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Original article

A pilot study on screening for gestational/congenital toxoplasmosis of pregnant women at delivery in the Eastern Province of Saudi Arabia

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ABSTRACT

Background: Globally, congenital toxoplasmosis remains a significant cause of morbidity and mortality, and outbreaks of T. gondii infection represent a major public health threat, especially in developing countries. Evidence in the literature indicates that only a few studies have been conducted on the incidence of maternal and congenital toxoplasmosis in Saudi Arabia. This prospective study aims to measure the overall incidence of congenital toxoplasmosis, both patent and 'silent' infection, among pregnant women in the Eastern Province of Saudi Arabia. The study would attempt to relate the cord blood results with the time of seroconversion in the mother, underlining the importance of early intervention in such cases.
Methods: Five hundred paired maternal/cord blood samples were tested for anti-Toxoplasma IgG or IgM antibodies. Samples were collected during delivery from mother and newborn (cord blood) from November 2011 to May 2012. Only positive for anti-Toxoplasma IgG or/and IgM cord blood was processed for real-time PCR for confirmation. The age of mothers ranged from 16 to 45 years.
Results: The sample subjects were tested during child delivery for specific IgG and IgM antibodies against Toxoplasmosis, of which 21.0% (n = 105) mother/baby pairs were found serologically positive for anti-Toxoplasma IgG antibodies. The rate of maternal seropositivity for anti-Toxoplasma IgM antibodies was found among 4 participants (0.8%), who were also seropositive for anti-Toxoplasma IgG antibodies. None of the children tested positive for anti-Toxoplasma IgM antibodies, even those born to mothers with IgM positive. All 105 cord blood tests in the study sample were confirmed negative by real-time PCR. The seroprevalence of Toxoplasma IgG antibodies increased with maternal age, parity, and was significantly higher in women who gave birth to children with congenital anomalies (p = 0.008).
Conclusion: The findings of the current study indicate a dire need to develop and implement preventive programs against Toxoplasma gondii infection, as well as a health education program on how to avoid toxoplasmosis for all seronegative women during pregnancy.

1. Background

Toxoplasmosis is an endemic zoonotic disease caused by the protozoan parasite *Toxoplasma gondii* (*T. gondii*), infecting all warm-blooded animals (e.g., members of the cat family Felidae), and humans who serve as intermediate hosts (CDC, 2020, NHS, 2020). *T. gondii* is an intracellular protozoan parasite, a member of the phylum Apicomplexa, class Coccidia. It is ubiquitous and characterized by its ability to infect a broad range of hosts and many dif-

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ferent hosts cell types. *T. gondii* parasite can persist in humans (and other animals) for long periods, possibly even throughout life (CDC, 2020, Singh, 2003, McAuley, 2013).

Globally, congenital *toxoplasmosis* remains a significant cause of morbidity and mortality, and outbreaks of *T. gondii* infection represent a major public health threat, especially in developing countries (CDC, 2020, Torgerson and Mastroiacovo, 2013, El Bissati et al., 2018). The most important estimate of the global incidence of congenital *toxoplasmosis* was a meta-analysis published in 2013 in the Bulletin of the World Health Organization. According to this report, the global annual incidence of congenital *toxoplasmosis* of 190,100 cases, equivalent to a disability burden of 1.20 million disability-adjusted life years (DALY) (95% CI: 0.76–1.90). Approximately one-third of the world's population is infected with this pathogenic parasite, including populations in Europe, South America, Africa and parts of Asia (Torgerson and Mastroiacovo, 2013, El Bissati et al., 2018).

There are four principal ways of human *toxoplasmosis* transmission: (1) eating undercooked, contaminated meat or shellfish; (2) unintentional ingestion of undercooked, contaminated meat or shellfish after handling them and not washing hands thoroughly; (3) eating food that was contaminated by knives, utensils, cutting boards, and other foods that have had contact with raw, contaminated meat or shellfish; (4) drinking water contaminated with T. gondii; (5) accidentally swallowing the parasite through contact with cat feces with Toxoplasma; (5) receiving an infected organ transplant or infected blood by transfusion, although this is rare; (5) Mother-to-child (congenital) transmission. Infected women can transmit the infection transplacentally to their unborn fetus (CDC, 2020, Singh, 2003, McAuley, 2013).

Toxoplasma can be transmitted from mother to fetus in approximately 40% case, when pregnant women are infected with *T. gondii* during pregnancy, resulting in abortion or fetal abnormalities (Kaye, 2011). The parasite can cross the intestinal epithelial barrier, disseminate throughout the body and localize in the placenta, producing progressively larger focal lesions (CDC, 2020, Montoya and Liesenfeld, 2004, Munoz et al., 2011). Those infected, have very few symptoms because the immune system of a healthy person usually prevents the parasite from causing illness. However, pregnant women and those with weakened immune systems should be careful; for them, *Toxoplasma* infection can cause serious health problems (Singh, 2003, McAuley, 2013).

The prevalence of positive serological tests for *T. gondii* varies in different regions and cultures. As such, in Saudi Arabia, prevalence studies have shown that 29.5–35.6% of pregnant women were infected with *T. gondii* during pregnancy (Al-Harthi et al., 2006). Data show that the *seroprevalence* (i.e., serological prevalence) among pregnant Saudi women varies regionally. The results of the study among pregnant women and newborn infants at the King Fahd Hospital in Al-Khobar (Eastern Province), showed that a very small number (1/175, 0.57%) of pregnant women were *seroconverted* during pregnancy. At the same time, the specific IgG positivity was significantly high (69/175, 39.4) (Al-Qurashi et al., 2001). While a study in the Al-Ahsa region found that of the 554 pregnant women, 51.4% were *seropositive* for IgG antibody, and 8.8% were IgM antibody (Al-Mohammad et al., 2010). According to the most recent study conducted among Saudi pregnant women from Riyadh (n = 250), *T. gondii* was prevalent with 32.5% for IgG and 6.4% for IgM antibodies (Alghamdi et al., 2016).

Studies investigating possible risk factors for *T. gondii* infection in pregnant Saudi women have shown that serological prevalence is highest among those with low educational levels in the Western Province (Al-Harthi et al., 2006). While the highest serological prevalence rates were among people living in rural areas in the Eastern Province (Al-Harthi et al., 2006). However, these data are outdated, and new research is needed to study and report on the current burden of *T. gondii* infection in pregnant Saudi women.

Until recently, only a few studies have been conducted on the incidence of maternal and congenital *toxoplasmosis* in Saudi Arabia. This prospective study aims to measure the overall incidence of congenital *toxoplasmosis*, both patent and 'silent' infection, among pregnant women in the Eastern Province of Saudi Arabia. Furthermore, the study would attempt to relate the cord blood results with the time of *seroconversion* in the mother, underlining the importance of early intervention in such cases. Since the detection of parasites in cord blood by PCR is a practical and cost-effective approach to the early neonatal diagnosis of congenital *toxoplasmosis*. In the Kingdom of Saudi Arabia, as in the United States, antenatal screening relies on a single serum sample taken during the antenatal period to detect antibodies to *toxoplasma*. A sample, which is often obtained either at the end of the first trimester or in the second or third trimester, is the only source of information about whether a fetus is at risk. Based on the results and findings, this study will serve as a basis for the development of neonatal screening policies in the Eastern Province of Saudi Arabia.

2. Materials & methods

Study Area and Participants: This study was conducted in King Fahad Military Medical City (KFMMC) in the Eastern Province.

Study Design and Samples selection*:* Prospective study of prenatal and postpartum diagnosis of gestational and congenital *toxoplasmosis*. A sample of n = 500 pregnant women at delivery, enrolled in this study from November 2011 to May 2012. The age of the participants ranged from 16 to 45 years old, with the mean age 29.2 ± 5.9 (median age 28 years). Paired blood samples were collected from both mother and newborn (cord blood) at birth. Samples were randomly selected from all pregnant women at delivery and without exclusion criteria.

Ethical consideration: All participant-mothers signed a written informed consent form after the purpose of the study was explained to them. The Research and Ethics committee approved the study of the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain, and the Research and Ethics Committee of KFMMC.

Data Collection. The questionnaire was administered among all selected mothers on their age, history of spontaneous abortion, stillbirth, congenital anomalies. The birth and clinical status of the newborn including gender, weight, gestational age, and head circumference at birth was obtained from the hospital's antenatal card.

Sample Collection and Laboratory Processing. Blood samples were collected in vacuum serum separation tubes to measure specific *anti-Toxoplasma* antibodies. Five milliliters of peripheral blood were taken from delivering mothers by venipuncture. Five milliliters of cord blood were similarly collected at the time of delivery under sterile conditions, accessing either an umbilical cord vessel or one of the fetal surfaces of the placenta. Blood was allowed to clot, centrifuged to sediment the clot, and serum aseptically transferred to a sterile 10 ml capped tube. Sera were then stored at − 60° C until assayed. Five milliliters of each baby's cord blood were also collected in Potassium EDTA for the real-time PCR detection of *Toxoplasma* DNA and refrigerated at 2–8 °C until assayed. Only cord blood found positive for *anti-Toxoplasma* IgG or/and IgM were processed for real-time PCR for confirmation.

2.1. Serological assays

Serum from the umbilical cord blood and maternal blood was analyzed in batches of 50 – 100 paired specimens for both *anti-Toxoplasma* IgM and IgG by chemiluminescent microparticle immunoassay (CMIA) technology in ARCHITECT i 2000 System using commercial kits (Abbott Diagnostics) with appropriate positive and negative controls provided by the manufacturer.

2.2. Detection of T. gondii B1 gene by real-time PCR

PCR has been performed following the manufacturer's instructions (QuantiTect SYBR® Green, Qiagen, Germany) on Applied Biosystems 7500 Real-Time PCR System with a 96-well plate with SYBR Green I dye, a double-stranded DNA binding dye, to detect PCR products as they accumulate during PCR cycles. Qualitative analysis of the PCR product was achieved by melt curve analysis that occurs post-PCR. *T. gondii* tachyzoites RH strain were provided by BRC *Toxoplasma*-lab parasitology-France and were used as a positive control. Detailed protocols sequences are available upon request. The target DNA for real-time PCR amplification is the published sequence of the 35-fold repetitive B1 gene of the *T. gondii* RH strain. B1 gene is highly conserved and one of the most sensitive and specific genes of *T. gondii*. The primer sequences received from Thermo Fisher Scientific were as follows:

Forward primer	TOXO-F: 5′-TCCCCTCTGCTGGCGAAAAGT-3′
Reverse primer	TOXO-R: 5′-AGCGTTCGTGGTCAACTATCGATTG-3′

- The total volume in the reaction tube was 20 μL, the primer concentrations used for SYBR Green I dye was 50 nM of forward and reverse primer.
- The reaction mixture was incubated for 10 min at 95 °C for initial denaturation followed by 40 cycles of 15 s at 95 °C, 1 min at 60 °C.

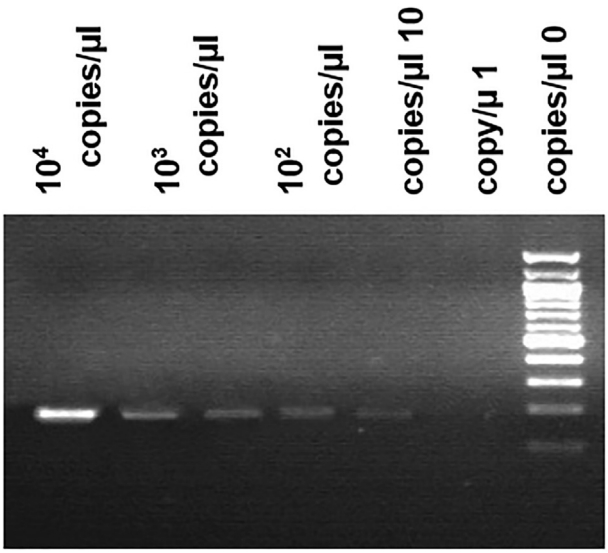

Fig. 1. Visualized bands under UV light. Analysis of PCR product by 1.5% agarose gel electrophoresis. PCR was based on the amplification of a 100 bp fragment of the B1 gene. Lane 1: molecular weight markers, lane 2: negative control, and lanes 3–7: positive results for the B1 gene.

- The products were loaded to 1.5% agarose gel with an 0.1 μg/ml ethidium bromide stain and electrophoresed at 80 V for 45 min, and the bands were visualized under UV light (Fig. 1).

2.3. Data analysis

Data compilation, tabulation, and statistical analysis were performed using Windows Excel and the Statistical Data for Social Sciences (SPSS) 20 packages. Statistical significance of the relationship between any possible variables used with appropriate p values and comparison of groups was carried out by Chi-square. All statistical tests were interpreted at the 5% level of significance ($p > 0.05$), not statistically significant.

3. Results

The average number of deliveries was 2.1 ± 1.94, with a median of 2 deliveries. The most frequent parity history was 2–4 deliveries (229; 45.8%). As summarized in Table 1, the prevailing number of mothers (n = 345, 69.0%) recruited in this study had no history of abortions. While most of the studied women had no history of abnormal pregnancy outcomes, intrauterine fetal death was recorded in n = 7 cases (1.4%), and neonatal mortality in n = 14 (2.8%), while congenital anomalies were seen in n = 4 (0.8%) of cases. Of n = 500 women, n = 478 (95.5%) had been tested for both specific *anti-Toxoplasma* IgG and IgM antibodies at various times during the pregnancy; n = 22 (4.4%) had remained untested during this antenatal period.

Of n = 500 women tested during delivery of specific IgG and IgM antibodies against *toxoplasmosis*, n = 105 (21.0%) were found to be serologically positive for *anti-Toxoplasma* IgG antibodies. The *seropositivity* rate for *anti-Toxoplasma* IgM antibodies was 0.8% (n = 4 mothers), who were also *seropositive* for *anti-Toxoplasma* IgG antibodies. Consequently, n = 395 (79.0%) of these Saudi women remained susceptible to acute *Toxoplasma* infection in childbearing age. According to real-time PCR, n = 105 cord blood

Table 1
General and obstetric characteristics of n = 500 pregnant women.

Obstetric Characteristic	No	%
Age group (year)		
16–20	30	6.0
21–30	284	56.8
≥31	186	37.2
Tested in trimester		
First	241	48.2
Second	108	21.6
Third	129	25.8
Not tested	22	4.4
Previous abortion		
None	345	69.0
One abortion	105	21.0
More than one abortion	50	10.0
Parity		
None	117	23.4
1	101	20.2
2–4	229	45.8
≥5	53	10.6
Previous intra-uterine fetal death (IUFD)		
None	493	98.6
Yes	7	1.4
	Previous Neonatal Death (NND)	
None	486	97.2
Yes	14	2.8
Previous congenital anomalies (CA)		
None	496	99.2
Yes	4	0.8

in the studied samples were confirmed negative. After six months, follow-up showed that all those children whose mothers were positive for *anti-Toxoplasma* IgM became negative for *Toxoplasma* IgG antibodies. This study found no evidence for congenital *toxoplasmosis* in children at birth when screened by serology or real-time PCR. Although the PCR results were negative and the serology results also turned negative, observation at six months of age may be helpful. The main advantage of PCR is its sensitivity in detecting very low levels of *T. gondii*.

It was observed that seropositivity for IgG antibodies increased with age, with the lowest rate of 10% among the youngest age group of 16–20 years old; it increased to 16.4% at the age of 21–30 years, and a maximum prevalence rate of 29.6% was seen in the age group of 31–45 years of pregnant women. These IgG seroprevalence rate differences between different age-groups were statistically significant (p = 0.001). The increment observed in the current study may be associated with a more considerable time risk of exposure with age. IgM seropositivity also increased with age, although the numbers were too small, no differences were statistically significant (Table 2). There is a probability that we would get statistical significance when we increase the sample size. However, the IgM positive cases are few in number though we have a sufficient sample size. Therefore, we cannot be certain that when we increase the sample size, there will be a significant difference since there may or may not be IgM positive cases to show significant results.

All samples were taken during delivery, thus there were no abortions during the study. The study cites the entire history of abortion. As shown in Table 3, women who had a history of abortion were more predisposed to *seropositive* for *anti-T. gondii* IgG. The level of *seropositivity* in women who had one abortion was 21.0%, and the highest rate of 32.0% was observed in women who had more than one abortion. There was no statistically significant association between prior abortion and the seropositivity of *T. gondii* IgG.

The lowest level of *seropositivity* – 19.4% was observed in women without a history of abortion. In women with a history of abortion, IgM *seropositivity* was not observed. IgG *seropositivity* increased again with parity with the lowest *seropositivity* rate – 15.4% among unborn babies and the highest – 34.0% among women with ≥ 5 previous births, and this trend was observed with increasing maternal age. The difference was statistically significant (X2 = 7.97, P = 0.046). The IgM *seropositivity* rate differences were not significant due to small numbers in different categories.

The IgG *seropositivity* rates showed no differences between women who had experienced neonatal death (21.4%) and those who had not (21.0%). *Seropositivity* rate was higher (28.6%) in women with a history of intrauterine fetal death compared with

Table 2
Distribution of IgG and IgM seroprevalence of toxoplasmosis amongst different age groups of Saudi women at delivery in the Eastern Province of Saudi Arabia.

Age group (year)	N tested	IgG Negative		IgG Positive		*P*	IgM negative		IgM positive		*P*
		No	%	No	%		No	%	No	%	
16–20	30	27	90.0	3	10	X^2 = 13.81	30	100.0	0	0.0	X^2 = 2.51
21–30	284	237	83.5	47	16.5	P = 0.001	283	99.6	1	0.4	P = 0.29
31–45	186	131	70.4	55	29.6	Significant	183	98.4	3	1.6	
Total	500	395	79.0	105	21.0		496	99.2	4	0.8	

X^2 = Pearson Chi-Square.

Table 3
Association of Toxoplasma seropositivity with obstetric history of women at delivery.

Obstetric History Abortion	No. tested	IgG positive			IgM positive		
		No	%		No	%	
0	345	67	19.4	X^2 = 4.166	4	1.2	X^2 = 1.812
1	105	22	21.0	P = 0.125	0	0.0	P = 0.404
≥ 2	50	16	32.0		0	0.0	
Total	500	105	21.0		4	0.8	
Parity		No	%	X^2 = 7.979	No	%	X^2 = 1.997
0	117	18	15.4	P = 0.046	1	0.9	P = 0.573
1	101	19	18.8		0	0.0	
2–4	229	50	21.8		3	1.3	
≥5	53	18	34.0		0	0.0	
Total	500	105	21.0		4	0.8	
NND*		No	%	X^2 = 0.002	No	%	X^2 = 0.116
None	486	102	21.0	p = 0.968	4	0.8	p = 0.733
Yes	14	3	21.4		0	0.0	
Total	500	105	21.0		4	0.8	
IUFD*		No	%	X^2 = 0.245	No	%	X^2 = 0.057
None	493	103	20.9	p = 0.620	4	0.8	p = 0.811
Yes	7	2	28.6		0	0.0	
	500	105	21.0		4	0.8	
CA*		No	%	X^2 = 7.087	No	%	X^2 = 0.033
None	496	102	20.6	p = 0.008	4	0.8	p = 0.857
Yes	4	3	75.0		0	0.0	
Total	500	105	21.0		4	0.8	

X^2 = Pearson Chi-Square
NND* neonatal death
IUFD* intrauterine fetal death
CA*congenital anomalies

those (20.9%) with no such history. There was a significantly higher *seroprevalence* rate (75.0%) among women with a history of congenital anomalies compared with those (20.6%) with no such history. This showed a significant association (X2 = 7.87, P = 0.008) with maternal *seropositivity* for *anti-Toxoplasma* IgG antibodies. In women with such a previous adverse obstetric outcome, IgM *seropositivity* was not observed.

The distribution of *seroprevalence* levels of *anti-Toxoplasma* IgG and IgM antibodies among mothers with different birth characteristics of their children is presented in Table 4. The *seropositivity* rates of *anti-Toxoplasma* IgG were similar among mothers who gave birth to boys (21.1%) or girls (21.8%). Similarly, the level of *seropositivity* for *anti-Toxoplasma* IgM was 0.8%, no matter whether mothers gave birth to boys or girls. The seropositivity for *anti-T. gondii* IgG in mothers classified according to their children's gestational age showed a 25.0% rate for gestation age $\geq$ 41 weeks; 20.0% for gestation age $\leq$ 34 weeks; and 21.0% for gestation age between 35 and 40 weeks. These differences were not statistically significant.

Four mothers who gave birth to babies with birth weight of $\leq$ 1899 g were all *seronegative* for *toxoplasmosis*. The seemingly higher rate of *seropositivity* (33.3%) was among mothers giving birth to babies with weight of $\geq$ 4000 g, which was not statistically significant.

Statistically significant association (X2 = 7.812, P = 0.020) was found between maternal *seropositivity* for *Toxoplasmosis* and large head circumference in newborns, as well as with other adverse outcomes include preterm and baby die due to multiple congenital anomalies (X2 = 8.1, P = 0.044), even though the number of babies born with such issues was small. As shown in Fig. 2, a statistical analysis of the results for risk factors revealed a significant relationship between the *seropositivity* of maternal *anti-Toxoplasma* IgG and life in rural or urban areas (P = 0,000), as well as women who were aware of the mode of disease transmission (p = 0.023). No significant association was found between *seroprevalence* of *anti-Toxoplasma* IgG antibodies and such risk factors as eating raw or undercooked meat or sheltering cats.

4. Discussion

Evidence in the literature indicates that only a few studies have been conducted on the incidence of maternal and congenital *toxoplasmosis* in Saudi Arabia. Furthermore, currently, there is no newborn screening strategy for the early diagnosis and treatment of infected children in the Kingdom. This prospective study is designed to pilot a neonatal screening program and use more sensitive serological and PCR techniques to detect *Toxoplasma* infection in both mother and child in a sample population in the Eastern Province of Saudi Arabia. The findings and results of the current study can serve as a basis for developing neonatal screening policies in the Eastern Province of Saudi Arabia, taking into account the health plan and the Kingdom's health system outlook.

The present study was undertaken to determine the *Toxoplasma* infection rates in a sample population of 500 Saudi national women who delivered at KFMMC between November 2011 and May 2012. The age of the participants ranged from 16 to 45 years old, with the mean age 29.2 ± 5.9 (median age 28 years). Paired blood samples were collected from both mother and newborn (cord blood) at birth. Samples were randomly selected from all pregnant women at delivery and without exclusion criteria. In the present study, a *seroprevalence* of 21.0% is reported in women which is lower than the rates previously reported from other parts of KSA. The 0.8% of women tested at delivery had both positive IgG and IgM antibodies. Findings have shown that similar to previous findings not all women with positive IgG and IgM test results may be said to have a recently acquired infection, as *anti-Toxoplasma* IgM antibodies may persist for several months, making it difficult to calculate the time of exposure (Pelloux et al., 1997).

We found a relatively low risk of mother-to-child transmission in women with untreated *Toxoplasma* infection during pregnancy. All mothers who may have become infected during pregnancy gave birth to babies with no evidence of a congenital *toxoplasmosis* infection. Thus, the incidence of congenital *toxoplasmosis* in our study population was in the range from 0% to 40% as in the UK

Table 4
Relationship of Maternal Seroprevalence of Toxoplasma gondii IgG and IgM antibodies with birth characteristics of their babies at delivery.

Characteristics	No tested	IgG positive		P value	IgM positive		P value
Gender		No	%		No	%	
Boy	239	48	20.1	X^2 = 0.232	2	0.8	X^2 = 0.008
Girl	261	57	21.8	P = 0.630	2	0.8	P = 0.930
Total	500	105	21.0		4	0.8	
Maturity		No	%		No	%	X^2 = 0.074
≤34	5	1	20.0	X^2 = 0.042	0	0.0	P = 0.964
35-40	491	103	21.0	P = 0.979	4	0.8	
≥41	4	1	25.0		0	0.0	
Total	500	105	21.0		4	0.8	
Birth weight (g)[1]		No	%		No	%	X^2 = 5.78
≤1899	4	0	0.0	X^2 = 4.52	0	0.0	P = 0.123
1900–2499	37	5	13.5	P = 0.211	1	2.7	
2500–3999	435	92	21.1		2	0.5	
≥4000	24	8	33.3		1	4.2	
Total	500	105	21.0		4	0.8	
Head circumference (cm)[1]		No	%		No	%	X^2 = 0.024
≤29	1	0	0.0	X^2 = 7.812	0	0.0	P = 0.988
30-40	497	103	20.7	P = 0.020	4	0.8	
≥41	2	2	100.0		0	0.0	
Total	500	105	21.0		4	0.8	
Infant status		No	%		No	%	X^2 = 0.049
Normal	494	102	20.6	X^2 = 8.1	4	0.8	P = 0.997
Congenital Anomalies	1	0	0.0	P = 0.044	0	0.0	
preterm	3	1	33.3		0	0.0	
Baby die due multiples CA	2	2	100.0		0	0.0	
Total	500	105	21.0		4	0.8	

X^2 = Pearson Chi-Square.

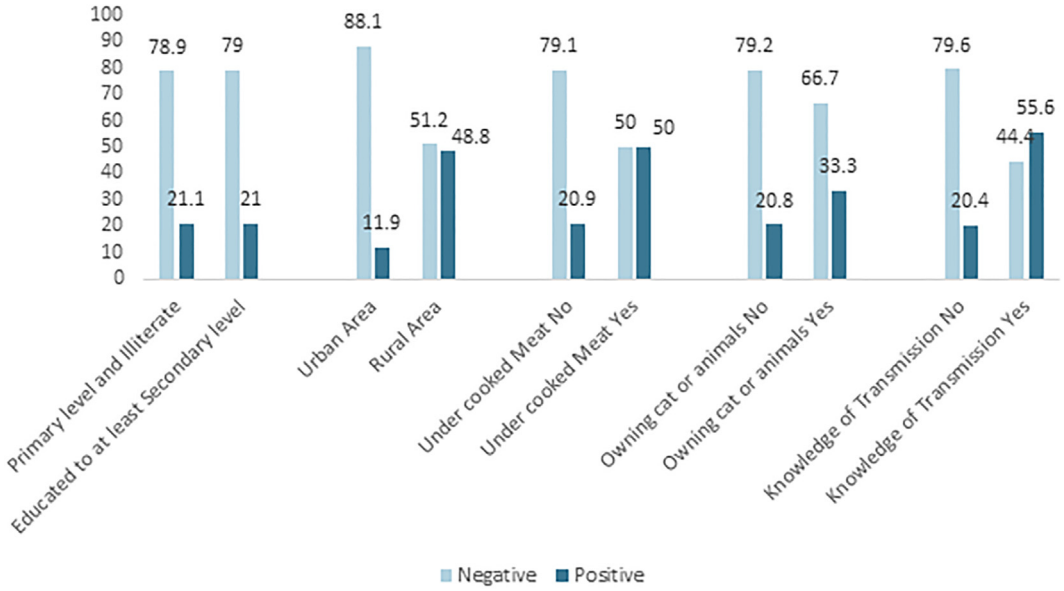

Fig. 2. Relationship between seroprevalence of *T. gondii* IgG antibodies and exposure to risk factor.

and from 0 to 100/10,000 live births elsewhere (Joynson, 1992, Mozzatto and Procianoy, 2003).

The results of our study confirm previous studies, i.e., the relationship of parity with *seropositivity*. Studies in Al-Ahsa and the southwestern region of Saudi Arabia have shown that IgG *seroprevalence* against *T. gondii* significantly correlates with increased parity. This may be due to an increase in exposure (Al-Mohammad et al., 2010, Almushait et al., 2014). Evidence shows that women, infected before pregnancy, become immune to reinfection, which does not pose a threat to any subsequent fetal development. However, gestational age during *seroconversion* is an essential factor in both the incidence of congenital infections and its severity. The likelihood of developing a placental infection increases with gestational age, while the severity of fetal damage is highest if transmission occurred in early pregnancy (Remington and Desmonts, 1990).

In our examination of previous obstetric outcomes and occurrence of congenital anomalies, we found a significant association of such outcomes with *T. gondii* infection. This is in agreement with the results of a recent Saudi study reported from Al-Ahsa, which showed adverse obstetric outcomes significantly associated with *T. gondii* infection (Al-Mohammad et al., 2010). Previous investigations on the association between obstetric outcomes and *T. gondii* infection were reported but not found to be of statistical significance (Almushait et al., 2014).

Further findings of current analysis showed that congenital *toxoplasmosis* was associated with an increased risk of premature birth and a shorter gestational period in women who seroconverted to 20 weeks, while we found no evidence of a significant association between congenital *toxoplasmosis* and low birth weight upon delivery. These observations are consistent with studies among members of the European Multicenter Study of congenital *toxoplasmosis* (Freeman et al., 2005), as well as study by Fochi et al. (2015), where comparison of birth weight with different serological profiles did not reveal statistically significant differences. There has been a relationship between the *seroprevalence* of *Toxoplasma* IgG and the head circumference of newborns. However, no evidence of a link between the *seroprevalence* of *Toxoplasma* infection and other growth characteristics of born children was found. Two out of 500 born children died during the first month of life due to multiple congenital anomalies, and both were born to mothers with a history of *toxoplasmosis*. Several risk factors influence prevalence, including age, cultural practices regarding hygienic eating habits, pets, and the environment (Tenter et al., 2001, Pappas et al., 2009). The known risk factors most likely account for the observed *seroprevalence* levels of differences within Saudi Arabia. In the current study, a statistically significant association was found between maternal *seropositivity* for *toxoplasmosis* and large head circumference in newborns, as well as with other adverse outcomes, including the premature death of the child and death from multiple congenital anomalies. Concerning the cause of death in infants from ether due to confirmed *toxoplasmosis*, the number was not statistically significant, therefore we cannot confirm this. In addition, it was difficult to conduct any analysis of the deceased infants.

Based on the results, we can suggest the frequent consumption of undercooked meat was not associated with an increased risk of chronic *T. gondii* infection. This is consistent with findings from the southwestern region of Saudi Arabia (Almushait et al., 2014). While studies in Al-Ahsa and Makkah (Al-Harthi et al., 2006, Al-Mohammad et al., 2010) show an association of often undercooked consumption meat with an increased risk of *T. gondii* infection during pregnancy. However, it is difficult to explain this regional disparity in Saudi Arabia. Considering these discrepancies in findings, we can confirm previous hypothesis that increased consumption of meat from animals raised indoors, and large-scale imports of frozen meat may explain the differences in accessibility, hence the discrepancies between the prevalence of the parasite in animals and humans in the region. The right temperature for cooking meat is critical to reducing the infection of *T. gondii*.

Several studies have shown that the prevalence and transmission of *T. gondii* are higher in rural than in urban (Ades and Nokes, 1993). Since the Eastern Province of Saudi Arabia, namely the city of Dhahran, is surrounded by villages and where the study hospital is allocated, many women from these rural areas came for delivery. In the present study, it was noted that women from rural or Bedouin communities were significantly more *seropositive* of *anti-T. gondii* IgG than from urban areas. Although, it is possible that these women once lived in rural areas and then moved to urban areas of the Eastern Province. These findings are consistent with the study by Al-Qurashi et al. (2001) who reported that the highest *seroprevalences* of *anti-T. gondii* IgG were found among peo-

ple who lived in rural areas, and Al-Mohammad et al. (2010) suggested that rural living may correlate with increased exposure to soil potentially contaminated with oocysts (Al-Qurashi et al., 2001, Al-Mohammad et al., 2010). Moreover, recent data by Rivera et al. (2019) confirmed that significant differences were found in serological prevalence analyzes between urban and *peri*-urban areas in Chascomus, Argentina. The authors, suggested that higher *seroprevalence* in *peri*-urban areas may be associated with poor socioeconomic conditions and/or poor *peri*-urban environments, which is a risk factor to be considered when planning health care for pregnant women (Rivera et al., 2019).

There was no significant difference in the level of *seropositivity* between illiterate and literate study participants regarding the relationship of *Toxoplasma seroprevalence* with education. This finding is supported by a similar study reported in Turkey (Ertug et al., 2005). However, Al-Harthi et al. (2006) indicated that the western region reported the highest *seroprevalences* of *anti-T. gondii* IgG among people with a low level of education (Al-Harthi et al., 2006). This may be since women in rural Saudi Arabia have fewer opportunities for formal education. Our study showed that there is a significant difference between the prevalence of *toxoplasmosis* infection in women who had knowledge and awareness of the transmission methods of *T. gondii* and those who did not. This is consistent with data from Jordan (Jumaian, 2005). In Saudi Arabia, antenatal screening depends on a single serum sample obtained during the antenatal period to detect *Toxoplasma*-antibodies. Thus, health education programs on how to avoid *toxoplasmosis*, such as eating habits and hygienic practices that are identified as risk factors during pregnancy, maybe a more cost-effective approach to preventing congenital *toxoplasmosis* and reducing the burden of infection than implementing a national program screening.

Nevertheless, the development of more sensitive and discriminatory methods is required to confirm the immunological status of people infected with *T. gondii*. The most cost-effective methods are those with the best combination of sensitivity and specificity and fewer equivocal results. We used a polymerase chain reaction to detect *T. gondii* DNA in the umbilical cord blood of mothers suspected of having an infection. Using this procedure, we were able to amplify and directly detect an individual organism's DNA from cord blood samples. This sensitivity level also allowed us to identify the BI gene from purified DNA samples containing 1 copy of DNA in cord blood. According to Burg et al. (1989) and James et al. (1996), PCR had the highest level of sensitivity and specificity in the diagnosis of (prenatal and postnatal) *toxoplasmosis* compared with serological tests (Burg et al., 1989, James et al., 1996). A single PCR-positive sample collected in the first half of pregnancy, combined with an IgG-positive test result might confirm a primary infection, even in the presence of serologic results that are difficult to interpret due to the lack of subsequent follow-up during pregnancy (Nimri et al., 2004). The sensitivity of PCR from amniotic fluid has been also shown to be affected by the stage of pregnancy in which maternal infection occurs. It is notable that testing amniotic fluid for *T. gondii* was found to be effective about 4 weeks following infection, which is the usual period for the parasitemic stage in the infected mother (Rorman et al., 2006). Conversely, a negative result by PCR testing amniotic fluid on prenatal diagnosis cannot rule out congenital infection (Abdul-Ghani, 2011). Amniotic fluid puncture for prenatal diagnosis should not be used as a routine procedure because of its associated risk of fetal loss. Observational studies reported that spontaneous abortion rate was 1.7% after amniocentesis and 0.7% in the control group (Tabor et al.,1986; Alfirevic, Sundberg, & Brigham, 2003).

Our study suggests that the detection of parasite DNA in cord blood using PCR is an important, cost-effective tool for the diagnosis of newborns probably infected with *T. gondii*. As there is no official notification of congenital *toxoplasmosis* infections within KSA, the true number of cases is not known. If a national prenatal antibody screening programs were introduced in KSA, 79% of non-immune pregnant women would have to be followed serologically until delivery; we concluded that repeated screening during pregnancy would be expensive. This test with a better sensitivity is relatively low cost compared to serological tests currently used in our diagnostic laboratories. However, the benefits of early treatment with the regimens currently available have not been evaluated in a randomized controlled trial, and the optimum duration of treatment is not known.

Based on this study's findings, we propose the need to establish preventive programs against *T. gondii* infection, a health education program regarding how to avoid *toxoplasmosis* for all *seronegative* women during pregnancy together with the National Board of Health screening for congenital *toxoplasmosis* of all neonates in Saudi Arabia, could, in theory, be a cost-effective approach to preventing and managing congenital *toxoplasmosis*. The potential benefit refers not only to the initiation of specific *anti-toxoplasmosis* treatment, but importantly identifies this susceptible group of infants who require full clinical evaluation and facilitates their enrollment in early intervention services as appropriate. Future studies can be developed regarding ToRCH (i.e., (toxoplasmosis, rubella, cytomegalovirus and Herpes simplex virus) co-infections and maternal/neonatal sequels.

5. Limitations

In addition to the study's main findings, several limitations were identified, that should be addressed in the future to confirm these findings and add additional data. As the part of the current research molecular and serology was performed. The current study did not include the avidity. However, Ig ELISA test can be developed in our future work. Although the PCR results were negative and the serology results also turned negative, observation at six months of age may be helpful. The main advantage of PCR is its sensitivity in detecting very low levels of T. gondii. Future research can be developed for bioassay. For the purposes of this study, we wanted to understand how important the history of the parameters with *seropositivity* was. In future research, we can categorize *seropositive* cases according to antibody titers, and assess their correlation with sequelae (e.g., history of abortions, etc.).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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