**In utero** and intra-partum HIV-1 transmission and acute HIV-1 infection during pregnancy: using the BED capture enzyme-immunoassay as a surrogate marker for acute infection

Edmore T Marinda,1,2 Lawrence H Moulton,3 Jean H Humphrey,1,3* John W Hargrove,5 Robert Ntozini,1 Kuda Mutasa1 and Jonathan Levin2,4

1ZVITAMBO Project, Harare, Zimbabwe, 2School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, 3Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 4MRC/UVI Uganda Research Unit on AIDS, Entebbe Uganda and 5DST/NRF Centre of Excellence in Epidemiological Modeling and Analysis (SACEMA), University of Stellenbosch, Stellenbosch, South Africa

*Corresponding author. ZVITAMBO project, #1 Borrowdale Rd., Borrowdale, Harare, ZIMBABWE.
E-mail: jhumphrey@zvitambo.co.zw

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**Objective** The BED assay was developed to estimate the proportion of recent HIV infections in a population. We used the BED assay as a proxy for acute infection to quantify the associated risk of mother-to-child-transmission (MTCT) during pregnancy and delivery.

**Design** A total of 3773 HIV-1 sero-positive women were tested within 96 h of delivery using the BED assay, and CD4 cell count measurements were taken. Mothers were classified according to their likelihood of having recently seroconverted.

**Methods** The risk of MTCT in utero and intra-partum was assessed comparing different groups defined by BED and CD4 cell count, adjusting for background factors using multinomial logistic models.

**Results** Compared with women with BED \( \geq 0.8/\text{CD4} \geq 350 \) (typical of HIV-1 chronic patients) there was insufficient evidence to conclude that women presenting with BED < 0.8/\text{CD4} \geq 350 (typical of recent infections) were more likely to transmit in utero [adjusted odds ratio (aOR) = 1.37, 96% confidence interval (CI) 0.90–2.08, \( P = 0.14 \)], whereas women with BED < 0.8/\text{CD4} 200–349 (possibly recently infected patients) had a 2.57 (95% CI 1.39–4.77, \( P \)-value < 0.01) odds of transmitting in utero. Women who had BED < 0.8/\text{CD4} < 200 were most likely to transmit in utero (aOR 3.73, 95% CI 1.27–10.96, \( P = 0.02 \)). BED and CD4 cell count were not predictive of intra-partum infections.

**Conclusions** These data provide evidence that in utero transmission of HIV might be higher among women who seroconvert during pregnancy.

**Keywords** BED, CD4, in utero, intra-partum, seroconversion, HIV
Introduction

Primary infection \(^1\)-\(^8\) is characterized by elevated viral load \(^9\)-\(^18\) which is a strong risk factor for sexual transmission \(^15\) and all modes of mother-to-child transmission (MTCT) of HIV. \(^19\) We recently reported data from the ZVITAMBO vitamin A trial demonstrating that breastfeeding-associated HIV transmission was 2.9–4.6 times higher among mothers who seroconverted post-natally compared with mothers who were already infected at delivery. Among 17 mothers who were seroconverted during delivery (i.e. their blood tested negative by HIV ELISA but positive by HIV RNA PCR), 75% of their infants died or became infected by 9 months post-partum. \(^20\) Thus within ZVITAMBO, maternal seroconversion during the intra-partum and post-natal periods was associated with high risk of MTCT.

Reports have been inconsistent, however, regarding whether maternal seroconversion during pregnancy increases in utero MTCT. \(^21\)-\(^24\) It is important to understand whether primary HIV infection during pregnancy increases transmission risk: firstly, because the risk of HIV acquisition may be higher during pregnancy than non-pregnancy; \(^25\)-\(^27\) secondly, to strengthen HIV prevention messages targeting reproductive-aged couples; and lastly, to determine whether repeat HIV testing and screening of antenatal women is warranted.

Within the ZVITAMBO trial we previously noted that women testing HIV-negative were younger and were more educated than those testing HIV-positive. Moreover, we noted that among HIV-positive women, those who transmitted in utero were younger, were more educated and also had a higher CD4 cell count than those who transmitted intra-partum or post-natally. \(^28\) We hypothesized that the group that transmitted in utero may have included a large number of women who seroconverted during pregnancy.

We used the BED capture enzyme-immunoassay (henceforth BED), a technically simple and relatively cheap assay, to measure plasma concentration of HIV-specific antibodies (IgG) that increase with time following primary infection. Individuals presenting with ‘low’ (<0.8) normalized optical density (ODn) values on BED are likely to have been infected within the past half year. \(^29\) Although the BED assay was designed to estimate the proportion of recently acquired HIV at the population level, in this analysis we used BED in combination with CD4 cell count as a proxy for acute infection to distinguish HIV-positive women who were likely to have acquired HIV during pregnancy from chronically infected women. We then compared in utero and intra-partum transmission rates between these groups to estimate the risk of in utero and intra-partum MTCT associated with primary HIV infection during pregnancy.

Methods

The ZVITAMBO project protocol and primary outcomes have been reported elsewhere. \(^28\)-\(^31\) In brief, 14 110 mother-infant dyads were enrolled within 96h of delivery between November 1997 and January 2000. Mother–infant pairs were eligible if both were free of acutely life-threatening conditions, the baby was a singleton with birth weight \(\geqslant 1500\) g and the mother planned to stay in Harare after delivery. Written informed consent was obtained.

Baseline data were collected by interview, medical record transcription or direct measurement. Gestational age was estimated using the Capurro method. \(^32\) Infant birthweight and maternal mid-upper arm circumference (MUAC) were measured using published methods. \(^33\) Mother–infant pairs were followed up at 6 weeks, 3 months and then every 3 months to 12 or 24 months. The trial preceded availability of HIV testing and anti-retroviral prophylaxis for antenatal women in Harare public sector facilities.

Laboratory procedures

At baseline, all mothers were tested for HIV using an algorithm that included two parallel enzyme-linked immunosorbent assays [HIV 1.0.2 ICE (Murex Diagnostics); GeneScreen HIV 1/2 (Sanofi Diagnostics Pasteur)] and Western blot [HIV Blot 2.2; (Genelabs Diagnostics)] where results were discordant. Haemoglobin (Hb) was measured for women enrolled from 1 October 1998 onwards (~60% of the total sample) (HemoCue, Mission Viejo, CA, USA). For HIV-positive women, CD4 cells were enumerated (FACSCount; Becton Dickinson) and plasma assayed by the Calypte HIV-1 BED Incidence EIA (BED-CEIA), (cat. No. 98003; Calypte Biomedical Corporation, Lake Oswego, OR, USA).

From infants born to HIV-positive mothers, cell pellets (Roche Diagnostics Systems, Alameda, CA, USA) and plasma were prepared from whole blood collected at baseline and follow-up visits and stored at \(-70^\circ \text{C}\). Following all patient contact, the last available sample from each infant was tested [pellet by Roche Amplicor version 1.5 qualitative DNA PCR assay (Roche Diagnostic Systems) for samples collected prior to 18 months; serum by GeneScreen ELISA for samples collected after 18 months]. If this sample was negative, the child was classified as HIV-negative; if it was positive, then earlier samples were tested to determine timing of infection.

The ZVITAMBO trial was approved by the Medical Research Council of Zimbabwe (MRC-Z), the Medicines Control Authority of Zimbabwe, the Johns Hopkins Bloomberg School of Public Health Committee on Human Research (CHR) and the Montreal General Hospital Ethics Committee (MGHEC). BED analysis of archived specimens was approved by MRC-Z, CHR, MGHEC and CDC Program Ethics Review Board, whereas the
University of the Witwatersrand Human Research Ethics Committee (Medical) approved the current research.

**Statistical analysis**

**Infant HIV infection groups**

HIV-exposed children were classified into one of three HIV status groups: (i) *in utero* infected (infant-tested PCR-positive at baseline), (ii) intra-partum infected (infant-tested PCR-negative at baseline and PCR-positive at 6 weeks) and (iii) not infected at 6 weeks (infant-tested PCR-negative at 6 weeks or later).34

**Maternal likelihood of recent seroconversion**

We used the BED assay together with CD4 cell count to categorize HIV-1-positive women according to their likelihood of having acquired HIV during pregnancy. Recently-infected individuals are likely to present with low BED values (ODn < 0.8)29 and relatively high CD4 cell counts, Figure 1.35 People with advanced stage HIV-1 may also present with low BED readings,36 but they usually have low CD4 cell counts. On fitting multinomial logistic regression models using three outcome levels, it was found that there was a significant interaction term (P-value = 0.03) between BED and CD4 cell count as continuous variables. We categorized women according to their BED and CD4 cell count values informed by this significant interaction term and the natural history of HIV infection as illustrated in Figure 1.35

Women were classified into one of six discrete groups according to their BED ODn and CD4 cell count interpreted as the following HIV duration and severity:

(i) BED ≥ 0.8/CD4 ≥ 350 cells/μl (characteristic of chronic asymptomatic infection).

(ii) BED ≥ 0.8/CD4 200–349 (characteristic of chronic intermediate stage infection).

(iii) BED ≥ 0.80/CD4 < 200 (characteristic of chronic end-stage infection).

(iv) BED < 0.8/CD4 ≥ 350 (characteristic of recent infection).

(v) BED < 0.8/CD4 200–349 (characteristic of the short interval of primary infection when CD4 cell count falls precipitously as illustrated in Figure 1).

(vi) BED < 0.8/CD4 < 200 (characteristic of very severe end-stage infection where BED declines due to depressed antibody production), Table 3.

The primary comparison was between group 1 and each of groups 4 and 5. We performed sensitivity analysis by combining groups (1 and 2) and (1, 2 and 3), and comparing these new groups with combined group (4 and 5). We assessed the effect of missing CD4 cell count data by comparing models with

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**Figure 1** Relationship among peripheral blood CD4+ T-cell count, plasma viremia and clinical disease progression. During the early period after primary infection, there is widespread dissemination of virus and a sharp decrease in the number of CD4 T cells in peripheral blood. An immune response to HIV ensues, with a decrease in detectable viremia followed by a prolonged period of clinical latency. The CD4 T-cell count continues to decrease during the following years, until it reaches a critical level below which there is a substantial risk of opportunistic diseases. (From Pantaleo G, Graziosi C, Fauci AS. The immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* 1993;328:327–35, with permission.)
complete data with models where CD4 was imputed. Maternal age, MUAC, Hb and plasma viral load were used to estimate for missing CD4 cell counts.

The data were analysed in STATA release 10 (STATACORP version 10; College Station, TX). Baseline characteristics were compared across infant infection status categories using ANOVA for continuous variables, except in the case of extreme non-normal variables where a Kruskal–Wallis test was used. Chi-squared tests were used for categorical variables. To compare the risk of in utero and intra-partum infection between recent infection and chronic infection, we fitted both unadjusted and adjusted multinomial regression models, using three outcome levels namely in utero, intra-partum or PCR-negative at 6 weeks. We adjusted for maternal factors: Hb level, MUAC, age, education, mode of delivery and duration from rupture of membranes to delivery. We also adjusted for the infant’s gender, gestational age and birthweight. We did not adjust for maternal viral load since the main question of interest was whether in utero or intra-partum transmission was associated with incident infection during pregnancy. Estimates were reported with 95% CIs.

Results
A total of 4495 women enrolled in the ZVITAMBO trial tested HIV positive at baseline; 382 and 508 of their neonates were infected during the in utero and intra-partum periods, respectively, 2883 tested PCR-negative at 6 weeks and the remainder (722) had an undefined or missing HIV status. As previously reported, mothers who transmitted HIV during the in utero period were younger and had better education than mothers who transmitted intra-partum or who did not transmit by 6 weeks, and mothers transmitting in utero had higher CD4 cell counts than mothers who transmitted intra-partum. A new finding not previously published, is that the in utero group also had higher plasma viral load (despite having higher CD4) compared with the intra-partum group, Table 1. Infants not included in the analysis due to missing timing of HIV infection had a lower mean birthweight (2.83 vs 2.93 kg), were born slightly earlier (gestational age 38.9 vs 39.2 weeks), their mothers had lower median CD4 cell count (382 vs 403 cells/μl) and their mothers’ plasma viral load was higher (log_{10} 4.20 vs 3.99 copies/μl) compared with infants included in the analysis.

Maternal baseline factors and BED assay results
Of the 4495 HIV-positive mothers, 4466 had a baseline BED reading and 509 (11%) of these had a low BED (<0.8 ODn) reading. Compared with mothers with BED values ≥0.8, mothers with a low BED were younger, had better education and higher CD4 cell counts (Table 2). Viral load values were more widely distributed among women with low BED readings than in those with high BED readings (IQR = 3.26–4.71 compared with 3.39–4.57). Similarly, the proportion of women with both undetectable viral load (<2.6 log_{10}) and very high viral load (>4.6 log_{10}) was greater among women with low BED readings.

Risk of in utero and intra-partum transmission according to BED and CD4 cell count
Among chronically infected women (BED ≥ 0.8/CD4 ≥ 350), 156 (9.1%) of their neonates tested PCR positive at birth compared with 34 (13.4 %) among women described as recently infected (BED < 0.8/CD4 ≥ 350), Table 3. Among women with BED ≥ 0.8/CD4 ≥ 200 (a more liberal definition of chronic infection), 225 (9.1%) neonates tested PCR positive at birth, and this percentage hardly changed (275 or 9.4%) when BED ≥ 0.8 readings irrespective of CD4 count were considered. Among women with BED < 0.8/CD4 ≥ 200, a broader definition of recent infections, 48 (14.9%) neonates tested PCR positive at birth. In the intra-partum groups, 180 (10.5%) and 19 (7.5%) were classified in the chronic and recently infected groups, respectively, and these percentages hardly changed when different definitions of chronic and recent infections were used.

In unadjusted models, a low BED (<0.8) reading was a risk factor for in utero transmission (OR = 1.68, 95% CI 1.26–2.25) and a protective factor for intra-partum transmission (OR = 0.63, 95% CI 0.44–0.90). The odds of in utero infection ranged from 1.37 (95% CI 0.90–2.08) to 2.64 (95% CI 1.37–5.06) times higher when comparing women with BED < 0.8/CD4 ≥ 350 and BED < 0.8/CD4 200–349 to women with BED ≥ 0.8/CD4 ≥ 350 in adjusted multinomial models, Table 4. In sensitivity analysis the odds of in utero transmission were 1.54 (95% CI 1.08–2.20; P = 0.02) and 1.47 (95% CI 1.03–2.09; P = 0.03) times higher when comparing a group with BED < 0.8/CD4 ≥ 200 (4 and 5) with BED ≥ 0.8/CD4 ≥ 350 (1 and 2) and BED ≥ 0.8/all CD4 groups (1, 2 and 3) in adjusted models respectively.

Based on multiple adjusted models, the odds of intra-partum infection ranged from 12% (95% CI –127; 66%) to 23% (–28; 54%) lower in recently infected women when compared with chronically infected, although the variances of the estimates were large. When the recently infected group (4 and 5) was compared with the two definitions of chronically infected groups (1 and 2; and 1, 2 and 3) in sensitivity analysis, the odds were 0.69 (95% CI 0.43–1.07; P = 0.10), and 0.60 (95% CI 0.38–0.93; P = 0.03), respectively.

In sensitivity analysis models with imputed CD4 cell count data, the aOR estimates for in utero infection were 2.85 (95% CI 1.55–5.26, P < 0.01) and 1.38
(95% CI 0.95–2.00, \(P = 0.09\)) for \(\text{BED} < 0.8 / \text{CD4} 200–349\) and \(\text{BED} \geq 0.8 / \text{CD4} 350\), respectively, compared with \(\text{BED} \geq 0.8 / \text{CD4} 350\). The estimates were \(0.98 \ (0.41–2.32, \ P = 0.96)\) and \(0.65 \ (0.41–1.04, \ P = 0.07)\) for intra-partum infection comparing \(\text{BED} < 0.8 / \text{CD4} 200–349\) and \(\text{BED} \geq 0.8 / \text{CD4} 350\) relative to \(\text{BED} \geq 0.8 / \text{CD4} 350\).

**Other factors associated with in utero and intra-partum transmission**

Other factors that were independently associated with *in utero* infection in multiple multinomial logistic models were: gender adjusted odds ratio (aOR) 1.56 times for females compared with males, birthweight aOR 0.50, 50% lower odds for every kilogram increase in birthweight, maternal age aOR 0.96, 4% lower odds for a year increase in mother’s age, mode of delivery aOR 0.54, 46% less odds for caesarean delivery compared with vaginal deliveries and maternal Hb aOR 0.36, 64% lower odds for HBs >11 mg/dl compared with HBs <7 mg/dl, Table 4. Gestational age aOR 0.92, 8% lower likelihood of intra-partum infection for a week increase in gestational age, mother’s education aOR 0.62, 38% less odds of infection comparing to females compared with males, birthweight aOR 0.50, 50% lower odds for every kilogram increase in birthweight, maternal age aOR 0.96, 4% lower odds for a year increase in mother’s age, mode of delivery aOR 0.54, 46% less odds for caesarean delivery compared with vaginal deliveries and maternal Hb aOR 0.36, 64% lower odds for HBs >11 mg/dl compared with HBs <7 mg/dl, Table 4. Gestational age aOR 0.92, 8% lower likelihood of intra-partum infection for a week increase in gestational age, mother’s education aOR 0.62, 38% less odds of infection comparing

**Table 1** Baseline characteristics of infant–mother dyads in which HIV transmission occurred intra-uterine and intra-partum and post-natal/did not get infected

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intra-uterine ((n = 382))</th>
<th>Intra-partum ((n = 508))</th>
<th>PCR negative at 6 weeks ((n = 2883))</th>
<th>Overall P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infant</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>153 (40.2)</td>
<td>250 (49.2)</td>
<td>1473 (51.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight, g [(\bar{x}(SD))]</td>
<td>2.79 (0.47)</td>
<td>2.88 (0.49)</td>
<td>2.96 (0.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age, weeks [(\bar{x}(SD))]</td>
<td>39.0 (1.6)</td>
<td>38.9 (1.6)</td>
<td>39.2 (1.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
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</tr>
<tr>
<td>Age, years [(\bar{x}(SD))]</td>
<td>24.8 (4.6)</td>
<td>26.3 (5.4)</td>
<td>25.7 (5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Schooling, years</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt;8</td>
<td>12 (3.2)</td>
<td>38 (7.5)</td>
<td>142 (4.9)</td>
<td></td>
</tr>
<tr>
<td>8 to &lt;12</td>
<td>170 (44.6)</td>
<td>245 (48.2)</td>
<td>1334 (46.3)</td>
<td></td>
</tr>
<tr>
<td>(\geq 12)</td>
<td>199 (52.2)</td>
<td>225 (44.3)</td>
<td>1402 (48.7)</td>
<td>0.016</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vaginal</td>
<td>351 (93.9)</td>
<td>465 (92.4)</td>
<td>2597 (90.6)</td>
<td>0.062</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>23 (6.1)</td>
<td>38 (7.6)</td>
<td>270 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Rupture of membranes to delivery (hours)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&gt;4</td>
<td>147 (39.8)</td>
<td>231 (47.4)</td>
<td>1052 (40.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MUAC, cm [(\bar{x}(SD))]</td>
<td>25.6 (2.9)</td>
<td>25.4 (2.6)</td>
<td>25.8 (3.0)</td>
<td>0.016</td>
</tr>
<tr>
<td>Hb, g/dl [(\bar{x}(SD))]</td>
<td>10.9 (2.2)</td>
<td>10.7 (2.0)</td>
<td>11.2 (1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4 cell count, cells/100(\mu)l</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median (IQR)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>400 (262–542)</td>
<td>322 (185–492)</td>
<td>418 (276–588)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(&lt;200)</td>
<td>56/328 (17)</td>
<td>123/436 (28)</td>
<td>317/2521 (13)</td>
<td></td>
</tr>
<tr>
<td>200–349</td>
<td>83 (25)</td>
<td>115 (26)</td>
<td>628 (25)</td>
<td></td>
</tr>
<tr>
<td>(\geq 350)</td>
<td>189 (58)</td>
<td>198 (45)</td>
<td>1576 (63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma viral load, (\log_{10}) copies/(\mu)l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.58 (4.00–5.09)</td>
<td>4.37 (3.90–4.90)</td>
<td>3.95 (3.42–4.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(&lt;3.38)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35/376 (9.3)</td>
<td>57/493 (11.6)</td>
<td>840/2830 (29.7)</td>
<td></td>
</tr>
<tr>
<td>3.38–4.6</td>
<td>164 (43.6)</td>
<td>238 (48.3)</td>
<td>1463 (51.7)</td>
<td></td>
</tr>
<tr>
<td>(&gt;4.6)</td>
<td>177 (47.1)</td>
<td>198 (40.2)</td>
<td>527 (18.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Undetectable viral load</td>
<td>6/376 (1.6)</td>
<td>13/493 (2.6)</td>
<td>245/2830 (8.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>All value are \(n\) (%) except where noted otherwise.

<sup>b</sup>Chi-squared/ANOVA/Kruskal–Wallis test as appropriate.

<sup>c</sup>Kruskal–Wallis test used.

<sup>d</sup><3.38 is below lower quartile (Q1), and >4.6 is above upper quartile (Q3).
women with ≥12 years of education with women with 0–7 years spent at school, mode of delivery 0.57, 43% lower odds for intra-partum infection for babies delivered by caesarean section compared with vaginal deliveries, prolonged duration of rupture of membranes aOR 1.49 times higher odds of infection intra-partum for duration of rupture to delivery of ≥4h compared with periods of <4h and maternal HBs aOR 0.39, 61% lower odds for HBs >11 mg/dl compared with <7 g/dl.

Discussion

Maternal acute HIV infection during the post-partum period has been associated with increased risk of MTCT in breastfeeding populations.11,20,38,39 Although 50–90% of individuals with primary HIV infections do present with symptomatic seroconversion illnesses, their symptoms are usually non-specific and are similar to many common febrile illnesses, with pregnancy further clouding the issue.40–42 Most
## Table 4 Risk of *in utero* and intra-partum infection for women with low BED and high CD4 count at delivery

<table>
<thead>
<tr>
<th>Risk Factors (levels)</th>
<th>Intrauterine transmission</th>
<th></th>
<th></th>
<th>Intrapartum transmission</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable</td>
<td>Multivariable OR (95% CI)</td>
<td>P-value</td>
<td>Univariable</td>
<td>Multivariable OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Infant factors</strong></td>
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<tr>
<td>Sex</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Males</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.08 (0.89–1.30)</td>
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<tr>
<td>Females</td>
<td>1.56 (1.25–1.94)</td>
<td>1.56 (1.21–2.00)</td>
<td>0.01</td>
<td>0.88 (0.83–0.94)</td>
<td>0.92 (0.84–0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>0.91 (0.85–0.98)</td>
<td></td>
<td></td>
<td>0.50 (0.33–0.61)</td>
<td>&lt;0.01</td>
<td>0.66 (0.54–0.82)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>0.44 (0.35–0.56)</td>
<td>0.50 (0.33–0.61)</td>
<td>&lt;0.01</td>
<td>0.66 (0.54–0.82)</td>
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<tr>
<td><strong>Maternal factors</strong></td>
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<tr>
<td>Age, years</td>
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<td></td>
</tr>
<tr>
<td>0–7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
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<tr>
<td>8–11</td>
<td>1.51 (0.82–2.78)</td>
<td></td>
<td></td>
<td>0.69 (0.47–1.01)</td>
<td>0.66 (0.42–1.04)</td>
<td>0.80</td>
</tr>
<tr>
<td>≥12</td>
<td>1.68 (0.91–3.08)</td>
<td></td>
<td></td>
<td>0.60 (0.41–0.88)</td>
<td>0.62 (0.39–0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>0.98 (0.94–1.02)</td>
<td></td>
<td></td>
<td>0.95 (0.92–0.99)</td>
<td></td>
<td></td>
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<tr>
<td>Hb, g/dl</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–10</td>
<td>0.58 (0.29–1.18)</td>
<td>0.53 (0.23–1.26)</td>
<td>0.15</td>
<td>0.60 (0.32–1.15)</td>
<td>0.59 (0.26–1.32)</td>
<td>0.20</td>
</tr>
<tr>
<td>≥11</td>
<td>0.40 (0.20–0.80)</td>
<td>0.36 (0.15–0.84)</td>
<td>0.02</td>
<td>0.40 (0.21–0.76)</td>
<td>0.39 (0.17–0.87)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean section</td>
<td>0.63 (0.41–0.98)</td>
<td>0.54 (0.30–0.97)</td>
<td>0.04</td>
<td>0.79 (0.55–1.12)</td>
<td>0.57 (0.36–0.92)</td>
<td>0.02</td>
</tr>
<tr>
<td>Prolonged rupture of membrane (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>1.08 (0.87–1.35)</td>
<td></td>
<td></td>
<td>1.47 (1.21–1.79)</td>
<td>1.49 (1.20–1.86)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>BED/CD4 interaction terms</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BED × CD4 (CD4 continuous)</td>
<td>0.87 (0.75–1.01)</td>
<td></td>
<td></td>
<td>0.93 (0.77–1.11)</td>
<td></td>
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</tr>
<tr>
<td>BED × CD4 both continuous</td>
<td>1.05 (1.01–1.10)</td>
<td></td>
<td></td>
<td>0.99 (0.95–1.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BED</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>≥0.8 (long-term)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.8 (recent)</td>
<td>1.68 (1.26–2.25)</td>
<td></td>
<td></td>
<td>0.63 (0.44–0.90)</td>
<td></td>
<td></td>
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<tr>
<td><strong>CD4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;200 (end-stage)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200–349 (intermediate stage)</td>
<td>0.76 (0.52–1.09)</td>
<td></td>
<td></td>
<td>0.47 (0.35–0.63)</td>
<td></td>
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</tr>
<tr>
<td>≥350 (early stage)</td>
<td>0.69 (0.50–0.95)</td>
<td></td>
<td></td>
<td>0.33 (0.25–0.42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group BED/CD4</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1. BED ≥ 0.8 and CD4 ≥ 350</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.02 (1.01–1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. BED ≥ 0.8 and CD4 200–349</td>
<td>1.45 (0.78–1.41)</td>
<td>1.10 (0.80–1.51)</td>
<td>0.56</td>
<td>1.45 (1.12–1.87)</td>
<td>1.45 (1.11–1.90)</td>
<td>0.01</td>
</tr>
<tr>
<td>3. BED ≥ 0.8 and CD4 &lt; 200</td>
<td>1.46 (1.04–2.06)</td>
<td>1.61 (1.12–2.32)</td>
<td>0.01</td>
<td>2.91 (2.23–3.79)</td>
<td>2.07 (2.03–3.60)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4. BED &lt; 0.8 and CD4 ≥ 350</td>
<td>1.49 (1.00–2.22)</td>
<td>1.37 (0.90–2.08)</td>
<td>0.14</td>
<td>0.72 (0.44–1.19)</td>
<td>0.77 (0.46–1.28)</td>
<td>0.31</td>
</tr>
<tr>
<td>5. BED &lt; 0.8 and CD4 200–349</td>
<td>2.57 (1.39–4.77)</td>
<td>2.64 (1.37–5.06)</td>
<td>&lt;0.01</td>
<td>0.80 (0.31–2.03)</td>
<td>0.88 (0.34–2.27)</td>
<td>0.80</td>
</tr>
<tr>
<td>6. BED &lt; 0.8 and CD4 &lt; 200</td>
<td>3.39 (1.19–9.64)</td>
<td>3.73 (1.27–10.96)</td>
<td>0.02</td>
<td>4.12 (1.62–10.45)</td>
<td>2.71 (0.93–7.86)</td>
<td>0.07</td>
</tr>
</tbody>
</table>
prevention of mother to child transmission (PMTCT) programmes in developing countries provide a single test sometime during the antenatal period. Mothers who test positive are then likely to be enrolled into PMTCT programmes. Major reductions have been reported in intra-partum and breastfeeding transmission (the two MTCT modes contributing the biggest portion of all MTCT) due to HIV prophylaxis and feeding options. It is thus likely that in utero transmission will contribute proportionately more to total MTCT compared with other transmission times. Women with low BED reading and a moderate CD4 cell count were more likely to transmit to their unborn children than women with high BED and high CD4 cell count (aOR = 2.64, P < 0.01), although the effect for women with a low BED reading and high CD4 cell count was smaller (aOR = 1.37, P = 0.14). Reproductive-aged women should be educated about this higher risk of transmitting to their unborn child associated with seroconversion during pregnancy.

We have previously reported a 2.90–4.60 times increased risk of post-natal MTCT among breastfed children if the mother seroconverted during this period. This risk is much higher than the 1.37–2.64 times risk reported here, suggesting that the transmission risk associated with maternal primary infection may be lower for placental than breastfeeding-associated transmissions. This is further supported by the fact that intra-partum and post-natal transmissions individually contribute more to total MTCT where anti-retroviral treatment is not available.

We acknowledge that our interpretation of HIV duration and severity for each BED/CD4 group is imperfect and may have resulted in misclassification, especially among women with BED < 0.8 and CD4 200–350, whose risk of in utero transmission was higher than that of chronically infected women. We judged that this combination of BED and CD4 cell count values reflected the nadir in CD4 count that occurs during the acute phase of HIV infection and therefore classified these women as having been recently infected. However, this BED/CD4 combination may also reflect a period of advanced disease when antibody production is depressed, but CD4 count has not yet dropped below 200. If any women in this BED < 0.8/CD4 200–350 group were truly chronically infected, our estimate of excess in utero transmission risk due to maternal primary infection during pregnancy would have been biased towards the null if the misclassified women had a lower risk compared with the truly acutely infected women.

In our previous publication, the infant death or infection rate among the 17 women whose blood sample near delivery tested HIV ELISA negative but HIV RNA PCR positive was 75%; clearly mothers who deliver during HIV seroconversion (when HIV antibodies remain undetectable and viral load is extremely high) are at very high risk of peri-partum transmission. In this report, women likely to have acquired HIV infection during pregnancy, based on their BED/CD4 group, were not at increased risk of intra-partum transmission compared with chronically infected women. Indeed, our risk estimates of intra-partum infection were consistently lower for women classified as recently infected compared with chronically infected women although all OR CIs overlapped 1 except in one comparison. This is probably because all the women included in the current report tested positive for HIV-antibodies at delivery, indicating they had completed the brief ‘window period’ of the ELISA assay (when HIV antibodies are undetectable but viral load is very high) and were now in a much lower risk period of early asymptomatic disease characterized by high antibody, high CD4, low viral concentrations.

In sensitivity analysis models with imputed CD4 cell count, adjusted estimates for in utero and intra-partum infections were consistent with those obtained with observed data, although they had smaller CIs (i.e. smaller standard errors).

Similar to other studies, the risk for in utero infection was inversely related with birthweight, with smaller babies at increased risk of infection. It is not clear whether an infected fetus fails to grow properly or fetal growth impairment predisposes the unborn child to increased risk of HIV infection. The proportion of infants with low birthweight was 16% overall in this study, 24% of those infected in in utero, 22% in intra-partum and 14% among infants who tested negative at 6 weeks. Prematurity has been cited as a risk factor for infection because of immature immune systems, permeability of neonatal mucosal barriers and low levels of maternal antibodies which are normally transferred in the latter half of pregnancy.

There was a 43–46% lower risk of infection among babies born by caesarean section than those delivered vaginally in both the in utero and intra-partum groups. Prolonged rupture of membranes before delivery resulted in a 50% excess risk in intra-partum infection as reported elsewhere. Among vaginal deliveries in our study, 37% had rupture-of-membranes-to-delivery durations of >4h, whereas among emergency caesarean sections, 72% had prolonged rupture of membranes and only 17% among elective caesarean sections. Babies are exposed to cervico-vaginal secretions during prolonged rupture of membranes thus increasing the likelihood of infection. Long duration of ruptured membranes before delivery and low CD4 cell count high viral load have been reported to increase risk of HIV transmission. It is unlikely that elective caesarean sections will ever become the choice of delivery in the most affected HIV regions because of the huge challenges in cost, personnel and equipment. Other efforts to reduce the risk of infection such as reducing viral load have to thus be scaled up in these areas.
Research on optimal and easy-to-apply anti-retroviral treatment protocols should continue.

In conclusion, our findings indicate that the risk of in utero HIV transmission is likely to be greater among women who have their primary HIV infection during pregnancy compared with women who were infected prior to conception. Our estimates of this excess risk ranged from 1.37 (95% CI 0.90–2.08) to 2.64 (95% CI 1.37–5.06), which are lower than the excess risk of transmission during breastfeeding among women who seroconvert during the breastfeeding period. This suggests that the placenta may be a more effective barrier to the high viral load of primary infection than the mammary gland.

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