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.

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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-
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 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 *T. gondii* during pregnancy, and 8.8% were IgM antibody (AI-Mohammad et al., 2010). According to the most recent study conducted among Saudi pregnant women from Riyadh (n = 250), *T. gondii* infection rate 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 pre-natal 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 delivery 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 ℃ for initial denaturation followed by 40 cycles of 15 s at 95 ℃, 1 min at 60 ℃.

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 | 486 | 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, IgG 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

| 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         | X2 = 13.81 | 30 100.0     | 0 0.0        | X2 = 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        |   |

X2 = Pearson Chi-Square.

| Obstetric History | No. tested | IgG positive | IgM positive |
|-------------------|------------|--------------|--------------|
| Abortion          | No %       | X2 = 4.166   | 4 1.2        | X2 = 1.812 |
| 0                 | 345        | 67 19.4      | 4 1.2        | X2 = 1.812 |
| 1                 | 105        | 22 21.0      | 0 0.0        | P = 0.404 |
| ≥ 2               | 50         | 16 32.0      | 0 0.0        |   |
| Total             | 500        | 105 21.0     | 4 0.8        |   |
| Parity            | No %       | X2 = 7.979   | No %         | X2 = 1.997 |
| 0                 | 117        | 18 15.4      | 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 %       | X2 = 0.002   | No %         | X2 = 0.116 |
| None              | 486        | 102 21.0     | 4 0.8        | P = 0.733 |
| Yes               | 14         | 3 21.4       | 0 0.0        |   |
| Total             | 500        | 105 21.0     | 4 0.8        |   |
| IUFD*             | No %       | X2 = 0.245   | No %         | X2 = 0.057 |
| None              | 493        | 103 20.9     | 4 0.8        | P = 0.811 |
| Yes               | 7          | 2 28.6       | 0 0.0        |   |
| Total             | 500        | 105 21.0     | 4 0.8        |   |
| CA*               | No %       | X2 = 7.087   | No %         | X2 = 0.033 |
| None              | 496        | 102 20.6     | 4 0.8        | P = 0.857 |
| Yes               | 4          | 3 75.0       | 0 0.0        |   |
| Total             | 500        | 105 21.0     | 4 0.8        |   |

X2 = 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 ($X^2 = 7.87, P = 0.008$) 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 ($X^2 = 7.812, P = 0.020$) 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 ($X^2 = 8.1, P = 0.044$), 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 | | | | | |
| 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 | | | | | | |
| $\leq$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 |
| $\geq$41 | 4 | 1 | 25.0 | 0 | 0.0 |
| Total | 500 | 105 | 21.0 | 4 | 0.8 |
| Birth weight (g)$^1$ | | | | | | |
| $\leq$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 |
| $\geq$4000 | 24 | 8 | 33.3 | 1 | 4.2 |
| Total | 500 | 105 | 21.0 | 4 | 0.8 |
| Head circumference (cm)$^1$ | | | | | | |
| $\leq$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 |
| $\geq$41 | 2 | 2 | 100.0 | 0 | 0.0 |
| Total | 500 | 105 | 21.0 | 4 | 0.8 |
| Infant status | | | | | | |
| Normal | 494 | 102 | 20.6 | $X^2 = 8.1$ | 4 | 0.8 | $P = 0.097$ |
| 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.
and from 0 to 100/10,000 live births elsewhere (Joynson, 1992, Mozzotto 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 Dhafran, 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 been 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; Alfieric, 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 sequelae.

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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