

Rubella virus, *Toxoplasma gondii* and *Treponema pallidum* congenital infections among full term delivered women in an urban area of Tanzania: a call for improved antenatal care

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Abstract

Background: A significant proportion of newborns in the developing countries are born with congenital anomalies.

Objective: This study investigated congenital infections due to Rubella virus, *Toxoplasma gondii*, *Treponema pallidum* among presumed normal neonates from full term pregnant women in Mwanza, Tanzania.

Methods: Sera from mothers were tested for *Treponema pallidum* and *Toxoplasma gondii* infection while newborns from mothers with acute infections were tested for *T. pallidum* and *T. gondii*, and all newborns were tested for Rubella IgM antibodies.

Results: A total of 13/300 (4.3 %) mothers had *T. pallidum* antibodies with 3 of them having acute infection. Two (0.7 %) of the newborns from mothers with acute infection were confirmed to have congenital syphilis. Regarding toxoplasmosis, 92/300 (30.7 %) mothers were IgG seropositive and 7 had borderline positivity, with only 1/99 (1%) being IgM seropositive who delivered IgM seronegative neonate. Only 1/300 (0.3 %) newborn had rubella IgM antibodies indicating congenital rubella infection.

Conclusion: Based on these results, it is estimated that in Mwanza city in every 100,000 live births about 300 and 600 newborns have congenital rubella and syphilis infections, respectively. Rubella virus and *T. pallidum* are likely to be among common causes of congenital infections in developing countries.

Keywords: Congenital infections, Mwanza, Tanzania.

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Background

Congenital and perinatal infections are the leading cause of childhood morbidity and mortality particularly in low

and middle-income countries (LMIC). Congenital infections acronymed as TORCH (*Toxoplasma gondii* (*T. gondii*); Other (e.g., *Streptococcus agalactiae*, *Listeria spp.*); Rubella virus; Cytomegalovirus (CMV) and Herpes simplex virus (HSV) proposed by Nahmias et al in 1970's has been recently expanded to include other infections acronymed as CHEAPTORCHES standing for C – Chickenpox and shingles; H – Hepatitis B, C, (D), E; E – Enterovirus; A – AIDS (HIV infection); P –Parvovirus B19; TORCH; E – Everything else sexually transmitted (*Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, Human

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papillomavirus); S – Syphilis². This expansion entails to incorporate a number of infections that can potentially cause congenital infections with serious consequences to the health of newborn soon after birth or later in life. Overwhelming evidence suggest that maternal infections are common in LMIC²⁻¹³ with limited information regarding congenital infections. Following a call by the World Health Organization (WHO) on the improvement on maternal and child health¹⁴, many high-income countries (HIC) embarked on improved health services to account for the consequences of infections which appears to be common worldwide. Despite the effort of different health authorities in LMIC, these infections are still the leading causes of prenatal and perinatal infections in these countries. The course of these infections depends on several factors such as mode of transmission, timing of infection in respect to gestational age, predilection of the pathogen, maturity of the fetal immune system and presence or absence of the maternal immunity at the time of infection¹⁵. Each of the pathogen can cause various clinical manifestations, however most of them might also lead to asymptomatic infection in the pregnant women and newborns. The WHO recommends antenatal and prenatal screening for most of these infections to prevent vertical transmission and possible consequences to the newborns. Maternal screening, treatment or vaccination where appropriate have been found to be effective strategies to significantly reduce the burden of these infections in HIC². However, the burden is still high in LMIC due to the financial constraints and health policy priorities. Apart from including the screening of few of these pathogens such as human immunodeficiency virus (HIV) and *T. pallidum* in most of these countries, there is no monitoring of the effectiveness of screening programmes, and other pathogens such Rubellavirus and *T. gondii* are not routinely screened. In addition, there is paucity of data regarding the magnitude of these infections among newborns who appear normal at birth in most of the LMIC. Previous studies^{3,4,6,9} in the city of Mwanza have reported Rubella virus, *T. gondii* and *T. pallidum* to be common among pregnant women with limited data of infections of these pathogens in presumed normal newborns. This study was designed to estimate the magnitude of congenital Rubella virus, *T. gondii* and *T. pallidum* infections among presumed normal live neonates from full term pregnant women

Methodology

Study design, duration, study area and study population

A cross-sectional hospital-based study was conducted at Sekou Toure Regional Hospital (STH) and the Bugando Medical Centre (BMC) between January and May 2015. A total of 300 women (114 from the BMC and 186 from STH labor wards) were enrolled.

Inclusion and exclusion criteria

All women aged 18 years and above with normal deliveries who consented to participate in the study were enrolled. In order to establish the magnitude of congenital infections in normal live births, all neonates with obvious congenital defects at birth were excluded.

Data and specimen collection

Socio-demographic information such as age, residence, occupation, marital status, education, and other relevant clinical information such as gravidity and gestation age were collected using a pre-tested structured data collection tool. Paired samples (about 4 mL of mother's venous blood and 4 mL of cord blood) were collected aseptically in a plain vacutainer tubes (Becton Dickinson (BD), Nairobi, Kenya). All specimens were transported to the Bugando Medical Centre where sera were separated from the whole blood by centrifugation at 3,000 rpm for 5 minutes. Sera were numbered accordingly and kept at -80°C until transportation to Germany for further analysis at the Institute of Medical Microbiology, Gottingen University Medical Centre, Germany.

Diagnosis of congenital infection

In order to establish congenital infections, different algorithms were followed for screening of these pathogens in the cord blood. *T. gondii* congenital infections was investigated by screening all neonates from mothers who tested positive for both *T. gondii* IgG and IgM antibodies. Congenital syphilis was established by testing neonates from mothers with recent *T. pallidum* infection while for congenital rubella infections all neonates were tested for Rubella IgM antibodies¹⁶⁻¹⁸.

Laboratory analysis of the samples

For the diagnosis of *T. gondii* infections¹⁷, sera were tested for anti-*T. gondii* antibodies using Enzyme linked Fluoro-

rescent Assay(ELFA) kits (VIDAS Toxo-IgG-II- (ELFA) and Toxo-IgM-ELFA for anti-*T. gondii*-specific IgG and IgM antibodies, respectively (bioMérieux, France). The cut-off values for detection of IgG antibodies were as follows: sera with IgG levels <4IU/mL were regarded as negative, IgG levels 4 -<8IU/mL were regarded as borderline and IgG levels \geq 8IU/mL were considered as positive. The cut-off values for detection of IgM were as follows: index value of <0.55 was considered as IgM negative, index value of 0.55 - <0.65 was borderline and index value of \geq 0.65 was termed as IgM positive. Rubella congenital infections was investigated using cord blood for the presence of specific rubella IgM antibodies^{16,19}. Detection was done by using AxSYM rubella virus IgM-microparticle enzyme immunoassay (MEIA) (Abbott, IL, USA); anti-rubella IgM of > 0.8 was considered as positive and anti-rubella IgM of <0.6 was considered as negative and IgM value between 0.6-0.8 was considered as borderline. The reversed syphilis algorithm diagnosis was employed in the detection of recent *Treponema pallidum* infection using *Serodia Treponema pallidum* particle assay (TPPA) (Fujirebio, Japan) and Venereal Disease Research Laboratory (VDRL) test (Omega Diagnostics, Germany)¹⁸. All laboratory methods were performed according to manufacturer instructions.

Data analysis

All data were entered by Microsoft Office Excel 2007 and transferred to STATA version 12 (College Station, Texas, USA) for cleaning and analysis. Categorical variables were summarized as proportions and were analyzed using the Pearson's Chi-square test to observe the statistical differences among the various groups. Continuous variables were summarized as median with interquartile range or

means with standard deviations. The differences in medians were statistically compared using Wilcoxon ranksum Mann-Whitney test. Univariate analysis and multivariate logistic regression models were performed to determine the predictors of *T. gondii* IgG seropositivity among pregnant women. Variables with p-value less than 0.2 on univariate analysis were fitted into the multivariate logistic regression analysis to establish independent predictors. A P value of <0.05 at 95% confidence interval was considered statistically significant.

Ethical considerations

The study protocol was approved by the CUHAS/BMC Research Ethics Review Committee (CREC) with ethical clearance number CREC/043/2014. An informed written consent was sought from each enrolled woman.

Results

Socio-demographic characteristics of the study participants

The median age of the 300 enrolled women was 24 (IQR: 20.5-29.5) years while the mean gestation age at delivery was 38 ± 1.44 weeks. A significant proportion 124 (41.3%) of participants were primigravida while 123 (41.0%) were housewives (Table 1). On Wilcoxon ranksum Mann-Whitney test, primigravida women had significantly younger age compared to multigravida women (20, IQR: 18-22 vs. 28, IQR: 24.4-32 years, P <0.001). Regarding education level, the majority of these women 215 (71.67%) had either no formal education or primary education. In addition, a significant proportion 72 (24%) of these women reported to have been drinking unboiled water while 14 (4.7%) reported to have been keeping cats at home (Table 1).

Table1: Socio-demographic characteristics of the 300 full term delivered women in the city of Mwanza

Study variable	Number of patients	Percent (%)/Median IQR/Mean±SD
Age (years)	300	24 20.5-29.5
Gestation age at birth	300	38±1.44
Marital status		
Married	262	87.3
Unmarried	38	12.7
Occupation		
Housewives	123	41.0
Employed	22	7.3
Small scale business	101	33.7
Peasant	54	18.0
Education level		
Primary/No formal	215	71.7
Secondary/tertiary	85	28.3
Gravidity		
Primigravida	124	41.3
Multigravida	176	58.7
Drinking unboiled water		
Yes	72	24.0
No	228	76.0
Keeping cats		
Yes	14	4.7
No	286	95.3

Prevalence of congenital infections and associated factors among full term delivered women in the city of Mwanza, Tanzania

Out of 300 enrolled women 13 (4.3 %) were TPPA positive indicating exposure to *T. pallidum*. Of these 13 TP-PA-positive women, 3 were VDRL positive indicating the overall prevalence of 1.0% of recent *T. pallidum* infection among women studied. Two (0.7 %) out 300 newborns were serologically confirmed to have congenital syphilis with no obvious symptoms observed, making about 600 cases of congenital syphilis infections in 100,000 live births. Increase in age was significantly associated with *T. pallidum* past infection (OR: 1.08, 95% CI: 1.00-1.16, P=0.031). Furthermore, the odds of having *T. pallidum* past infection was significantly higher among multigravida women than in primigravida women (OR: 3.76, 95% CI: 1.06-13.27, P=0.040).

Regarding *T. gondii*, 92/300 (30.7 %) women were IgG seropositive with titers ≥ 8 IU/mL and 7 had borderline positivity i.e. titers between 4 and 8IU/ml. Out of 92 mothers who were IgG seropositive and 7 who had borderline titres, only 1/99 (1%) was IgM seropositive; however, her newborn was not infected. On multivariate logistic regression analysis, being a small-scale business/employed woman (OR=1.69, 95%CI: 1.01-2.8, P=0.044) and drinking unboiled water (OR=1.78, 95%CI: 1.01-3.1, P=0.046) independently predicted *T. gondii* IgG seropositivity.

Rubella congenital infections (presence of IgM antibodies in cord blood) was detected in 1 (0.3 %) out of the 300 newborns tested, making about 300 cases of rubella congenital infections in 100,000 live births.

Table2: Seroprevalence of *T. gondii*, *Treponema pallidum* and Rubella infections among mothers and full term delivered newborns in the city of Mwanza, Tanzania

	<i>T. pallidum</i>		<i>T. gondii</i>		Rubella	
	Test	Positive	Test	Positive		Positive
Mothers (n=300)	TPPA	13 (4.3%)	IgG	92 (30.7%)	IgG	NT
Mothers (n=300)	VDRL	3 (1.0%)	IgM	1 (0.3%)	IgM	NT
Newborns(n=300)	VDRL	2 (0.7%)	IgM	0 (0.0%)	IgM	1 (0.3%)

NT = not tested

Table 3: Factors associated with *T. gondii* IgG seropositivity among women with full term delivery in the city of Mwanza, Tanzania

Characteristics	IgG sero-positivity (N, %)	Univariate		Multivariate	
		OR (95% CI)	P value	OR (95% CI)	P value
Marital status					
Married	79(30.2)	1			
Unmarried	13(34.2)	0.83(0.403-1.70)	0.613		
Gravidity					
Primigravid	33(26.6)	1			
Multigravida	59(33.5)	1.39(0.83-2.3)	0.202		
Education					
Secondary/Tertiary	24(28.2)	1			
Primary/No formal education	68(31.6)	0.85(0.48-1.47)	0.566		
Drinking unboiled water					
No	62(27.2)	1			
Yes	30 (41.7)	1.92(1.1-3.32)	0.021	1.78(1.01-3.1)	0.046
Keeping cat					
No	85(29.7)	1			
Yes	7(50.0)	2.3(0.8-6.9)	0.118	2.2(0.73-6.75)	0.159
Occupation					
Others	47(26.5)	1			
Small scale business/employed women	45(36.6)	1.58(0.97-2.6)	0.171	1.69(1.01-2.8)	0.044
HIV status					
Negative	11(25.6)	1			
Positive	2(14.3)	0.34(0.075-1.58)	0.171	0.283(0.06-1.34)	0.113
Unknown	79(32.5)	0.71(0.34-1.48)	0.369	0.612(0.286-1.3)	0.207

Discussion

Prenatal and perinatal infections have been commonly associated with congenital anomalies and mortality particularly in low and middle income countries (LMIC)². Many high income countries (HIC) have improved maternal and child health services to prevent the consequences of these infections following a call by the world health organization (WHO)¹⁴. In LMIC efforts have been made, however effectiveness of screening for these infections during antenatal period and after birth have not been well emphasized. Here, we report the prevalence of selected infections with a potential risk for vertical transmission^{3,4,6,8,9} in the city of Mwanza among full term delivered women and their presumed normal newborns. The magnitude reported in this study among presumed normal newborns are considerably high calling for the need of considering these infections as an important agenda in the efforts to improve maternal and child health in LMICs.

In the current study, the prevalence of *T. pallidum* specific antibodies and its associated congenital infection was high signifying the importance of effective screening programmes. Our findings are in line with previous studies^{20,21}, which also reported high prevalence of congenital syphilis. Despite the current programme of screening *T. pallidum* infections during antenatal visits across the country, there is a need to consider re-screening during the course of pregnancy to account for the re-infections which has been documented to be 2.7 %⁹. The current study confirms what has been reported previously that increase in age and multigravidity predict *T. pallidum* infections^{22,23}. This could be explained by the fact that most of women enrolled in this study had advanced age which entails more exposure to *T. pallidum* infection as compared to young women.

Regarding *T. gondii* IgG seropositivity, the findings from the current study are comparable to the previous studies^{3,24,25} indicating the endemicity of toxoplasmosis in Tanzania. In addition, the IgM seropositivity found in this study is also comparable with a previous study²⁴ and lower compared to another report²⁶. These findings suggest that there is possibility of the ongoing vertical transmission of *T. gondii* infections in this setting resulting to *T. gondii* associated adverse pregnancy outcomes. This calls for the need to introduce screening and treatment programme among women attending antenatal clinics in

Tanzania and other countries where *T. gondii* infection is endemic. In the current study, being formally employed or a small-scale business woman predicted *T. gondii* IgG seropositivity. This was also observed in a previous study³ in the same setting. It has been noted that living in town with reasonable income entails high socioeconomic status (SES). High SES can allow women to frequently afford meat dishes that can expose them to *T. gondii* infections^{27,28}.

Concerning rubella congenital infection, the findings from this study are comparable with previous studies in Tanzania and Sudan^{29,30}. Rubella virus is endemic in Tanzania and other African countries with a significant proportion of women at high risk of contracting primary rubella virus infection that can lead to vertical transmission^{4,31}. This was confirmed in the previous study which reported a significant number of pregnant women had acute rubella virus infection hence a potential risk to transmit it to the fetuses⁶. This calls for the need to emphasize on the antenatal screening and vaccination of child bearing women as suggested previously³².

Overall the current study highlights the importance of effective antenatal screening of these infections which will improve maternal and child health. Despite the inclusion of *T. pallidum* in antenatal screening, there is a paramount need of emphasizing re-screening during the course of pregnancy and /or at delivery. Being common in Tanzania *T. gondii* screening and treatment will improve antenatal care services across the country. In case of rubella, despite a strategy of vaccinating children, there is a need to consider additional strategy of vaccinating child bearing aged women in developing countries.

Competing interest

Authors declare that they have no competing interest. All authors have read and approve the final version of the manuscript to be submitted

Authors' contributions

All authors participated in the study design. MMM and SEM participated in the collection of the samples and clinical data. UG did laboratory analysis of all samples. MMM and SEM analysed and interpreted the data. MMM wrote the first draft of the manuscript. SEM and UG did critical review of the manuscript. All authors read and approved the final version of the manuscript.

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