

Full Length Research Paper

Seroepidemiology of Toxoplasmosis among Pregnant Women in Osogbo, Southwestern, Nigeria

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Infection with *Toxoplasma gondii* during pregnancy represents a risk of congenital infection. Simple hygienic practices due to adequate knowledge about the parasite is one of the major keys to avoiding this preventable infection among pregnant women and women of child bearing age. The present cross sectional study was carried out to investigate the seroprevalence of anti-*Toxoplasma* Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies, associated risk factors of infection and knowledge about *T. gondii*, its transmission, treatment and prevention among pregnant women in Osogbo, Southwestern Nigeria. A total of 391 serum samples were collected from consenting pregnant women aged 16-50 years attending ante-natal clinic in four major hospitals within Osogbo between October and December, 2015. The samples were screen for toxoplasmosis using specific Toxo IgG and IgM Enzyme-Linked Immunosorbent Assay (ELISA) kits. Information on sociodemographic data, associated risk factors and knowledge about the *T. gondii* were obtained from the women using a structured questionnaire. The results indicated that 30.44% and 17.14% of the women had anti-*Toxoplasma* IgG and IgM antibodies respectively. Seropositivity of *Toxoplasma* infection was found to increase positively with the age of the pregnant women and decreases with increase in level of education. Logistic regression analysis showed a significant association between participant's age, habit of tasting meat while cooking and cat ownership. A generally low knowledge of *T. gondii* was observed in this study whereby majority (90.76%) of the pregnant women claimed not to have any knowledge about toxoplasmosis while none of the participant admitted to been previously tested for the disease.

Key words: Seroprevalence, Infection, pregnant women, IgG and IgM antibodies, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular coccidian parasite found in many species of animals in virtually all parts of the world (Ishaku et al., 2009) except the Antarctica (Uttah et al., 2013) and approximately one

third of the world's population is infected with the parasite (Tenter et al., 2000). *T. gondii* infection is asymptomatic in the immunocompetent but causes a severe clinical complication in immunocompromised individuals and to

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the fetuses of mothers suffering from primary infection during pregnancy (Torda, 2001; Remington et al., 2001). Humans are infected majorly through consumption of contaminated foods, fruits, vegetables and water while pregnant women can transfer the infection through placenta to unborn fetuses (Remington et al., 2001). Maternal infection acquired before pregnancy does not affect foetus (Montaya & Liesenfeld, 2004) but those acquired during pregnancy can lead to complication for the foetus most especially if primary infection is acquired during the first trimester when the disease causes severe neurological damage, miscarriage or death. Foetal infection during second and third trimesters may result in either congenital disease or subclinical infection (Remington et al., 2005). Most congenitally infected babies appear normal at birth, however, serious sequelae such as neurological impairment and chorioretinitis can manifest in the second or third decades of life (Dunn et al., 1999). Seroconversion of susceptible women of child-bearing age are preventable by simple precautions if such women are well informed. These precautions include consumption of well cooked meat, avoidance of ingesting fruits and vegetable contaminated with *T. gondii* oocyst and observance of improved personal hygiene in the handling of cat litters and in gardening.

Routine screening for toxoplasmosis among pregnant women is the key to early detection of infection which can assist in early chemotherapy to avert any impending damage to the foetus. This practice is the norm in some European countries including France, Germany and Austria but this has not been introduced / included in the tests mandated for pregnant women attending ante-natal care clinic in Nigeria.

It is very germane that studies on the seroepidemiology of the parasite partially, knowledge about the parasite, its transmission, treatment and prevention are necessary in Nigeria as they would provide a baseline on the disease thereby justifying the need for inclusion of toxoplasmosis tests in the routine screening of pregnant women and the need for health care providers to step up sensitization of pregnant women about the devastating consequence of toxoplasmosis in Nigeria. Here lays the significance of the present study.

MATERIALS AND METHODS

A cross sectional study was conducted between October and December 2015 to determine the seroprevalence and knowledge about *T. gondii* among pregnant women attending ante-natal care clinic in two major hospitals and two primary health care centre in Osogbo, Southwestern Nigeria. Osogbo is the capital of Osun State and lies on coordinates, 07° 46' N and 04° 34' E with elevation of 320 m above the sea level. According to the NPC, (2006) the city has a population of about 250,951 people and a landmass of 47 km². Osogbo has tropical climate with an annual average temperature and precipitation of 26.1°C and 1, 241 mm respectively (Yusuf, 2016). The vegetation of the area is described as derived savannah characterized by a gallery of forest and tall grasses with scattered perennial trees (Ola and Adewale, 2014). The inhabitants

are predominantly Yoruba speaking ethnic extraction of the Southwest Nigeria with a few representations of people from other ethnic groups of Nigeria.

Consent and Ethical Issues

Ethical clearance was sought and collected from Osun State Ministry of Health and the Ethical Boards of the hospitals involved in this study before the commencement of blood sample collection. The pregnant women who were recruited in this study were adequately enlightened on the purpose and importance of the study. Participants who consented were assured of confidentiality.

Questionnaire administration

A well-structured, pre-tested and closed-ended questionnaire was administered orally to extract from participants their socio-demographic (sex, age, place of residence) and socio-economic (occupation, literacy, ethnic group) information/details, risk factors associated with toxoplasmosis acquisition including habit of tasting of meat while cooking, owning of cats and knowledge about the parasite, its transmission, treatment and prevention. Subjects' local language/ dialect was used to extract information and their responses were recorded by ticking the appropriate boxes in the questionnaire.

Collection of blood samples

5 ml of venous blood was collected from each participant by a qualified phlebotomist following the standard procedure for blood collection. Each blood sample was collected into a plain vacutainer and was allow to clot. The clots were removed and the sample was centrifuged at 2000 rpm for 10 min. The resulting clear serum samples was carefully collected in a vial and kept at -20°C in the Parasitology Laboratory, Department of Zoology, Obafemi Awolowo University Ile Ife prior to serological examination.

Each serum was tested for the presence of anti-*Toxoplasma* IgG and IgM antibodies in duplicate using commercially procured Enzyme Linked Immunosorbent Assay Elisa Kit (Microwell Toxo Kit) (Bio-Inteco Diagnostic Ltd. UK) designed for this purpose and strictly following the manufacturer's instruction. The mean value for each sample was calculated by dividing the mean absorbance values of duplicate wells of each sample by the cut off calibrator mean value.

Statistical analysis

All statistical analyses were performed using SPSS for windows version 20.0. Relationship between antibodies seroprevalence was determined by Pearson chi-square test at 95% confidence interval and 0.05 level of significant. Bivariate logistic regression was further carried out to assess the predictive effect of the various risk factors on the seroprevalence of *T. gondii* in this study.

RESULTS

A total of 391 pregnant women were recruited from four hospitals in Osogbo metropolis between October and December 2015. Most of the pregnant women (153, 39.1%) were from State Hospital, Asubiaro followed by

Table 1. Socio-demographical profile of the participant pregnant women.

Socio-demographic variables	N (N=391)	%
Age group(years)		
≤20	22	56.63
21-30	246	62.92
31-40	120	30.69
41-50	3	0.77
Marital Status		
Single	55	14.07
Married	305	78.01
Divorced	20	5.12
Widowed	11	2.81
Occupation		
Civil servant	59	15.09
Trading	167	42.71
Artisan	109	27.88
Farmer	10	2.56
Student	24	6.14
Applicant	22	5.63
Educational level		
Primary	53	13.55
Secondary	226	57.80
Tertiary	105	26.85
No education	7	1.79
Gestation Period		
1 st trimester	48	12.28
2 nd trimester	103	26.34
3 rd trimester	240	61.38

Atelewo Primary Healthcare (104, 26.6%), Oke-Baale Primary Healthcare (80, 20.5%) and Ladoke Akintola University Teaching Hospital (54, 13.8%) (Table 1).

Knowledge about the parasite, its transmission, causes, treatment and prevention

Table 2 summarizes response response regarding their knowledge, transmission, treatment and prevention of *T. gondii*. Thirty-six (9.21%) claimed to have knowledge about the existence of the parasite while majority (355, 90.79%) of the pregnant women claimed not to know anything about the parasite. None of the participants indicated that they had ever been tested for toxoplasmosis; however approximately 40% were uncertain if they had been tested before. Fourteen (3.58%) participants indicated that they heard about toxoplasmosis on radio programme while 47 (12.02%) believed that bacteria was the etiologic agent of toxoplasmosis. There was under spread ignorance on the mode of transmission, treatment and preventive measures

of toxoplasmosis among population understudy. This is because 30 (7.67%) indicated that the disease could be contracted by insect bites while 280 (71.61%) did not have an idea on how the parasite is contracted. Seventeen (4.34%) claimed toxoplasmosis does not have cure while 47.47% indicated that it could be cured by chemotherapy. Majority (53.96%) of the participant do not know any preventive measures to adopt in order to avoid contracting toxoplasmosis while 28.13% claimed that improved personal hygiene can prevent toxoplasmosis.

Seroprevalence of *T. gondii* antibodies among pregnant women in Osogbo

The overall seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies recorded in this study were 30.43 and 17.14% respectively (Table 3). The highest prevalence of both IgG (36.67%) and IgM (19.17%) antibodies were recorded among pregnant women aged 31-40 years while least prevalences of both IgG (13.64%) and IgM (9.09) were recorded among the ≤20 years age group.

Table 2. Knowledge about Toxoplasmosis among Pregnant women studied.

Questions on Knowledge about Toxoplasmosis	N (n=391)	%
Have you heard about Toxoplasmosis?		
Yes	36	9.21
No	355	90.79
Have you been tested for Toxoplasmosis before?		
Yes	0	0.00
No	235	60.10
No idea	156	39.90
Sources of information about Toxoplasmosis		
Internet	9	2.30
Newspaper/ magazines/ books	8	2.05
Radio	14	3.58
T.V	5	1.28
No idea	355	90.79
Pathogenic organism of Toxoplasmosis		
Virus	38	9.72
Bacteria	47	12.02
Protozoan	45	11.51
No idea	261	66.75
Ways of contacting disease/ Risk factors		
Contaminated food and drinks	10	2.56
Insect bites	30	7.67
Eating of raw/undercooked meat	16	4.09
Mother to child (vertical transmission)	5	1.28
Owing cats	13	3.33
Gardening / farming	18	4.61
Contaminated fruits and vegetables	19	4.86
No idea	280	71.61
Treatment of Toxoplasmosis		
Chemotherapy	170	43.48
Vaccination	25	6.39
No cure	17	4.35
No idea	179	45.78
Prevention of Toxoplasmosis		
Avoid contact with cat faeces	16	4.09
Consumption of properly cooked meat	10	2.56
Wash fruits and vegetable properly before eating	18	4.61
Wash hands after handling raw meat	15	3.84
Wash knives and kitchen utensil properly	11	2.82
Increase in personal hygiene	110	28.13
No idea	211	53.96

None of the pregnant women at age group 41-50 years had anti-*Toxoplasma* IgM antibodies. The seroprevalence of both IgG and IgM antibodies increased as the age of the women increased. There was a significant relationship between age distribution and seropositivity to anti-*Toxoplasma* IgG and IgM antibodies among the pregnant

women. ($P < 0.05$).

It was observed from the result (Table 3) that significantly high seroprevalence to anti-*Toxoplasma* antibodies was recorded in all pregnant women based on marital status except the widowed who recorded the least prevalence of IgG (9.09%) and IgM (9.09%) antibodies in

Table 3. Seroprevalence of *T. gondii* IgG and IgM antibodies among pregnant women.

Parameter	Number examined	IgG Number (%) positive	IgM Number (%) positive	P-value
Age group (years)				
≤20	22	3(13.64)	2(9.09)	$\chi^2=5.433$ P=0.043 Significant
20-30	246	71(28.86)	42(17.07)	
31-40	120	44(36.67)	23(19.17)	
41-50	3	1(33.34)	0(0.00)	
Marital status				
Single	55	17(30.91)	9(16.36)	$\chi^2=2.591$ P=0.015 Significant
Married	305	94(30.83)	54(17.70)	
Divorced	20	7(35.00)	3(15.00)	
widowed	11	1(9.09)	1(9.09)	
Occupation				
Civil servant	59	18(30.51)	12(20.34)	$\chi^2=3.282$ P=0.657 NS
Trading	167	50(29.94)	29(17.36)	
Artisan	109	38(34.86)	20(18.35)	
Farmer	10	3(30.00)	1(10.00)	
Student	24	4(16.67)	1(4.17)	
Applicant	22	6(27.27)	4(18.18)	
Educational level				
Primary	53	18(33.96)	11(20.76)	$\chi^2=0.810$ P=0.847 NS
Secondary	226	65(28.76)	39(17.26)	
Tertiary	105	34(32.38)	17(16.19)	
No education	7	2(28.57)	0(0.00)	
Gestation period				
1 st trimester	48	15(31.25)	9(18.75)	$\chi^2=0.021$ P=0.990 NS
2 nd trimester	103	31(30.10)	21(20.39)	
3 rd trimester	240	73(30.42)	37(15.42)	
Cat ownership				
Yes	22	5(22.73)	3(13.64)	$\chi^2=0.654$ P=0.055 Significant
No	369	114(30.89)	64(17.35)	
Cat in the premises				
Yes	145	34(23.45)	19(13.11)	$\chi^2=5.792$ P=0.419 NS
No	216	73(33.79)	42(19.45)	
No idea	30	12(40.00)	6(20.00)	
Meat tasting while cooking				
Yes	279	89(31.90)	49(17.56)	$\chi^2=0.987$ P=0.011 Significant
No	112	30(26.79)	18(16.07)	
Backyard gardening				
Yes	172	48(27.91)	25(14.53)	$\chi^2=0.927$ P=0.336 NS
No	219	71(32.42)	42(19.18)	
Rodent/ cockroaches				
Yes	334	104(31.14)	60 (17.96)	$\chi^2=0.019$ P=0.889 NS
No	57	15(26.32)	7(12.28)	

the category ($P < 0.05$).

Considering the effect of occupational affiliations on seropositivity of *Toxoplasma* infection among the women studied, a non-significantly higher seroprevalence of anti-*Toxoplasma* antibodies was recorded among the artisans than any other in the category $P > 0.05$. Considering the influence of level of educational attainment on the prevalence of anti-*T. gondii* antibodies, results indicate that seropositivity to IgM antibodies decreased as the level of education increases ($P > 0.05$). High seroprevalence of anti-*Toxoplasma* antibodies were recorded in all the pregnant women in all gestational ages in this study; highest IgG (31.25%) and IgM (20.39%) were recorded among those in their first and third trimesters respectively ($P > 0.05$).

High seroprevalence of anti-*Toxoplasma* IgG (31.90) and IgM (17.56%) antibodies was recorded among pregnant women that engaged in habit of tasting raw or undercooked meat while cooking than their counterparts who do not engaged in tasting of undercooked meat while cooking ($P > 0.05$). Effect of backyard gardening was assessed with respect to seropositivity of *Toxoplasma* infection; the result shows that higher prevalence of infection was recorded among pregnant women that do not engaged in backyard gardening than their counterpart that engaged in backyard gardening ($P > 0.05$). Majority of the pregnant women in the study admitted to harbouring of rodents/cockroaches in their household; consequently highest prevalence of anti-*Toxoplasma* IgG (31.14%) and IgM (17.96%) antibodies were recorded among them ($P > 0.05$). Also, a higher seroprevalence of *T. gondii* infection was recorded among pregnant women that are unaware of any cat visiting their premises ($P > 0.05$).

Potential risk factors were identified in this study, after adjusting for the effect of individual variables. Toxoplasmosis infection can be explained by three of the variables entered into the model (Table 4). These are the participant's age, cat ownership and tasting of raw/undercooked meat. The risk factors analysis showed that the odds of being infected with toxoplasmosis are more likely among younger pregnant women within the child bearing age. Pregnant women whose ages are ≤ 20 years and 21-30 years were approximately 4.0 and 2.5 times more likely to be infected with *T. gondii* than their older counterpart's age (41-50 years). The risk factors also showed that pregnant women who owned cat were 3 times more likely to be infected with *T. gondii* than those who did not own a cat. According to habit of tasting raw/ undercooked meat among the pregnant women, the odd of being infected with *T. gondii* was observed to be 4 times more likely in pregnant women that engaged in the habit than those who did not.

DISCUSSION

Several reports on the seroprevalence of toxoplasmosis

among pregnant women has been reported from various part of the world (Dubey, 2004) but this current study is one of the few from Nigeria that explore the seroprevalence and knowledge about *T. gondii*, its transmission, causes and prevention among pregnant women who stands the risk of congenitally infected babies if and when they contract the disease.

In this study, the overall seroprevalence of *T. gondii* antibodies among pregnant women was 40.59%. High seroprevalence of IgG (30.44%) and IgM (17.15%) representing chronic/ latent and recently acquired toxoplasmosis respectively were recorded among the sampled pregnant women.

The result of IgG antibodies recorded in this study is comparable to 31.5% reported elsewhere in Nigeria by Emmanuel et al. (2011) and 35.1% reported in Qatar by Abu Madi et al. (2010), but higher than 27.9% reported in Palestine (Nijem and Al-Amleh, 2009).

The varied prevalence rates been reported by various authors might be due to differences in geographical location, levels of exposure, methodology, sample size and behavioral attitudes of the population (Yusuf et al., 2016).

The result of this study suggest that considerable number of pregnant women (69.56%) sampled in Osogbo, Southwest Nigeria are seronegative that is they have not been exposed to *T. gondii*. This group is at risk of becoming infected later in life if they come in contact with the parasite. According to Moura et al. (2013) guidance on primary preventive measures and serological monitoring of pregnant women in the at-risk (seronegative) group are important measures for preventing congenital toxoplasmosis.

An increase in seropositivity of anti-*Toxoplasma* antibodies was observed with increasing age in this study. This is in agreement with other studies (Bobic et al., 1998; Ertug et al., 2005; Ishaku et al., 2009; Zeneme et al., 2012). Findings also suggest that low level of education was associated with higher rate of toxoplasmosis infection among pregnant women in this study. This is in agreement with reports of previous studies (Jones et al; 2001; Hajssoleimani et al., 2012; Ataenia and Tadavon, 2008; Varella et al., 2003; Ishaku et al., 2009). Pregnant women that were artisan and civil servants recorded a non-significantly higher prevalence in anti-*Toxoplasma* antibodies while lower seropositivity of *T. gondii* antibodies was observed among pregnant women that were students. This is in line with the findings of Zemene et al. (2012) and Ahmed et al. (1988) who recorded higher seropositivity to *T. gondii* antibodies among employed pregnant women. The explanation for this might be that employed pregnant women are financially stable with incomes that enable them buy meat compared to students who essentially dependent financially on either parents or guardian. A non-significant association was recorded between *T. gondii* infection and gestational age in this study. This result is in

Table 4. Factors associated with *T. gondii* infection among pregnant women in Osogbo, Southwestern Nigeria.

Parameter	Number Examined	Odd ratio(95%CL)	P-value
Age group(years)			
≤20	22	3.944(0.245-63.61)	0.017
20-30	246	2.433(2.899-6.984)	0.023
31-40	120	1.938(2.767-3.675)	0.102
41-50 ^b	3		
Marital Status			
Single	53	0.152 (0.017-1.375)	0.194
Married	305	0.227 (0.028-1.861)	0.167
Divorced	20	0.184 (0.018-1.858)	0.151
Widowed ^b	11		
Occupation			
Civil servant	59	0.942(2.232-4.865)	0.621
Trading	167	0.797(2.776-3.519)	0.129
Artisan	109	0.587(0.896-3764)	0.223
Farmer	10	0.678(2.007-6.986)	0.675
Student	24	2.023(2.112-2.099)	0.113
Applicant ^b	22		
Educational level			
Primary	53	3.034(1.098-3.678)	0.211
Secondary	226	1.822(3.887-9.735)	0.121
Tertiary	105	0.231(3.985-534)	0.150
No education ^b	7		
Gestation Period			
1 st trimester	48	1.543(1.234-4.454)	0.231
2 nd trimester	103	1.210(0.364-0.389)	0.474
3 rd trimester ^b	240		
Cat ownership			
Yes	22	5.182(0.268-2.902)	0.009
No ^b	369		
Presence of cat in the premises			
Yes	145	3.346(4.215-9.218)	0.219
No	216	1.680(0.623-4.528)	0.115
No idea ^b	30		
Habit of tasting raw/ undercooked meat			
Yes	279	4.784(0.444-1.384)	0.001
No ^b	112		
Backyard Gardening			
Yes	172	1.030(0.617-1.720)	0.109
No ^b	219		
Presence of rodent/ cockroaches in the household			
Yes	334	3.654(0.320-1.338)	0.245
No ^b	57		

95% CL =95% Confidence limit; ^bReference group.

agreement with studies in Saudi Arabia (Al-Harhi et al., 2006) and Addis Ababa (Gelaye et al., 2014) but in contrary to a previous study by Aqeely et al. (2014). Pregnant women in their first and second trimester recorded high prevalence of IgG (chronic) and IgM (recently acquired infection) respectively. Infection of pregnant women with *T. gondii* in their first trimester according to Dunn et al. (1999) could lead to miscarriage or foetal death while infection acquired during the second trimester may result in either congenital disease or subclinical infection (Remington et al., 2005). Contact with cat as resulting from cat ownership was assessed in this study and cat contact was significant associated with *T. gondii* infection. These findings are in agreement with results obtained in Taiwan (Lin et al., 2008), Ethiopia (Zemene et al., 2012), France (Baril et al., 1999) and Brazil (Moura et al., 2013). Backyard gardening, tasting of raw/undercooked meat and presence of rodent/cockroaches in the households has been reported to be potential sources of *T. gondii* infection (Sroka et al., 2010; Koskiniemi et al., 1989; Alvarado et al., 2011; Moura et al., 2013), this probably explains why a significant relationship was recorded among pregnant women that are habitual taster of raw/undercooked meat in this study and *T. gondii* infection. Although in Nigeria, proper cooking of meat is the norm but the present study recorded a significantly higher prevalence of toxoplasmosis among pregnant women who engaged in the habit of tasting meat while cooking than their counterparts who do not. This result agrees with previous findings of Cook et al. (2000), Spalding et al. (2005) and Ishaku et al. (2009). Tasting of meat while cooking may serve as a route of ingesting tissue cysts that have not been killed thus causing toxoplasmosis infection.

Also, logistic regression model showed that participant's age, cat ownership and habit of meat tasting were significantly associated with toxoplasmosis infection among pregnant women. The odds of being infected by younger pregnant women were more likely than in the older ones. This observation is similar to the findings of Ertug et al. (2005) in Turkey, Esquivel et al. (2006) in Mexico and Walle et al. (2013) in Ethiopia. Consumption of raw/undercooked meat is common among some women who engaged in tasting of meat in the course of checking if the meat is well cooked thereby increasing the risk of infection. The odds of infection were more among those that taste undercooked meat while cooking than non-taster. This result is not surprising because consumption of undercooked meat is one of the risk factors for *T. gondii* transmission therefore The finding is in line with the previous studies of Gelaye *et al.* (2012) and Endris *et al.* (2014). According to Dubey, (2004) who reported that cats are definitive hosts that shed millions of oocysts within short period of time and play a major role in transmitting *T. gondii* infection, ownership of cat was found to be significantly associated with *T. gondii* infection. This is in agreement with other results reported from Jimmah town, Ethiopia (Zemene et al., 2012), Dabre

Tabor, Ethiopia (Agmas et al., 2015) and Thailand (Nissapatorn et al., 2011)

The results of the present study showed that there was absolutely low knowledge about the parasites, its transmission, causes and prevention among the pregnant women in the study area. Majority (355, 90.79%) of the women claimed not to have any knowledge about the parasite, a condition that put all these women at a great risk of infection as they will be expose them to the infection and other risk factors in the environment. Similar findings were recorded by Miller et al. (2014) among pregnant and postpartum women in Brazil where over 72% claimed not to have heard about the parasite. There was a relatively low knowledge about the role of contact with cats, consumption of raw/ undercooked meat and other risk factors in the study area which conforms with the observation of Jones et al. (2003).

Conclusion

The findings of this study showed a high seroprevalence of toxoplasmosis and a very low level of awareness and understanding about the parasite (*T. gondii*), its transmission, causes and prevention among the study population.

RECOMMENDATION

There is a need for the inclusion of mandatory screening for toxoplasmosis in ante-natal care and the need to sensitize women of child bearing age about the disease and the risk factors that could predispose them to contracting the parasite, thereby, preventing maternal and congenital infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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