

Research Article



High Rate of Sexually Transmitted Infections (STIs) among Asymptomatic Pregnant Women in a Resource-poor Setting in the Middle Belt Zone of Nigeria

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Abstract | Sexually transmitted infections (STIs) can complicate pregnancy and may be associated with adverse health outcomes for both the woman and her developing baby. Despite the serious health consequences of STIs, information is lacking regarding the magnitude of the disease in the study setting. This study investigated the prevalence of five major STIs (HIV, HBV, HCV, HSV-2 and syphilis) in asymptomatic women in Anyigba. Sera samples from a cross-section of 250 consenting pregnant women on antenatal visits to five health facilities were screened for HIV, HBV, HCV and HSV-2 using commercially available immunochromatographic based kits and for syphilis using non-treponemal and treponemal antibody test. Socio-demographic and obstetric parameters were obtained using structured questionnaires. Sixty-four (25.6%) out of 250 women had at least one STI. The overall prevalence of syphilis, HIV, HBV, HCV and HSV-2 were 28(11.2%), 21(8.4%), 10(4.0%), 5(2.0%) and 0% respectively. Women who were married, unemployed, aged 15–35 years and of low-income earners had a higher prevalence of STIs. Factors such as a history of STIs in women and husbands, blood transfusions, genital ulcers and trimesters were significantly related to a higher predisposition to STIs. Conclusively, a high prevalence of STIs was observed among asymptomatic pregnant women in the study area. Our findings highlight a concern with economic consequences. Hence intervention programs including comprehensive screening of pregnant women with increased access to health care and treatment of confirmed cases at an affordable cost should be promoted to reduce STIs thereby reducing associated morbidities and mortalities.

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Introduction

Sexually Transmitted Infections (STIs) remain a serious health-related challenge and the global burden of the disease is reportedly on the increase (Williams et al., 2018). Sexually transmitted diseases (STDs) constitute significant medical, social and

economic problems in both the pregnant and non-pregnant women globally. It can complicate pregnancy and may have serious adverse consequences for both women and her developing babies such as stillbirth, spontaneous abortions, low-birth weight, post-partum endometritis and premature rupture of fetal membranes (CDC, 2020; Neerja et al.,

2012). Although bacteria (e.g., *Treponema pallidum* and *Neisseria gonorrhoeae*), protozoal (*Trichomonas vaginalis*) and Fungal (e.g., *Candida albicans*) can lead to these consequences, most adverse pregnancy outcomes have been associated with viral infections (e.g., HIV, HSV-2, HCV, HBV). A mother can transmit the infection to her baby during antepartum, intrapartum and postpartum periods (US Panel Report, 2012). For HIV for example, the transmission may occur during pregnancy via the placenta, labor and delivery or after birth through breastfeeding. However, it is possible to reduce the risk of transmission to less than 2% when HIV is diagnosed before and during pregnancy and correct preventive steps are taken (US Panel Report, 2012). In addition to adverse perinatal outcome, STIs may lead to serious reproductive health consequences such as infertility, cervical cancers or chronic pelvic infections (WHO, 2020, 2019).

More than 30 different infectious agents are known to be transmitted majorly via sexual contact. Most important of these are *Treponema pallidum*, *Herpes Simplex Virus* (HSV), *Hepatitis B virus* (HBV) and *Human immunodeficiency virus* (HIV) which are reportedly associated with a greater incidence of STDs (WHO, 2020). Globally, more than 1million STIs are acquired each day with most cases asymptomatic or only showing mild symptoms that are difficult to recognize as an STI (WHO, 2020).

In Nigeria, pregnant women attending antenatal clinics are routinely screened for HIV and Syphilis while care for other STIs such as HSV and HBV usually depends on symptoms. Thus, the lack of a comprehensive screening program for the detection of STIs in asymptomatic pregnant women constitutes a management challenge in this resource-poor setting. Most epidemiological studies on STIs in pregnant women population were among those manifesting symptoms of STIs and /or bad obstetric history (NACO, 2006). One common practice in this locality is that people do not report to the hospital until they have started presenting with symptoms. Since early recognition and treatment are needed to prevent adverse outcomes that are major causes of infant morbidity and mortality (Andrews et al., 2000), current study becomes imperative to appraise the current burden of STDs among pregnant women in the study area to generate information that could be extrapolated to the general population of sexually active women. Since there is a dearth of information

on STDs prevalence among pregnant women in Anyigba, the current study was therefore designed to assess the prevalence of HIV, *Treponema pallidum*, HBV, HCV, and HSV-2 infections and also identify predisposing risk factors to STI.

Materials and Methods

Study area

This study was conducted in Anyigba (7°15'–7°29" N, 7°11'–7°32" E) located in Dekina Local Government Area of Kogi State, Nigeria. The population of Anyigba estimated to be about 130,000 inhabitants comprised of major ethnic groups in Nigeria. The climate of Anyigba falls within tropical hinterland and the guinea savanna. The study area experiences hot and dry climate and has a mean annual temperature and rainfall of 25° C and 1600mm respectively. Farming is the major occupation of inhabitants. The inhabitants reportedly indulge in practices such as early age of sexual debut, traditional rituals (tribal marks), intravenous drug use, use of unscreened blood and sexual promiscuity (Omatola et al., 2019).

Study population/design

This was a cross-sectional hospital-based study in which 250 apparently healthy pregnant women at different ages and trimesters were consecutively recruited from five most utilized health facilities in Anyigba (Kogi State University Teaching Hospital, Grimmer Catholic Hospital, Amazing Grace Specialist Hospital, Maria Goretti Hospital, and Christ the Good-shepherd Hospital). Women were adequately counseled and those who granted both oral and written informed consent were successively recruited into the study. With the assistance of hospital personnel in each hospital, blood samples were drawn from consented participants. At the same time, information regarding demographic, social, behavioral characteristics and obstetric performance were obtained with a structured questionnaire. Those hospitals run a biweekly (Mondays and Thursdays) antenatal care services. The study was carried out between October 2019 and January 2020. A sample size of 250 was calculated using the sample size formula $N = Z^2pq/d^2$ and a previous STIs prevalence of 10.0% from a similar study in the study setting (Omatola et al., 2019). For each clinic, the sample size was determined from the proportion of women on a daily visit to the clinic. Ethics approval for the study was obtained from the Institutional Ethical Review Board (IRB)

Table 1: Socio-demographic characteristics of patients with sexually transmitted infections.

Variable	No. tested	HIV+(%)	P-Value	HBV+(%)	P-Value	HCV+(%)	P-Value	Syphilis+(%)	P-Value
Age group									
15 – 20	39	1(2.6)	0.19	0	0.28	1(2.6)	0.56	5(12.8)	0.75
21 – 25	75	4(5.3)		6(8.0)		1(1.3)		9(12.0)	
26 – 30	79	10(12.7)		3(3.8)		1(1.3)		6(7.6)	
31 – 35	32	5(15.6)		0		2(6.3)		4(12.5)	
36 – 40	22	1(4.5)		1(4.5)		0		4(18.2)	
>40	3	0		0		0		0	
Total	250	21(8.4)		10(4.0)		5(2.0)		28(11.2)	
Education									
None	5	0	0.26	0	0.44	0	0.80	0	0.20
Primary	32	0		0		0		3(9.4)	
Secondary	143	14(9.8)		8(5.6)		3(2.1)		21(14.7)	
Tertiary	70	7(10.0)		2(2.9)		2(2.9)		4(5.7)	
Family									
Nuclear	191	15(7.9)	0.58	8(4.4)	0.78	5(2.6)	0.21	22(11.5)	0.77
Extended	59	6(10.2)		2(3.4)		0		6(10.2)	
Occupation									
Self employed	108	12(11.1)	0.71	7(6.5)	0.28	3(2.8)	0.12	16(14.8)	0.12
Student	25	1(4.0)		0		2(8.0)		2(8.0)	
Housewife	38	3(7.9)		0		0		3(7.9)	
Farming	34	2(5.9)		2(5.9)		0		0	
Unemployed	45	3(6.7)		1(2.2)		0		7(15.6)	
Income level									
Low	128	13(10.2)	0.31	8(6.3)	0.06	4(3.1)	0.19	18(14.1)	0.14
High	122	8(6.6)		2(1.6)		1(0.1)		10(8.2)	
Marital status									
Single	55	2(3.6)	0.40	0	0.33	2(3.6)	0.79	6(10.9)	0.30
Married	188	19(10.1)		10(5.3)		3(1.6)		21(11.2)	
Divorced	5	0		0		0		0	
Widowed	2	0		0		0		1(0.5)	

of Kogi State University Teaching Hospital and it was in accordance with the Declaration of Helsinki Code of Ethics of the World Medical Association (IRB Approval No. /004/VOL.1). This approval covers the studies in the other four hospitals.

Sample collection, processing and storage

Five (5) milliliters of venous blood was obtained from each of the 250 consented participants into a well-labeled sterile vacutainer tube. Samples were spun with GreatMed® PRP centrifuge at 1000 revolutions per minute for 5 minutes to separate sera from whole blood. Sera were then stored at -20°C in line with manufacturer's specification until screened serologically for HIV, syphilis (VDRL and TPHA), HCV, HBV and HSV-2.

Serological assays

Screening for HIV antibodies was performed using two rapid HIV test methods; Determine HIV1/2 (Abbot Laboratories-USA) and Uni-Gold Recombinant® HIV ½ (Trinity Biotech, Ireland) test kits in line with the national HIV standard diagnosis algorithm (FMoH, 2010). Test for treponemal antibodies was done using ACON rapid immunochromatographic VDRL (ACON Laboratories, Inc., San Diego, CA, USA) test kits. Reactive sera were subjected to confirmation using the Treponema pallidum Hemagglutination test, TPHA (Teco Diagnostics, USA). Screening for HSV-2 virus-specific antibodies was done using the Biopanda membrane-based rapid immunochromatographic test kit (Biopanda Diagnostic and Reagents Limited®, United Kingdom).

Table 2: *Distribution of sexually transmitted infections in relation to putative risk factors.*

Variable	No. tested	HIV+(%)	P-Value	HBV+(%)	P-Value	HCV+(%)	P-Value	Syphilis+(%)	P-Value
Knowledge of STDs									
Yes	27	3(11.1)	0.59	0	0.26	1(3.7)	0.50	4(14.8)	0.53
No	223	18(8.1)		10(4.5)		4(1.8)		24(10.8)	
History of STDs									
Yes	38	9(23.7)	0.00	1(2.6)	0.64	1(2.6)	0.76	12(31.6)	0.00
No	212	12(5.7)		9(4.2)		4(1.9)		16(7.5)	
History of STDs in partner									
Yes	41	8(19.5)	0.01	0	-	3(7.3)	0.008	14(34.1)	0.00
No	209	13(6.2)		10(4.8)		2(1.0)		14(6.7)	
History of blood transfusion									
Yes	59	12(20.3)	0.001	1(1.7)	0.30	3(5.1)	0.05	8(13.6)	0.51
No	191	9(4.7)		9(4.7)		2(1.0)		20(10.5)	
Presence of genital ulcers									
Yes	29	7(24.1)	0.001	3(10.3)	0.06	2(6.9)	0.04	19(65.5)	0.00
No	221	14(6.3)		7(3.2)		3(1.4)		9(4.1)	
Age at first pregnancy									
15 – 20	86	6(7.0)	0.83	2(2.3)	0.15	3(3.5)	0.36	13(15.1)	0.33
21 – 25	128	12(9.4)		8(6.3)		1(0.8)		11(8.6)	
≥26	36	3(8.3)		0		1(2.8)		4(11.1)	
Gestation period									
1 – 12 weeks	91	4(4.4)	0.11	1(1.1)	0.01	2(2.2)	0.98	16(17.6)	0.05
13 – 28 weeks	111	10(9.0)		9(8.1)		2(1.8)		9(8.1)	
29 – 42 weeks	48	7(14.6)		0		1(2.1)		3(6.3)	
Parity									
0 – 3	174	14(8.0)	0.48	8(4.6)	0.74	3(1.7)	0.83	23(13.2)	0.15
3 – 6	72	6(8.3)		2(2.8)		2(2.8)		4(5.6)	
≥7	4	1(25.0)		0		0		1(25.0)	
History of abortion									
Yes	50	4(8.0)	0.91	2(4.0)	1.00	3(6.0)	0.02	8(16.0)	0.23
No	200	17(8.5)		8(4.0)		2(1.0)		20(10.0)	
Contraceptive use									
Yes	41	6(14.6)	0.12	0	0.15	1(2.4)	0.83	6(14.6)	0.45
No	209	15(7.2)		10(4.8)		4(1.9)		22(10.5)	

This qualitative assay detects anti-HSV-2 IgM and IgG antibodies which bind to conjugated recombinant HSV antigen on nitrocellulose membrane. Sera were tested for HBsAg using Coschesic strips which are coated with monoclonal anti-HBs capture antibody and also tested for anti-HCV antibodies using ARIA HCV-Ab test kit (CTK Biotech, Inc., San Diego, CA). Coschesic strips have >99.6% sensitivity and 98.6% specificity while the ARIA HCV-Ab test kit has diagnostic sensitivity and specificity of > 99% and 98% respectively. All screening tests and interpretation of results were carried out in accordance with the kit manufacturer's specifications.

Statistical analysis

Data generated in the study were analyzed using statistical packages for social sciences (SPSS) version 16 for windows. Categorical variables were summarized as proportions and were analyzed using the Chi-square tests. A p-value of less than 0.05 was set as the level of statistical significance.

Results and Discussion

Of the 250 sera screened for STIs, 64(25.6%) tested positive for syphilis, HIV, HBV, HCV, or a combination of one or more of the STIs (Table

1). None of the women tested positive for HSV. Syphilis/HIV, syphilis/HBV, syphilis/HCV, HIV/ HBV, HCV/HIV dual infections were 6(9.4%; $p=0.008$), 4(6.3%; $p=0.003$), 1(1.6%; $p=0.53$), 2(3.1%; $p=0.18$), and 2(3.1%; $p=0.01$) while 1(2.1%; $p=0.00$) were simultaneously seropositive for syphilis/HIV/ HBV. Accordingly, 28(11.2%), 21(8.4%), 10(4.0%), 5(2.0%) and 0% were the overall prevalence rates for syphilis, HIV, HBV, HCV and HSV respectively. Ninety percent (90%) of women who participated in the study were ≤ 35 years and had greater exposure to STIs (HIV=36.2%; HBV=11.8%; HCV=11.5% and syphilis=44.9%) compared to the older ages. HIV seropositivity rates increase progressively with increasing age until ages 31-35 with the highest seropositivity rate (15.6%) and decrease afterward. There was no statistically significant difference between the age of acquiring infection and HBsAg, anti-syphilis, anti-HIV or anti-HCV antibody seropositivity ($p>0.05$) (Table 1). Except for HIV and HCV, participants with a secondary level of education were more seropositive for HBV and syphilis infection. Occupationally, students and unemployed women had higher HCV (8.0%) and syphilis (15.6%) while the self-employed category had greater exposure for HIV (11.1%) and HBV (6.5%). Though the distribution of STIs in relation to the subject's occupation was not significant statistically ($p>0.05$). Generally, an income level of subjects was inversely correlated to the seropositivity rate. Low income earners had higher STIs (HBV=6.3%; HCV=3.1%; HIV=10.2%; syphilis=14.1%) compared to the higher income earners with HBV, HCV, HIV and syphilis seropositivity rates of 1.6%, 0.8%, 6.6% and 8.2% respectively. Married subjects with seroprevalence rates of 10.1%, 5.3%, and 11.2% respectively for HIV, HBV and syphilis were more exposed to STIs than the other marital groups. Though the difference between marital status and infection was not significant ($p>0.05$) (Table 1). Women who reported a history of STIs and in partners, as well as those who had genital ulcers, were significantly more seropositive for HIV, HCV and syphilis ($p<0.05$). Subjects in their first and second trimester of pregnancy respectively had significantly higher syphilis ($p=0.05$) and HBV ($p=0.01$) than the other gestational levels. The difference between previous receipts of transfused blood and anti-HIV ($p=0.05$) as well as anti-HCV ($p=0.001$) seropositivity was also statistically significant. Women who aborted one or more pregnancies in the past significantly had higher HCV infection ($p=0.02$). Generally, women

with higher births were more predisposed to STIs compared to those with lower parity. Similarly, subjects with a history of contraceptive use tend to be more seropositive for HIV, HCV and syphilis (14.6%, 2.4%, and 14.6%) than those without such history with HIV, HCV and syphilis prevalence of 7.2%, 1.9% and 10.5% respectively. Though, the difference between parity as well as contraceptive use in relation to syphilis, anti-HIV, and anti-HCV antibody positivity was not significant ($p>0.05$) (Table 2).

Findings from our study showed that 64(25.6%) out of the 250 asymptomatic pregnant women tested positive to one of the sexually transmitted pathogens (HIV, HBV, HCV, HSV and *Treponema pallidum*). In contrasts to prevalence rates reported for STIs among asymptomatic prenatal women from a middle (4.8% India) (Neerja et al., 2012) and a high (5.8% Brazil) (Lima and Viana, 2009) income countries, findings from the current study and previous Tanzanian's report of 15.6% by Ng'wamkai et al. (2019) support World health report (WHO, 2012) which posited that STIs is highly prevalent among asymptomatic pregnant women in low-income countries. Variations from reported rates in high-income countries may be accounted for by differential in quality of health care systems such as the approach used in identifying and managing STIs in pregnancy as well as risk profiles of the populations included in the study.

The overall prevalence of 11.2% reported for syphilis in this study is higher than 2.0% and 3.1% reported in Nigeria (Okoroiwu et al., 2018; Olowe et al., 2014), 1.8% in Ethiopia (Zinabie et al., 2018) and 2.2% in Zambia (Berrueta et al., 2017). This considerably high prevalence rate among asymptomatic women calls for serious concern because syphilis increases the risks of acquisition of other STIs and mother-to-child transmission of HIV in cases where the mother is co-infected. Difficulty in affording the cost of antenatal screening and the biweekly/monthly follow-up as well as possible re-infection from an untreated sexual partner may have contributed to the high rate of syphilis observed in this study. The significantly high difference between syphilis and positive HIV and HBV serology in the current study may justify earlier reports of (Fleming and Wasserheit, 1999) that syphilis increases the chances of contracting other STIs. Similar to the outcome of our study, Ng'wamkai et al. (2019) and Mutagoma et al. (2017) also reported a significant association between HIV and *T. pallidum*

infection.

The overall anti-HIV positivity rate of 8.4% observed in this study compares favorably with 8.5% earlier reported among apparently healthy pregnant women in the study area (Omatola et al., 2019). This finding suggests that the burden of the disease has not changed over the past two years. The current HIV seropositivity rate is substantially higher than the overall prevalence of 3.3% reported for Kogi State during the 2014 national sentinel survey (FMOH, 2015). The outcome of this study does not reflect the decline in the national HIV prevalence from 2.8% in 2018 to 1.4% in 2019 in which 1.9% was for women within the reproductive years (15-46years) (9). It also does not support findings from other studies in the sub-Saharan African countries (Manyahi et al., 2017; Ng'wankai et al., 2019) which also reported a declining trend in HIV in the population of pregnant women. While also highlighting the significance of regional variation, findings of this study underscore the need for sustained efforts in high-risk areas despite encouraging national trends. The national and global decline in the burden of STIs in contrast to findings from the present study could be due to the outcome of various intervention programs that have been implemented over the years. However, the risen trend of infection in our study may be due to a lack of full access to current prevention programs on STIs and more involvement in risky behaviors. Thus, with more pregnant women becoming seropositive for HIV in the study area, high perinatal transmission is not unexpected. Because mother-to-child transmission of HIV represents the principal route of transmission of HIV infection in children, there is, therefore, the need to increase the momentum of reaching out to this vulnerable population for a focused delivery and increased access to antenatal screening and counseling, HIV prevention, treatment and care services to rural settings.

HCV prevalence rate of 2.0% obtained in this study is three-fold higher than previous reports of 0.5% positivity rate among asymptomatic pregnant women in the study area (Omatola et al., 2019). Lower prevalence rates have also been reported from Tanzania and another part of Nigeria (6, 19). The significantly high rate of HIV/HCV co-infection in the present study could point to the fact that both viruses share transmission routes and because the population included in the study share lots of attributes, factors

that predispose one to either infection are likely to lead to infections by both viruses. No woman tested positive for HSV-2 IgM or IgG in this study. This finding is contrary to previous reports (2.0%, 33.3%) by Neerja et al. (2012) and Anaedobe and Ajani (2019) in India and Nigeria respectively. The reasons for this observation may be related to differences in the sensitivity of the diagnostic assays as well as the sample size. Despite the HSV negative serostatus observed in the current study, there is the need for continuous screening of asymptomatic women who may acquire genital herpes near the time of delivery thereby placing the baby at risk of neonatal herpes as earlier posited by JAMA report (JAMA, 2014). In comparison to other seroprevalence studies, the HBsAg seropositivity rate of 4.0% obtained in our study is similar to the 4.25% and 3.8% in Delhi (Chakravarti et al., 2005) and Mwanza city (Mirambo et al., 2016) respectively. Earlier et al. (2012) in India and Omatola et al. (2019) in the study area reported a lower prevalence of 2.4% and 1.0% respectively among asymptomatic women. A higher rate of 8.5% was reported in Ankpa, Nigeria (Omatola et al., 2020). The increasing trend in the prevalence of HIV, HBV, and /or HCV in the current study does not support previous epidemiological reports from the study setting which indicated lower prevalence rates nay the World Health position (WHO, 2013) on global reduction of STIs. This observation may be suggestive of endemicity due to increased exposure to the risk of occurrences.

In this study, the prevalence of STIs increased progressively until age 35 years but decline afterward. This age of greater infection corresponds with the peak age of sexual activity thus given credence to the role of sexual intercourse in disease transmission. This finding justifies World Health Reports (WHO, 2006) that age group 15-30 years of greater sexual activity are most infected by one STD or the other and that incidence decreases after age 35 years.

Socioeconomic levels were evaluated in our study using a wealth index. A lower-income level was correlated with increased STIs and this is indicative of poverty. This observation is consistent with a previous report of Ekanem et al. (2012) who reported a similarly high burden of STIs among asymptomatic pregnant women in a Nigerian state. The fact that women of low-income earners are likely to experience more difficulty in affording the cost of antenatal follow-

up programs, as well as treatment when they and/ or their partner is infected, could explain the reason for the higher infection. A higher rate of infection among the unemployed and self-employed women compared to the other occupational groups may further support this observation in the current study.

The high prevalence of STIs observed among the married women in the study agrees with previous reports in India (Neerja et al., 2012). Being married had also been previously linked to higher HIV and HBV infection in North-central Nigeria (Omatola et al. 2020, 2019, 2017). Marriage provides means of sexual intercourse and high-risk sexual activity by any of the spouses could lead to a predisposition to STIs. Contrasting reports from Nigeria (Ekanem et al., 2012), however, showed that divorcees and widows had a higher predisposition to STIs than the married and singled women. Possible geographical variation occasioned by differences in sexual behaviors and economic dimensions may partly explain this difference.

In this study, the significantly higher rate of STIs observed in relation to the history of STIs in husbands, receipt of transfused blood and presence of genital ulcers have been previously documented (National AIDS Reports, 2006; Neerja et al., 2012). The significantly higher infection of women by *T.pallidum* and HBV in the first and second trimesters respectively necessitate the need for early screening and proper management at this early stage of pregnancy to reduce the risk of mother-to-child transmission, adverse perinatal outcomes and /or community burden of infection.

In conclusion, findings from this study indicate a high burden of STs among asymptomatic pregnant women in the study area. Some factors such as a history of STIs in partners, income levels and previous receipt of transfused blood are linked to increased acquisition of STIs. Thus, to actualize the global objective on the reduction of STIs, there is the urgent need for targeted interventions of prevention services through education on the benefit of routine screening for all pregnant women, proper treatment of confirmed cases, contact tracing and treatment of sexual partners. The government of Nigeria should make routine screening for STIs mandatory for all pregnant women while also ensuring that the costs

of antenatal follow-ups program are reduced to encourage the economically poor ones to key into it. This study has certain limitations. Our findings may not be representative of the entire Anyigba pregnant population since only hospital-based cases were considered. Also, the qualitative immunochromatographic techniques employed in the study might not have ruled out false-negative results especially for latently infected subjects or those within the window period possibly resulting in underestimation of the current burden of STIs in the study area. Despite these limitations, findings from the current study are important as it would complement the scarce epidemiological data on STIs in the study area. It reaffirms the importance of serological screening during prenatal care and information generated could influence government policy vis-a-vis the need to strengthen the existing STIs surveillance and interruption program to contain the spread and also reduce risks of mother-to-child transmission.

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Authors' Contributions

CAO designed the study and obtained ethical approval; SDI and SJA collected samples; CAO, SDI, and SJA ran assay; CAO, MOO, PQA, and CKM analyzed the data; CAO and CKM wrote the draft manuscript. All authors read and approved the final manuscript.

Statement of conflict of interest

The authors have declared no conflicts of interest.

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