Month 4 : 72.7 % Month 5 : 34.1 % Month 6 : 22.7 %
Information from published papers	Jamieson et al, 2012	EBF, MF, or FF		
	Alvarez Uria et al, 2012	EBF, MF or FF	Yes, but no denominators were given	Not given
	Coovadia et al, 2012	No	No	Not given
	Thomas et al, 2011	EBF, MF	No	87%
	Marazzi et al, 2009	No	No	Not given
	Kilewo et al, 2009	No	No	90%

GRADE PROFILE

An evaluation of the quality of the studies considered in this analysis is given in Table 7. The assessment of quality was based on study limitations/risk of bias as per the evidence from the Newcastle-Ottawa Scale (Table 3, Appendix 5). We also considered inconsistency, indirectness and publication bias. All studies are rated very low quality.

Initially, all studies were scored low quality due to being observational and were downgraded for indirectness because their research areas were not directly in line with the PICO question. Where a pooled analysis was undertaken and a pooled estimate provided, studies were further downgraded for inconsistency. In all groups of studies there was at least one study with a risk of bias pertaining to lack of detailed information on feeding leading to further downgrading as did the substantial heterogeneity in transmission rate estimates between studies.

Table 7. GRADE evidence profile

Question: HIV transmission in breastfed infants of mothers on ART [1]

Setting: Zambia, Nigeria, Malawi, South Africa, Tanzania, Uganda and Zimbabwe, India, Kenya, Mozambique

Quality assessment							№ of patients	Effect	Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	[intervention]	Rate of transmission % (95% CI)		
Overall transmission at 6 months (Perinatal and Postnatal transmission)										
6	observational studies[2]	very serious [3]	very serious [4]	serious [5]	not serious	none	3175	Range from **0.70** (95%CI 0.20-1.20) to **7.90** (95%CI 6.20-9.90)	⊕◯◯◯ VERY LOW [2 3 4 5]	IMPORTANT
Postnatal transmission at 6 months										
6	observational studies [6]	very serious [7]	very serious [8]	serious [9]	not serious	none	2109	rate 1.08% (0.32 to 1.85)	⊕◯◯◯ VERY LOW [7 8 9]	CRITICAL
HIV transmission at 9 months										
1	observational studies[10]	serious[11]	not serious	serious [12]	not serious	none	532	rate **1.8** (0.7 to 4.8)	⊕◯◯◯ LOW[11 12]	CRITICAL
Overall transmission at 12 months - including peripartum transmission										
5	observational studies [13]	serious [14]	serious [15]	not serious [16]	not serious	none	1493	rate **4.23** (2.97 to 5.49)	⊕◯◯◯ VERY LOW [14 15 16]	CRITICAL
Postnatal transmission at 12 months (excluding peripartum transmission)										
2	observational studies [17]	serious [18]	serious [19]	serious [20]	not serious	none	833	rate **2.93** (0.68 to 5.18)	⊕◯◯◯ VERY LOW [18 19 20]	CRITICAL
Postnatal transmission at 18 months (lifelong ART, including all transmission)										
1	observational studies [21]	not serious [22]	not serious	serious[23]	not serious	none	211	rate **4.1** (2.2 to 7.6)	⊕◯◯◯ LOW [23]	CRITICAL

1. There was no comparison that was in line with the PICO question. In all studies the recommendation was exclusive breastfeeding for the first six months of life and the majority of mothers breastfed their children. Transmission according to mixed feeding was not provided by any of the studies.
2. Studies included: Ngoma et al, 2015; Sagay et al, 2015; Jamieson et al, 2012; Thomas et al, 2011; Marazzi et al, 2009 and Kilewo et al, 2009. Of these, Jamieson et al (2012) and Thomas et al (2011) were cohorts embedded in randomized control trials, while Ngoma et al, (2015), Sagay et al (2015), Marazzi et al (2009) and Kilewo et al (2009) were cohort studies
3. Risk of bias: We downgraded once due to potential selection bias for lack of detailed feeding history in Sagay et al, 2015 and Marazzi et al. 2009. Adherence to ART was not provided in Sagay et al, 2015; Marazzi et al, 2009 and Kilewo et al, 2009. Sagay et al, 2015 and Jamieson et al, 2012 did not provide a rate of transmission, but the rate of transmission was calculated from the number of children at risk provided in the paper. Sagay et al, 2015 is a retrospective study, and only one denominator was provided for all rates provided in the study. Ngoma et al, 2015 and Marazzi et al, 2009 did not provide confidence interval, which was calculated for both studies according to the formula provided in Appendix 6.
4. Inconsistency: We downgraded twice due to substantial heterogeneity in rates of transmission provided or calculated. Rates ranged from 0.7% (95% CI 0.20%-1.20%) in Sagay et al, 2015, which was a retrospective cohort, to 7.9% (95% CI 6.2%-9.90%) in Jamieson et al, 2012
5. Indirectness: We downgraded once as the studies' questions were not in line with the PICO question and covered different types of additional interventions. In Ngoma et al, 2015, Sagay et al, 2015, Marazzi et al. 2009 and Kilewo et al. 2009 the only intervention was ART. Jamieson et al. 2012 compared transmission rates in children whose mothers were on ART, infants on NVP and a control group and the three groups were further divided into those who were on a maternal nutrition supplement and those that were not.
6. Studies included were: Coovadia et al, 2012, Jamieson et al, 2012, Alvarez-Uria et al, 2012, Thomas et al, 2011, Marazzi et al, 2009 and Kilewo et al, 2009. Coovadia et al (2012), Jamieson et al (2012) and Thomas et al (2011) were cohorts embedded in randomized control trials, while Alvarez-Uria et al (2012), Marazzi et al (2009) and Kilewo et al (2009) were cohort studies.
7. Risk of bias: We downgraded once due to potential selection bias for lack of detailed feeding history in Marazzi et al, 2009. Adherence to ART was not provided in Marazzi et al, 2009 and Kilewo et al, 2009. There were some differences regarding age in weeks as endpoint considered perinatal transmission. Coovadia et al (2012), Alvarez-Uria et al (2012), Thomas et al (2011) and Kilewo et al (2009) excluded children positive at 6 weeks, Jamieson et al (2012) excluded at 2 weeks and Marazzi et al (2009) at 4 weeks. Jamieson et al (2012), Thomas et al (2011) and Kilewo et al (2009) did not provide a rate of transmission, but the rate was calculated based on the number of children at risk provided in the paper. In Marazzi et al (2009) a confidence interval was not provided but was calculated using the formula in Appendix 6.
8. Inconsistency: We downgraded due to substantial heterogeneity in rates of transmission provided or calculated (I^2=66.4%). Rates ranged from 0.24% (95% CI 0.0% to 1.40%) in Coovadia et al, 2012 to 3.10% (95% CI 1.20% to 7.80%) in Alvarez-Uria et al, 2012, the latter study had a very wide confidence interval due to the small sample of 127.
9. Indirectness: We downgraded once as the studies' questions were not in line with the PICO question and covered different types of co-interventions. In Alvarez-Uria et al., 2012, Marazzi et al. 2009 and Kilewo et al. 2009 the only intervention was ART. Jamieson et al. 2012 compared HIV-free survival in children whose mothers were on ART, infants on NVP and a control group and the three groups were further divided into those who were on a maternal nutrition supplement and those that were not. In Coovadia et al, infants were randomized to receive extended Nevirapine or placebo until 6 months of life. In Alvarez-Uria et al. 2012 all women were on ART but newborns were also given prophylaxis, the study also compared transmission rates between infants being breastfed and receiving formula feeding. In Thomas et al. 2011 all mothers were on ART but all infants received a single dose of NVP within 72 hours. Studies also varied with regard to initiation of maternal ART.

10. Single study included: Peltier et al, 2009, which was a cohort study.
11. Risk of bias: We downgraded once due to potential selection bias since this was a non-randomized intervention cohort in which the mother could choose the type of feeding for the infant. Only mothers with low CD4 count (<350 cells/µl or WHO clinical stage 4) were eligible for lifelong ART. The study had good adherence to ART and ascertainment of feeding. Only 15 mothers were reported to be mixed feeding their children, but no children were infected.
12. Indirectness: We downgraded once as the study research question was not in line with PICO questions and although the study compares breastfeeding and formula feeding, it did not study the effect of ART by feeding.
13. Studies included were: Giuliano et al, 2013, Alvarez-Uria et al, 2012, Thomas et al, 2011, Marazzi et al, 2009 and Kilewo et al, 2009. Thomas et al (2011) was a cohort embedded in a randomized control trial, while Giuliano et al (2013), Alvarez-Uria et al (2012), Marazzi et al (2009) and Kilewo et al (2009) were cohort studies
14. Risk of bias: We downgraded once due to potential selection bias for lack of detailed information regarding feeding in Marazzi et al, 2009.
15. Inconsistency: We downgraded once due to heterogeneity in rates of transmission (I^2 =39.9%), which varied from 2.8% (95%CI 1.40% to 5.50%) in Marazzi et al, 2009 to 5.80% (95% CI 3.60% to 8.00%) in Kilewo et al, 2009.
16. Indirectness: We downgraded once as the studies' research questions were not in line with PICO questions and covered different types of co-interventions. In Giuliano et al, 2013, Alvarez-Uria et al, 2012, Marazzi et al. 2009 and Kilewo et al. 2009 the only intervention was ART. Giuliano et al (2013) offered lifelong ART for mothers with low CD4 count. In Alvarez-Uria et al. 2012 all women were on ART but newborns were also given prophylaxis. In Thomas et al. 2011 all mothers were on ART but all infants received a single dose of NVP within 72 hours. Studies also varied with regard to initiation of maternal ART.
17. Studies include were: Coovadia et al, 2012 and Jamieson et al, 2012. Both studies were cohorts embedded in a randomized control trial.
18. Risk of bias: We downgraded once due to potential selection bias for lack of detail of feeding practices and adherence to ART in Coovadia et al, 2012. Jamieson et al, 2012 did not provide rate of transmission. The studies also varied in the age in weeks as endpoint for peripartum transmission, Coovadia et al, 2012 excluded infants identified as infected at 6 weeks and Jamieson et al, 2012 at 2 weeks.
19. Inconsistency: We downgraded twice due to high heterogeneity in the pooled estimate (I^2= 71.2%), with rates varying from 1.70% (95% CI 0.30% to 4.10%) in Coovadia et al, 2012 and 4.0% (95%Ci 1.94% to 4.29%) in Jamieson et al, 2012..
20. Indirectness: We downgraded once because the studies' research question was not in line with the PICO question and covered different types of co-interventions. . In Coovadia et al. 2012, infants were randomised to receive either extended Nevirapine prophylaxis or placebo until 6 months or until breastfeeding cessation. Jamieson et al, 2012 compared HIV-free survival in children whose mothers were on ART, infants on NVP and a control group and the three groups were further divided into those who were on a maternal nutrition supplement and those that were not.
21. Single study included: Ngoma et al, 2015.
22. Risk of bias: The study was not downgraded because the study had high adherence to ART, all mothers were receiving lifelong ART, and feeding was well-evaluated.
23. Indirectness: We downgraded once as the study's research questions were not in line with the PICO question, and although all mothers were on lifelong ART, and ascertainment of ART and breastfeeding were adequate, outcome was not stratified by feeding, and the number of infants who were mixed fed was not provided.

References

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3. Eayres D: **Technical Briefing 3. Commonly used public health statistics and their confidence intervals**. In. National Centre for Heath Outcomes Development; 2008.
4. Coovadia HM, Brown ER, Fowler MG, Chipato T, Moodley D, Manji K, Musoke P, Stranix-Chibanda L, Chetty V, Fawzi W *et al*: **Efficacy and safety of an extended nevirapine regimen in infant children of breastfeeding mothers with HIV-1 infection for prevention of postnatal HIV-1 transmission (HPTN 046): a randomised, double-blind, placebo-controlled trial**. *Lancet (London, England)* 2012, **379**(9812):221-228.
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6. Jamieson DJ, Chasela CS, Hudgens MG, King CC, Kourtis AP, Kayira D, Hosseinipour MC, Kamwendo DD, Ellington SR, Wiener JB *et al*: **Maternal and infant antiretroviral regimens to prevent postnatal HIV-1 transmission: 48-week follow-up of the BAN randomised controlled trial**. *Lancet* 2012, **379**(9835):2449-2458.
7. Thomas TK, Masaba R, Borkowf CB, Ndivo R, Zeh C, Misore A, Otieno J, Jamieson D, Thigpen MC, Bulterys M *et al*: **Triple-antiretroviral prophylaxis to prevent mother-to-child HIV transmission through breastfeeding--the Kisumu Breastfeeding Study, Kenya: a clinical trial**. *PLoS Med* 2011, **8**(3):e1001015.
8. Alvarez-Uria G, Midde M, Pakam R, Bachu L, Naik PK: **Effect of Formula Feeding and Breastfeeding on Child Growth, Infant Mortality, and HIV Transmission in Children Born to HIV-Infected Pregnant Women Who Received Triple Antiretroviral Therapy in a Resource-Limited Setting: Data from an HIV Cohort Study in India**. *ISRN Pediatr* 2012, **2012**:763591.
9. Marazzi MC, Nielsen-Saines K, Buonomo E, Scarcella P, Germano P, Majid NA, Zimba I, Ceffa S, Palombi L: **Increased infant human immunodeficiency virus-type one free survival at one year of age in sub-saharan Africa with maternal use of highly active antiretroviral therapy during breast-feeding**. *Pediatr Infect Dis J* 2009, **28**(6):483-487.
10. Kilewo C, Karlsson K, Ngarina M, Massawe A, Lyamuya E, Swai A, Lipyoga R, Mhalu F, Biberfeld G: **Prevention of mother-to-child transmission of HIV-1 through breastfeeding by treating mothers with triple antiretroviral therapy in Dar es Salaam, Tanzania: the Mitra Plus study**. *Journal of acquired immune deficiency syndromes (1999)* 2009, **52**(3):406-416.
11. Peltier CA, Ndayisaba GF, Lepage P, van Griensven J, Leroy V, Pharm CO, Ndimubanzi PC, Courteille O, Arendt V: **Breastfeeding with maternal antiretroviral therapy or formula feeding to prevent HIV postnatal mother-to-child transmission in Rwanda**. *AIDS (London, England)* 2009, **23**(18):2415-2423.
12. Ngoma MS, Misir A, Mutale W, Rampakakis E, Sampalis JS, Elong A, Chisele S, Mwale A, Mwansa JK, Mumba S *et al*: **Efficacy of WHO recommendation for continued breastfeeding and maternal cART for prevention of perinatal and postnatal HIV transmission in Zambia**. *Journal of the International AIDS Society* 2015, **18**:19352.
13. Sagay AS, Ebonyi AO, Meloni ST, Musa J, Oguche S, Ekwempu CC, Oyebode T, Ejeliogu E, Imade GE, Agbaji OO *et al*: **Mother-to-Child Transmission Outcomes of HIV-Exposed Infants Followed Up in Jos North-Central Nigeria**. *Curr HIV Res* 2015, **13**(3):193-200.
14. Giuliano M, Andreotti M, Liotta G, Jere H, Sagno JB, Maulidi M, Mancinelli S, Buonomo E, Scarcella P, Pirillo MF *et al*: **Maternal antiretroviral therapy for the prevention of mother-**

to-child transmission of HIV in Malawi: maternal and infant outcomes two years after delivery. *PLoS One* 2013, **8**(7):e68950.

Appendix 1 Search words for identification of studies

The search words in PubMed were:

(((Maternal[Title/Abstract] OR mother*[Title/Abstract]) AND (Antiretroviral therapy[Title/Abstract] OR Antiretroviral*[Title/Abstract] OR ART[Title/Abstract] OR ARV[Title/Abstract] OR HAART[Title/Abstract]) AND (HIV[Title/Abstract] OR Transmi*[Title/Abstract] OR Transmission[Title/Abstract] OR Infec*[Title/Abstract]) AND (Breastfeeding[Title/Abstract] AND Postnatal[Title/Abstract] OR Breast*[Title/Abstract] OR Feed*[Title/Abstract] OR Mixed[Title/Abstract])) AND ("2005"[Publication Date] : "2015"[Publication Date]))

Appendix 2 Excluded papers [1]with reason for exclusion, after full text screening

No.	Reference	Reason for Exclusion
1.	Cournil A, Van de Perre P, Cames C, de Vincenzi I, Read JS, Luchters S, Meda N, Naidu K, Newell ML, Bork K: Early infant feeding patterns and HIV-free survival: findings from the Kesho-Bora trial (Burkina Faso, Kenya, South Africa). Pediatr Infect Dis J 2015, 34(2):168-174	It was not provided transmission at 6 months for this paper or other Kesho Bora papers
2.	Cohan D, Natureeba P, Koss CA, Plenty A, Luwedde F, Mwesigwa J, Ades V, Charlebois ED, Gandhi M, Clark TD et al: Efficacy and safety of lopinavir/ritonavir versus efavirenz-based antiretroviral therapy in HIV-infected pregnant Ugandan women. Aids 2015, 29(2):183-191	It was not provided transmission at 6 months.
3.	Lawani, L. O., Onyebuchi, A. K., Iyoke, C. A., Onoh, R. C., & Nkwo, P. O. (2014). The challenges of adherence to infant feeding choices in prevention of mother-to-child transmission of HIV infections in South East Nigeria. Patient preference and adherence, 8, 377	Does not give rates for transmission. Just explain about mix feeding.
4.	Okafor I, Ugwu E, Obi S, Odugu B: Virtual Elimination of Mother-to-Child Transmission of Human Immunodeficiency Virus in Mothers on Highly Active Antiretroviral Therapy in Enugu, South-Eastern Nigeria. Annals of medical and health sciences research 2014, 4(4):615-618.	It was not provided transmission at 6 months.
5.	Shapiro RL, Kitch D, Ogwu A, Hughes MD, Lockman S, Powis K, Souda S, Moffat C, Moyo S, McIntosh K et al: HIV transmission and 24-month survival in a randomized trial of HAART to prevent MTCT during pregnancy and breastfeeding in Botswana. AIDS (London, England) 2013, 27(12):1911-1920.	It was not provided transmission at 6 months.
6.	Thistle P, Bolotin S, Lam E, Schwarz D, Pilon R, Ndawana B, Simor AE, Silverman M: Highly active anti-retroviral therapy in the prevention of mother-to-child transmission of HIV in rural Zimbabwe during the socio-economic crisis. Med Confl Surviv 2011, 27(3):165-176.	It was not provided transmission at 6 months.
7.	Homsy J, Moore D, Barasa A, Were W, Likicho C, Waiswa B, Downing R, Malamba S, Tappero J, Mermin J: Breastfeeding, mother-to-child HIV transmission, and mortality among infants born to HIV-Infected women on highly active antiretroviral therapy in rural Uganda. J Acquir Immune Defic Syndr 2010, 53(1):28-35.	It was not provided transmission at 6 months.
8.	Tonwe-Gold B, Ekouevi DK, Viho I, Amani-Bosse C, Toure S, Coffie PA, Rouet F, Becquet R, Leroy V, El-Sadr WM et al: Antiretroviral treatment and prevention of peripartum and postnatal HIV transmission in West Africa: evaluation of a two-tiered approach. PLoS Med 2007, 4(8):e257	It was not provided transmission at 6 months.
9.	Seth A, Chandra J, Gupta R, Kumar P, Aggarwal V, Dutta A. Outcome of HIV exposed infants: experience of a regional pediatric center for HIV in North India. Indian J Pediatr. 2012;79(2):188-93	Very small number of mothers on ART, and not provided information if those mothers were breastfeeding or not.
10.	Azcoaga-Lorenzo, A., Ferreyra, C., Alvarez, A., Palma, P. P., Velilla, E., & Del Amo, J. (2011). Effectiveness of a PMTCT programme in rural Western Kenya. AIDS care, 23(3), 274-280.	No transmission was given according to feeding and ART
11.	Oladokun, R. E., Awolude, O., Brown, B. J., Adesina, O., Oladokun, A., Roberts, A., ... & Kanki, P. (2010). Service uptake and performance of the prevention of mother-to-child transmission (PMTCT) programme in Ibadan, Nigeria. African journal of medicine and medical sciences, 39(2), 81-87.	It was not provided transmission at 6 months.
12.	Tchendjou, P., Same-Ekobo, C., Nga, A., Tejiokem, M., Kfutwah, A., Nlend, A. N., ... & Dabis, F. (2010). Effectiveness of multidrug antiretroviral regimens to prevent mother-to-child transmission of HIV-1 in routine public health services in Cameroon. PLoS One, 5(4), e10411.	Outcome was not discriminated for mothers on ART
13.	Nyandiko WM, Otieno-Nyunya B, Musick B, Bucher-Yiannoutsos S, Akhaabi P, Lane K, et al. Outcomes of HIV-exposed children in western Kenya: efficacy of prevention of mother to child transmission in a resource-constrained setting. Journal of acquired immune deficiency syndromes (1999). 2010;54(1):42-50	Not possible to extract transmission and death for ART and BF together.

[1] Studies are based on published papers, which were screened based on the search criteria in Appendix 1. Some studies are additional outputs of larger studies that produced further papers and reports not considered in this study.

14.	Noel, F., Mehta, S., Zhu, Y., Rouzier, P. D. M., Marcelin, A., Shi, J. R., ... & Pape, J. W. (2008). Improving outcomes in infants of HIV-infected women in a developing country setting. PloS one, 3(11), e3723.	Mothers on short course ART and ART, no transmission according to ART or feeding
15.	SaoundeTemgoua, E. M., Nkenfou, C. N., Zoung-Kanyi, B. A., Fokam, J., Billong, S. C., Sosso, S. M., ... & Colizzi, V. (2015). HIV-1 Early Infant Diagnosis is an Effective Indicator of the Prevention of Mother-to-Child Transmission Program Performance: Experience from Cameroon. Current HIV research.	Paper not found
16.	Kumela, K., Amenu, D., & Chelkeba, L. (2015). Comparison of anti-retroviral therapy treatment strategies in prevention of mother-to-child transmission in a teaching hospital in Ethiopia. Pharmacy Practice, 13, 539.	No discrimination of analysis by HAART
17.	Boerma, R. S., Wit, F. W., Orock, S. O., Schonenberg‐Meinema, D., Hartdorff, C. M., Bakia, A., & Hensbroek, M. B. (2015). Mortality risk factors among HIV‐exposed infants in rural and urban Cameroon. Tropical Medicine & International Health, 20(2), 170-176.	The study is about mortality, and transmission according to ARV is not accessed
18.	Mwendo EM, Mtuy TB, Renju J, Rutherford GW, Nondi J, Sichalwe AW, et al. Effectiveness of prevention of mother-to-child HIV transmission programmes in Kilimanjaro region, northern Tanzania. Tropical medicine & international health : TM & IH. 2014;19(3):267-74	Almost 50% of losses on the first PCR test, and only 7 children completed 18 months follow-up.
19.	Ibeto, M., Giddy, J., & Cox, V. (2014). Closing the gaps: Steps towards elimination of mother-to-child transmission of HIV. Southern African Journal of HIV Medicine, 15(3), 107-109.	Feeding is not accessed at the same time as type of ART
20.	Derebe G, Biadgilign S, Trivelli M, Hundessa G, Robi ZD, Gebre-Mariam M, et al. Determinant and outcome of early diagnosis of HIV infection among HIV-exposed infants in southwest Ethiopia. BMC research notes. 2014;7:309	No rates for breastfeeding and ART together were provided.
21.	Noubiap, J. J. N., Bongoe, A., & Demanou, S. A. (2014). Mother-to-child transmission of HIV: findings from an Early Infant Diagnosis program in Bertoua, Eastern Cameroon. Pan African Medical Journal, 15(1).	Feeding is not accessed at the same time as type of ART, and no transmission given at 6 months.
22.	Koye, D. N., & Zeleke, B. M. (2013). Mother-to-child transmission of HIV and its predictors among HIV-exposed infants at a PMTCT clinic in northwest Ethiopia. BMC public health, 13(1), 398.	Feeding is not accessed at the same time as type of ART, and no transmission given at 6 months.
23.	Ugochukwu, E. E., & Kalu, S. O. (2010). Early infant diagnosis of HIV infection in Southeastern Nigeria: prevalence of HIV infection among HIV-exposed babies. West African journal of medicine, 29(1).	Feeding is not accessed at the same time as type of ART, and no transmission given at 6 months.
24.	Simpore J, Pietra V, Pignatelli S, Karou D, Nadembega WM, Ilboudo D, et al. Effective program against mother-to-child transmission of HIV at Saint Camille Medical Centre in Burkina Faso. Journal of medical virology. 2007;79(7):873-9	Very small number of breastfed children.
25.	Binagwaho A, Pegurri E, Drobac PC, Mugwaneza P, Stulac SN, Wagner CM, et al. Prevention of mother-to-child transmission of HIV: cost-effectiveness of antiretroviral regimens and feeding options in Rwanda. PLoS One. 2013;8(2):e54180	Mothers are on short course ART, and does not provide transmission
26.	Chi BH, Musonda P, Lembalemba MK, Chintu NT, Gartland MG, Mulenga SN, et al. Universal combination antiretroviral regimens to prevent mother-to-child transmission of HIV in rural Zambia: a two-round cross-sectional study. Bulletin of the World Health Organization. 2014;92(8):582-92.	Does not provide total number of mothers on ART or transmission only by ART.
27.	Kagaayi J, Gray RH, Brahmbhatt H, Kigozi G, Nalugoda F, Wabwire-Mangen F, et al. Survival of infants born to HIV-positive mothers, by feeding modality, in Rakai, Uganda. PLoS One. 2008;3(12):e387	Mothers on different type of antiretroviral therapy. Not possible to identify HIV transmission by mothers on ART.
28.	Leroy V, Ekouevi DK, Becquet R, Viho I, Dequae-Merchadou L, Tonwe-Gold B, et al. 18-month effectiveness of short-course antiretroviral regimens combined with alternatives to breastfeeding to prevent HIV mother-to-child transmission. PLoS One. 2008;3(2):e1645	Mothers were not on ART, but on dual ARV with single dose NVP on labour.
29.	Magoni M, Bassani L, Okong P, Kituuka P, Germinario EP, Giuliano M, et al. Mode of infant feeding and HIV infection in children in a program for prevention of mother-to-child transmission in Uganda. AIDS (London, England). 2005;19(4):433-7	Mother receiving short course ART.
30.	Minniear TD, Girde S, Angira F, Mills LA, Zeh C, Peters PJ, et al. Outcomes in a cohort of women who discontinued maternal triple-antiretroviral regimens initially used to prevent mother-to-child transmission during pregnancy and breastfeeding--Kenya, 2003-2009. PLoS One. 2014;9(4):e93556	Mothers discontinued ART after labour.
32.	Nlend AEN, Ekani BB. Preliminary assessment of breastfeeding practices in HIV 1-infected mothers (prior to weaning) under the Djoungolo programme on the prevention of mother-to-child transmission of HIV. Journal of tropical pediatrics. 2010;56(6):436-9	The paper focus on breastfeeding, mastitis and transmission, and assessment of transmission done at 13 weeks.

33.	Shah M, Johns B, Abimiku Al, Walker DG. Cost-effectiveness of new WHO recommendations for prevention of mother-to-child transmission of HIV in a resource-limited setting. AIDS (London, England). 2011;25(8):1093-102.	Study based on models of % of adherence in Nigeria.
34.	Taha TE, Li Q, Hoover DR, Mipando L, Nkanaunena K, Thigpen MC, et al. Postexposure Prophylaxis of Breastfeeding HIV-Exposed Infants With Antiretroviral Drugs to Age 14 Weeks: Updated Efficacy Results of the PEPI-Malawi Trial. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2011;57(4):319-25	Mothers not on ART, comparison among dual therapy
35.	Torpey K, Kabaso M, Weaver MA, Kasonde P, Mukonka V, Bweupe M, et al. Infant feeding options, other nonchemoprophylactic factors, and mother-to-child transmission of HIV in Zambia. Journal of the International Association of Physicians in AIDS Care (Chicago, Ill : 2002). 2012;11(1):26-33	No data for mother on ART and breastfeeding together
36.	Torpey K, Kasonde P, Kabaso M, Weaver MA, Bryan G, Mukonka V, et al. Reducing pediatric HIV infection: estimating mother-to-child transmission rates in a program setting in Zambia. Journal of acquired immune deficiency syndromes (1999). 2010;54(4):415-22	No data for mother on ART and breastfeeding together
37.	van Lettow M, Bedell R, Landes M, Gawa L, Gatto S, Mayuni I, et al. Uptake and outcomes of a prevention-of mother-to-child transmission (PMTCT) program in Zomba district, Malawi. BMC Public Health. 2011;11:426	Very small number of mothers on ART

Appendix 3 Included papers: Descriptive information of studies providing information on breastfeeding and ART

First Author/Study	Place of study	Randomised for		Feeding	Beginning/ end ART	Breast-feeding duration	Time evaluation	N	Extracted information	
		ARV	Other						Transmission 6 mo	Exclude peripartum
Cohorts embedded on RCTs										
HPTN046 trial (Fowler, 2014; Coovadia, 2012)	South Africa, Tanzania, Uganda and Zimbabwe	ART or not	Infant NVP or not	All BF	From first antenatal visit/ 6 mo	6 mo	12 mo	1527	Given	Postnatal: excluding 6 weeks
Jamieson, 2012	Antenatal clinics in Malawi	ART† or infant NVP	Nutritional intervention	Majority BF	30wks or less/ 6 mo	6 mo	12 mo	849	Calculated	Both measures. Provides number of infected children before and after 2 weeks
Kisumu Breastfeeding Study (Okanda, 2014; Thomas, 2011)	Kenya (antenatal clinics)	ART		All BF	34 wks/ 6 mo‡	6 months	6 weeks, 6, 12 mo	502	Given	Include peripartum. Postnatal calculated.
Thakwalakwa[a], 2014	Malawi (Thyolo District Hospital)	Only ART	Nutritional intervention	All BF	From first antenatal visit/ lifelong	6 mo	12 mo	248	Not given	Gives only number of infected children with no denominator
Observational studies										
DREAM study (Giuliano, 2013; Palombi, 2007)	Malawi (two ante natal clinics)	ART*		All BF	1st tremester and lifelong (CD4+<350) or week 25/ 6 mo or end BF	4.5 mo	12 mo	300	Not given	Gives only number of infected children with no denominator
Alvarez-Uria[a], 2012	India (3 hospitals in Antapur)	ART		BF and RF	From first antenatal visit / 6 mo (BF), post labour (NBF)	6 mo	6 and 12 mo	318	Given	Exclude 6 weeks
Peltier[a], 2009	Rwanda (four government-run health facilities)	ART		BF and RF	28 wks / 7 mo‡	6 mo	9 mo	532	Not given	Provides Overall transmission including peripartum
Marazzi, 2009	Mozambique	ART		All BF	15 wks/ 6 mo‡	5 mo	6 weeks, 6	341	Given	Both, provides rates

							and 12 mo			including and excluding peripartum (exclude 4 wks)
Kilewo, 2009	Tanzania (Dar es Salam)	ART		All BF	34 wks/ 6 mo	6 mo	6 weeks, 6, 9, 12, 18 mo	441	Given	Include peripartum. Postnatal calculated.
Sagay, 2015	Nigeria	ART		All BF	lifelong	1 year	18 mo	856	Calculated	Include peripartum.
Ngoma, 2015	Zambia	ART		All BF	14 wks-lifelong	1 year	6 weeks, 6 and 12 mo	231	Given	Include peripartum

[a] Studies performed in rural environment

†Mothers with clinical stage 4 or CD4 <200 cells/mm^3 were excluded

‡Mothers with CD4 count <200 cells/mm3 or stage III or IVdisease remained on ART throughout the study, and those who subsequently met the criteria after stopping ARVs were restarted, or when CD4 cell counts were ≤350 cells/mm^3 (Peltier, Marazzi)

*Mothers on ART based on disease progression or low CD4+ count

Appendix 4 Modified NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. The item Comparability of cohorts assesses whether exposed and non-exposed individuals are matched in the design and risk for the exposure of interest is adjusted for confounders. In this study all mothers are exposed to ART, and the outcome is HIV transmission rate (not relative risk or odds ratio), which is not controlled for confounders or covariates, therefore item comparability was not applied in the quality assessment.

Selection

1) Representativeness of the exposed cohort

Assesses whether the women on ART in the study are representative of women on ART in general

a) truly representative of the average women on ART in the community ✵

b) somewhat representative of the average woman on ART in the community ✵

c) selected group of users

d) no description of the derivation of the cohort

2) Ascertainment of exposure (ART)

a) secure record (eg clinical records) ✵

b) structured interview ✵

c) written self report

d) no description

3) Adherence to ART

a) Adherence reported in sufficient detail and adherence rates at end of study are high ✵

b) Adherence reported in sufficient detail, non-adherence <20% and unlikely to introduce bias✵

c) Not described

4) Ascertainment of exposure (BF)

a) secure record (eg clinical records, close follow-up) ✵

b) structured interview ✵

c) written self report

d) no description

5) Duration of BF

a) Clear report of Exclusive breastfeeding up to 6 months and continue BF for 1 year ✵

b) Breastfeeding cessation at maximum 6 months

c) not described in detail

Outcome

1) Assessment of outcome

Outcome is infant transmission at 6 months. This item assesses whether the information regarding infection was assessed per protocol visit and laboratory procedures.

a) independent assessment ✵

b) record linkage with HIV clinical programmes ✵

c) self-report

d) no description

2) Type of outcome provided

Infection rate should be provided, including the number of infections and number of child at risk at 6 months. Where HIV transmission does not provide number of children at risk, or it cannot be defined, and/or studies which only report numbers of infections do not score on this item.

a) Rate of transmission including a denominator of children at risk ✵

b) Number of HIV transmission

3) Outcome stratified by feeding type

a) Yes ✵

b) No

4) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for ✵

b) subjects lost to follow up unlikely to introduce bias - small number lost - <20%✵

c) follow up rate < 20% and no description of those lost

d) no statement

Appendix 5. NOS Evaluation

Study	SELECTION																	
	Representativeness				Ascertainment ART				Adherence to ART			Ascertainment to BF				Duration of BF		
	Truly	*Somewhat*	*Selected*	*ND*	*Secure record*	*Structured interview*	*self report*	*ND*	*Majority*	*Some, no bias*	*ND*	*Secure record*	*Structured interview*	*self report*	*ND*	*EBF 6 mo up to 1 y*	*BF for 6 mo only*	*ND*
Giuliano **		*			*						-			-			-	
Marazzi **		*			*						-			-			-	
Ngoma *****		*			*					*			*			*		
Thakwalakwa *		* (govern)						-			-				-		-	
Peltier ****		*			*				*				*				-	
Thomas ****		* (low income)			*				*			*					-	
Kilewo ***		*			*						-	*					-	
Sagay ***		*			*						-				-	*		
Jamieson ****		*			*					*			*				-	
Alvarez-Uria **		* (says)			*						-				-		-	
Coovadia **		*				*					-				-		-	

Study	OUTCOME										
	Assessment of outcome			Type of outcome		Stratified by feeding time		Adequacy of follow-up			
	Independent assessment	*Record linkage*	*ND*	*Rate or denominator*	*Only numbers*	*Yes*	*No*	*Complete*	*Small, no bias*	*High follow up*	*ND*
Giuliano ***	*				-		-		*		
Marazzi ***	*				-				*		
Ngoma **	*				-		-		*		
Thakwalakwa **	*				-		-		*		
Peltier ***	*			*	-	*		*			
Thomas ***	*			*			-		*		
Kilewo ***	*			*			-	*			
Sagay **	*				-		-		*		
Jamieson **	*			*			-		*		
Alvarez-Uria ****	*			*		*		* 4%			
Coovadia *	*			*			-				-
Cournil ***	*				-	*			*		

Appendix 6: Formula used to calculate confidence intervals described by Eayres, 2008

The 100(1−α)% confidence interval limits for the proportion p are given by:

$$p_{lower} = \frac{\left(2O + z^2 - z\sqrt{z^2 + 4Oq}\right)}{2\left(n + z^2\right)}$$ **Formula 2a**

$$p_{upper} = \frac{\left(2O + z^2 + z\sqrt{z^2 + 4Oq}\right)}{2\left(n + z^2\right)}$$ **Formula 2b**

where:
q = (1−p) is the proportion without the specified characteristic;
z is the 100(1− α/2)th percentile value from the Standard Normal distribution. For example for a 95% confidence interval, α = 0.05, and z = 1.96 (i.e. the 97.5th percentile value from the Standard Normal distribution).

where:
O is the observed number of individuals in the sample/population having the specified characteristic;
n is the total number of individuals in the sample/population.