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Serological and Molecular Diagnosis of Acute Feto-Maternal Toxoplasmosis in the Southwestern Region of Saudi Arabia

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Abstract: Primary toxoplasma infection during pregnancy carries a risk of fetal damage. The most frequent challenge encountered is how to determine if a pregnant woman acquired the acute infection during gestation. This study aimed to determine the incidence of acute maternal toxoplasmosis by a reliable method and to estimate the rate of intrauterine transmission. A total of 487 Saudi pregnant women who had attended the three major hospitals in Southwestern region, Saudi Arabia between January 2008 and August 2010 were included in the study. Two blood samples were collected from each woman. Anti-*Toxoplasma gondii* antibodies (IgM and IgG) were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Cord blood samples were collected from the umbilical cord after delivery and anti-*T. gondii* IgM and IgA in these samples were also determined by ELISA. Maternal and fetal seropositive samples were confirmed by Polymerase Chain Reaction (PCR). The incidence rate of acute maternal toxoplasmosis during pregnancy was 2%. Anti-*T. gondii* IgM was found in 2.4% (4/168) while anti-*T. gondii* IgA was not detected in any. Infants born to mothers who developed acute toxoplasmosis during pregnancy had significantly higher risk (31 fold) of developing congenital toxoplasmosis. Diagnosis of acute maternal toxoplasmosis during pregnancy should rely on the detection of seroconversion or else PCR is mandatory for confirmation. The incidence of acute maternal toxoplasmosis was considerable in this region of Saudi Arabia. Infants born to those mothers had significantly higher risk of developing congenital toxoplasmosis.

Key words: Acute toxoplasma, pregnancy, seroconversion, diagnosis, Saudi Arabia

INTRODUCTION

Although, toxoplasmosis is considered a benign illness in non-pregnant women, it is potentially harmful to a woman's fetus (Giannoulis *et al*., 2008). Transplacental transmission of toxoplasmosis has been shown to occur in acutely infected pregnant women and to be more predominant in women who acquire their primary infection during gestation (Montoya and Remington, 2008). Depending on parasite virulence, the mother's immune response and the pregnancy stage, fetal toxoplasmosis may cause serious damage (e.g., visual and neurological impairment) and malformation even death *in utero* (Varella *et al*., 2009).

Diagnosis of toxoplasma infection by clinical signs is difficult in pregnant women and it is estimated that primary infection in pregnant women goes unrecognized by clinical signs in about 90% of toxoplasmosis cases. The most frequent and important challenge that physicians encounter, however is how to determine if a pregnant woman acquired the acute infection during gestation (Remington *et al*., 2004). Currently, toxoplasmosis detection is based on serological screening (Wong and Remington, 1994). Testing for maternal IgM antibodies is the most common method used worldwide to determine if and when a pregnant woman has experienced an acute toxoplasma infection (Montoya *et al*., 2002). Although, IgM antibodies appear at the time of infection, they may also persist for years afterwards and thus may lead to erroneous interpretations of serological test results.

The fetus and newborns also respond to maternally-transmitted toxoplasma with a detectable humoral immune response (Remington *et al*., 1995). Detection of IgM and IgA antibodies in neonate samples is essential for neonatal diagnosis of toxoplasmosis (Decoster *et al*., 1988). Furthermore, the presence of neonatal IgM and IgA isotypes has been shown to

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depend on the time of maternal seroconversion (Bessieres *et al*., 1992). Toxoplasmosis seroconversion is thus defined by IgG antibodies appearing after IgM antibodies.

In Saudi Arabia, there are no systematic serological screening programs for toxoplasmosis in pregnant women. Currently, serological testing is performed one time with only a single sample of the mother's serum, thereby placing the fetus at risk of congenital infection. This practice strategy is different from France where women who initially present as seronegative continue to be tested at regular intervals throughout gestation (Berger *et al*., 2009). It is important to note that serological test results can be inconclusive. Direct detection of the parasite by Polymerase Chain Reaction (PCR) is then used to diagnose the infection (Dupouy-Camet *et al*., 1993).

Precise diagnosis facilitates early treatment of acute maternal toxoplasmosis and reduces the risk of transplacental transmission. A pharmacologic regimen that has been shown to efficiently reduce the burden of disease in newborns includes 16 weeks of the spiramycin macrolide antimicrobial agent from the time of diagnosis followed by at least 4 weeks of combination treatment with the pyrimethamine antiprotozoal drug, the sulfadiazine antibiotic and folinic acid along with a standardized follow-up program (Hotop *et al*., 2012). While this treatment strategy is used in Saudi Arabia, it currently only benefits those women who are diagnosed by the one time test.

This study was designed to estimate the incidence of acute maternal toxoplasmosis and rate of intrauterine transmission in the South Western region of Saudi Arabia that will aid in the development of an effective screening program.

MATERIALS AND METHODS

A total of 487 pregnant women were recruited for study from three hospitals located in the Southwestern region of Saudi Arabia: King Faisal Armed Forces Hospital (252, 51.7%), Abha General Hospital (184, 37.8%) and Maternal and Child Hospital (51, 10.5%). The study was carried out with approval by the Ethical Committee of King Khalid University and a written consent was provided by all study participants.

For each woman, two samples (8-10 mL venous blood) were collected. Each sample was equally divided into two tubes including an empty Falcon tube (to obtain sera for serology) and a tube containing EDTA (for extraction of the parasite's DNA for performing PCR). The first sample was obtained upon the first visit to the antenatal clinic and the second sample was obtained at the time of labor. The 2 mL blood samples were collected from the umbilical cord after delivery. Each sample was divided equally into two tubes as described above for sera separation and DNA extraction. Anti-*T. gondii* IgM and IgG antibodies were detected using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Human GMBH, Wiesbaden, Germany) and using the manufacturer's protocol. Anti-*T. gondii* IgA antibodies were detected using commercially available double-sandwich ELISA kits (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's instructions.

All cases showing positive serology were assessed by PCR using the procedure described by Burg *et al*. (1989) to confirm the toxoplasma-infected status. DNA was extracted from the EDTA-blood samples using a commercial purification system (Qiagen, Hilden, Germany) and following the manufacturer's protocol. The extracted DNA was resuspended in 30 μL of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2) and stored at -70°C until use.

The highly conserved repetitive *B1* gene was amplified according to the method previously described by Burg *et al*. (1989) with slight modifications. The primers used in the first round of PCR (inner primers) were 5'-GGAACTGCATCCGTTCATGAG-3' and 5'-TCTTTA AAGCGTTCGTGGTC-3' which correspond to nucleotides 694-714 and 887-868 of the *B1* gene, respectively. The primers used in the second round (outer primers) were 5'-TGCATAGGTTGCAGTCACTG-3' and 5'-GGCG ACCAATGTGCGAATAGACC-3' which correspond to nucleotides 757-776 and 853-831, respectively.

Genomic DNA extracted from the brain of mice experimentally-infected mice with RH strain (GenBank No. 383379) was amplified as the positive control. A PCR mixture without DNA and with DNase-free water was amplified as the negative control. The amplified fragments were resolved on agarose gel, stained with ethidium bromide and visualized under UV light. Amplicons of the *B1* gene from the first and second PCR rounds were 193 base pair (bp) and approximately 96 bp, respectively. The molecular markers used were the Gelpilot 50 bp ladder (Cat No. 239025; Qiagen).

Data were coded, validated and analyzed using SPSS PC+Version 13 statistical software package (Chicago, IL, USA). Data are presented as frequency and percentages. Statistical significance of differences between groups (at the 5% level) was determined by the χ^2-test. Risk of developing congenital toxoplasma was assessed by calculating the Odds Ratio (OR) and concomitant 95% Confidence Interval (CI).

RESULTS AND DISCUSSION

The anti-toxoplasma IgG seroprevalence was 38.8% (189/487) while anti-toxoplasma IgM seroprevalence was 6.2% (30/487). Table 1 shows the seroconversion of the 201 pregnant women in the study who completed follow-up till delivery. Only four of the 201 women seroconverted, giving a crude incidence rate of 2%. None of the women showed a 3 fold increase in anti-*T. gondii* IgG. Table 2 presents the serodiagnosis of congenital toxoplasmosis. Of the 201 pregnant women who completed follow-up, 168 cord blood samples were taken and screened. Anti-*T. gondii* IgM was found in 2.4% (4/168) while anti-*T. gondii* IgA was not detected in any cord blood samples. Infants born to mothers who developed acute toxoplasmosis during pregnancy had 31 fold higher risk of developing congenital toxoplasmosis as evidenced by detection of *T. gondii* IgM in the cord blood samples (OR = 31.6; 95% CI = 4.0-247.6; Table 3). Of the samples that were positive for anti-*T. gondii* IgM at first admission to the antenatal clinic, 50.0% were positive by PCR (15/30; Fig. 1a and b). In contrast, of the samples that were positive for anti-*T. gondii* IgG at first admission to the antenatal clinic, PCR showed positivity in 26.5% (50/189). Of the samples taken at labor that showed anti-*T. gondii* IgM seropositivity, seven (including the four samples that seroconverted) were positive by PCR (70%, 7/10). Of the samples showing anti-*T. gondii* IgG seropositivity, 21.3% showed positive PCR (20/94). All of the serologically positive cord blood samples were also found to be positive by PCR (Table 4).

Table 1: Seroconversion during pregnancy detected at labor (N = 201)

	IgM at labor							
	Negative		Positive		Equivocal		Total	
Anti-toxoplasma IgMat admission	N	%	N	%	N	%	N	%
Negative	168	83.6	4	2	3	1.5	175	87
Positive	2	1.0	4	2	4	2.0	10	5
Equivocal	5	2.5	2	1	9	4.5	16	8
Total	175	87.1	10	5	16	8.0	201	100

Table 2: Serological diagnosis of congenital toxoplasmosis (N = 168)

	Negative		Positive		Total	
Anti-*T. gondii* IgM and IgACord blood	N	%	N	%	N	%
Anti-*T. gondii* IgM	164	97.6	4	2.4	168	100
Anti-*T. gondii* IgA	168	100.0	0	0.0	168	100

Table 3: The relation between acute maternal infection (IgM seroconversion) and fetal infection (IgM detection) (N = 167)

	IgM cord						
	Negative		Positive				
IgM labor	N	%	N	%	p-value	OR	95% CI
Negative (n = 147)	145	98.6	2	1.4	<0.001*	31.6	4.0-247.6
Positive (n = 7)	5	71.4	2	28.6			
Equivocal (n = 13)	13	100.0	0	0.0			
Total (n = 167)	163	97.6	4	2.4			

*Statistically significant

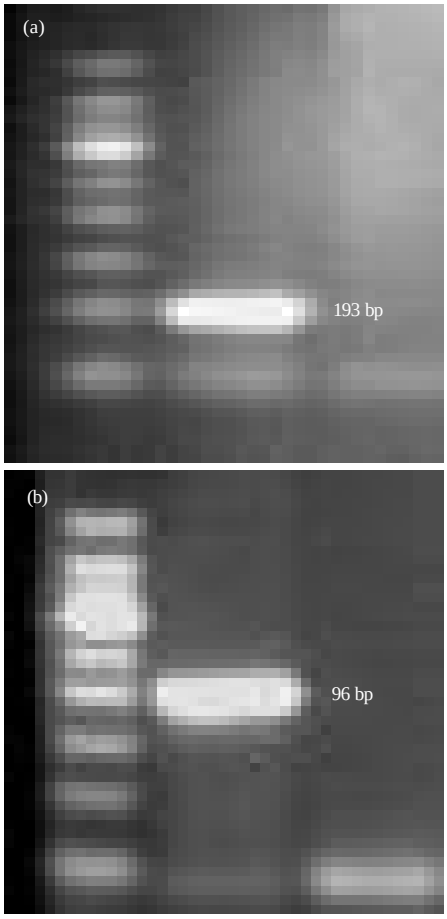

Fig. 1: PCR products resolved on 2% agarose/Tris-borate-EDTA gels products of a) first and b) second PCR rounds using the outer and inner pairs of primers targeting the *B1* gene, respectively. Lanes: 1: Molecular marker; 2: Positive case; 3: Negative case

Table 4: Comparison of anti-*T. gondii* IgM and IgG detection by ELISA and PCR

			Positive PCR for seropositive samples	
Ig	Sample	Seropositive samples	N	%
IgM	Pregnancy	30	15	50.0
	Labor	10	7*	70.0
	Cord	4	4	100.0
	Total	44	20	56.8
IgG	Pregnancy	189	50	26.5
	Labor	94	20	21.3
	Total	270	70	25.9

*These include four samples that showed IgM seroconversion and one sample that was also *T. gondii* IgM positive at the first antenatal visit

It is crucial to ascertain whether the *T. gondii* infection was acquired before or after conception because only primary infection during pregnancy carries a risk of fetal damage. Conventional serology (IgG and IgM) performed on only a single sample can not distinguish between acute progressive infection and chronic infection because IgM antibodies which emerge early after infection have a tendency to persist beyond a year, sometimes at high titers (Herbrinck *et al*., 1987). The only reliable way to determine when the infection was acquired is by documenting seroconversion during pregnancy (Emna *et al*., 2006).

In this study, ELISA detection of a population of pregnant women in the South Western region of Saudi Arabia showed that anti-*T. gondii* IgG seropositivity was 38.8% (189/487) and that anti-*T. gondii* IgM seroprevalence was 6.2% (30/487). In earlier similar studies carried out in other regions of Saudi Arabia, *T. gondii* IgG seroprevalence was reported to vary from 25.0-42.1% (Tonkal, 2008; Al-Mulhim and Al-Qurashi, 2001).

Generally, active toxoplasma infection is diagnosed by detecting toxoplasma-specific IgM antibodies and/or by detecting a 3 fold increase in IgG and/or IgM in paired sera samples (Iqbal and Khalid, 2007). In this study, acute toxoplasma infection during pregnancy was diagnosed by the appearance of *T. gondii* IgM (shown after testing paired sera) that was detected at labor (seroconversion), however researchers did not detect any case showing a 3 fold increase in *T. gondii* IgG in the paired serum samples. Researchers did not rely on a single positive IgM sample detected at the first antenatal visit for diagnosis of acute maternal toxoplasma infection during pregnancy since this result may have indicated an infection that had occurred prior to the pregnancy. The fact that IgM antibodies appear at the same time of infection, remain for at least a few months and may persist for years may have led to erroneous interpretations based on a single sample as well (Decoster *et al*., 1988). These pitfalls were obvious in the study, as researchers found IgM positivity in 6.2% (30/487) of women at their first antenatal visit but only 50% (15/30) of those cases were confirmed by PCR with the rest defined as false positive serological results. On the other hand, for IgM positive samples at labor (including the six cases that seroconverted), PCR was positive for 70% (7/10 including all seroconverted cases). This finding indicates that the detection of *T. gondii* IgM seroconversion is reliable for diagnosis of acute maternal Toxoplasmosis.

For samples that were *T. gondii* IgG positive at first admission and at labor, PCR was positive in only 26.5 and 21.3% of cases, respectively. This finding emphasizes that for diagnosing acute toxoplasmosis, a single *T. gondii* IgG positive sample must be confirmed by PCR as described by Nimri *et al*. (2004). In another Saudi study that was conducted in Jeddah, Tonkal reported 25.0% of the eight pregnant women studied showed positive IgG by both ELISA and PCR.

In the current study, the incidence rate of acute maternal toxoplasmosis (determined by seroconversion) was 2.0% (4/201 pregnant women) which is higher than the very low level of seroconversion rate (0.6%, 1/175) reported in Al-Khobar, an eastern province of Saudi Arabia by Al-Mulhim and Al-Qurashi (2001). While a simple explanation for the differences reported for regional distributions might be the various techniques used for diagnosing infection. Also, differences in toxoplasma seroprevalence in each area may play a role or even differences in the origin of the studied pregnant women since seroprevalence is well known to vary according to ethnicity (Pappas *et al*., 2009). Studies from across the globe support this theory. The rate of seroconversion during pregnancy was reported as 0.03% in Greece (Antoniou *et al*., 2007) and 8.6% in Goiania, Brazil (the highest to date) (Avelino *et al*., 2003).

Although, the detection of IgM and/or IgA antibodies in neonate samples is essential for the neonatal toxoplasmosis diagnosis (Bessieres *et al*., 1992), the absence of these congenital disease markers (IgM and IgA) in newborns does not constitute an exclusion criterion for toxoplasmosis (Rodrigues *et al*., 2009). *In utero*, the IgM antibodies are produced first followed by the IgG and IgA antibodies. Thus, the IgM antibodies produced *in utero* may have disappeared by the time of birth and the subsequently produced IgA antibodies may still be present. Moreover, the increase in total IgA and/or IgM immunoglobulins in infected newborns would be due to active synthesis of specific antibodies by the fetus (Pinon *et al*., 1985).

In the current study, congenital toxoplasmosis was diagnosed serologically by the presence of IgM and/or IgA in the cord blood according to the method described by Bessieres *et al*. (2001). Researchers detected toxoplasma IgM in the cord blood of 2.4% (4/168) of the delivered babies but did not detect toxoplasma IgA in any cord blood samples. The detection results were higher than those reported by a similar study carried out in Eastern Saudi Arabia which showed a congenital transmission rate of only 0.6% (1/175) (Pappas *et al*., 2009). This difference may be explained by the higher seroconversion rates among pregnant women in the community researchers studied. Intriguingly, the immunoglobulin detection results were lower than those reported for the French and Brazilian populations both of

which indicated high seroprevalence and seroconversion rates. Bessieres *et al.* (2001)'s study of French pregnant women infected during pregnancy detected specific IgM in 50% (21/42) of infected newborns, specific IgA in 60% of infected newborns (25/42) and both Ig isotypes in 29% (12/42) of infected newborns. However, the differences between these results and the own are likely due to the fact that the French study exclusively investigated pregnant women who had seroconverted (i.e., cases at high-risk of transplacental transmission). The study in Brazil detected IgM in 39.3% (11/28) of infected infants and IgA in 21.4% (6/28) (Rodrigues *et al*., 2009).

The absence of IgA in the cord blood samples of the study may be explained by the timing of seroconversion in the patient population. It is known that specific IgM antibodies are more frequently detected in the blood of a neonate whose mother has undergone seroconversion in the third trimester and IgA antibodies are more frequently found when seroconversion occurs in the first or second trimester. In the study, researchers took the first samples at the time of booking during the first or second trimester while the second samples were taken at the time of delivery. Maternal seroconversion occurred and was detected at the time of labor. However, all seropositive cord blood samples were also found to be positive by PCR. Researchers found a significant correlation between maternal seroconversion during pregnancy and fetal infection. That is to say, babies born to mothers who were seroconverted during pregnancy were found to have a 31 fold higher risk of developing toxoplasma infection. However, acute maternal infection is not always associated with fetal infection. Hohlfeld *et al.* (1994) studied 100 pregnant French women who acquired infection during the 2 weeks after their last menstrual period and found that toxoplasma infection was not transmitted to any of their fetuses.

The presence of toxoplasma DNA in maternal blood probably indicates a recent infection or apparent parasitemia which is likely to be clinically significant. The clearance time for toxoplasma DNA from the blood of patients with acute toxoplasmic lymphadenopathy has been estimated as 5.5-13 weeks (Guy and Joynson, 1995). In agreement with this timeline, Nimri *et al.* (2004) reported that the presence of both specific antibodies and DNA indicate the possibility that *T. gondii* infection occurred during the current pregnancy if the serum sample was collected within the 1st 20 weeks of gestation.

CONCLUSION

Researchers found that diagnosis of acute maternal toxoplasmosis during pregnancy should rely on the detection of seroconversion and that the PCR test is sufficient for confirming a diagnosis of acute infection based upon a single positive serological test in pregnant women. The incidence rate of acute maternal and congenital toxoplasmosis is considerable in this region of Saudi Arabia. Infants born to mothers who developed acute toxoplasmosis during pregnancy had significantly higher risk of developing congenital toxoplasmosis. The findings of this study would necessitate the implementation of routine screening and follow-up of the pregnant women who are seronegative for toxoplasmosis.

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REFERENCES

Al-Mulhim, A.A. and M.A. Al-Qurashi, 2001. Sero-prevalence of toxoplasmosis in pregnant mothers and new born infants in Eastern Province, Saudi Arabia. Saudi Soc. Family Commun. Med., 8: 45-53.

Antoniou, M., H. Tzouvali, S. Sifakis, E. Galanakis, E. Georgopoulou and Y. Tselentis, 2007. Toxoplasmosis in pregnant women in Crete. Parassitologia, 49: 231-233.

Avelino, M.M., D. Jr. Campos, J. do Carmo Barbosa de Parada and A.M. de Castro, 2003. Pregnancy as a risk factor for acute toxoplasmosis seroconversion. Eur. J. Obstet. Gynecol. Reprod. Biol., 108: 19-24.

Berger, F., V. Goulet, Y. Le Strat and J.C. Desenclos, 2009. Toxoplasmosis among pregnant women in France: Risk factors and change of prevalence between 1995 and 2003. Rev. Epidemiol. Sante Publique, 57: 241-248.

Bessieres, M., C. Roques, A. Berrebi, V. Barre, M. Cazaux and J.P. Seguela, 1992. IgA antibody response during acquired and congenital toxoplasmosis. J. Clin. Pathol., 45: 604-608.

Bessieres, M.H., A. Berrebi, M. Rolland, M.C. Bloom and C. Roques *et al.*, 2001. Neonatal screening for congenital toxoplasmosis in a cohort of 165 women infected during pregnancy and influence of in utero treatment on the results of neonatal tests. Eur. J. Obstet. Gynecol. Reprod. Biol., 94: 37-45.

Burg, J.L., C.M. Grover, P. Pouletty and J.C. Boothroyd, 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase china reaction. J. Clin. Microbiol., 27: 1787-1792.

Decoster, A., F. Darcy, A. Caron and A. Capron, 1988. IgA antibodies against P30 as markers of congenital and acute toxoplasmosis. Lancet, 2: 1104-1107.

Dupouy-Camet, J., S.L. de Souza, C. Maslo, A. Paugam and A.G. Saimot *et al*., 1993. Detection of *Toxoplasma gondii* in venous blood from AIDs patients by polymerase chain reaction. J. Clin. Microbiol., 31: 1866-1869.

Emna, S., A. Karim, K. Mohammed and B. Aida, 2006. Difficulty in dating primary infections by *Toxoplasma gondii* in pregnant women in Tunisia. Tunis. Med., 84: 85-87.

Giannoulis, C., B. Zournatzi, A. Giomisi, E. Diza and I. Tzafettas, 2008. Toxoplasmosis during pregnancy: A case report and review of the literature. Hippokratia, 12: 139-143.

Guy, E.C. and D.H. Joynson, 1995. Potential of the polymerase chain reaction in the diagnosis of active *Toxoplasma* infection by detection of parasite in blood. J. Infect. Dis., 172: 319-322.

Herbrinck, P., A.M. van Loon, J.P. Rotmans, F. van Knapen and W.C. van Dijk, 1987. Interlaboratory evaluation of indirect enzyme-linked immunosorbent assay, antibody capture enzyme-linked immunosorbent assay and immunoblotting for detection of immunoglobulin M antibodies to *Toxoplasma gondii*. J. Clin. Microbiol., 25: 100-105.

Hohlfeld, P., F. Daffos, J.M. Costa, P. Thulliez, F. Forestier and M. Vidaud, 1994. Prenatal diagnosis of congenital toxoplasmosis with a polymerase-chain-reaction test on amniotic fluid. N. Engl. J. Med., 331: 695-699.

Hotop, A., H. Hlobil and U. Gross, 2012. Efficacy of rapid treatment initiation following primary *Toxoplasma gondii* infection during pregnancy. Clin. Infect. Dis., 54: 1545-1552.

Iqbal, J. and N. Khalid, 2007. Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis. J. Med. Microbiol., 56: 1495-1499.

Montoya, J.G. and J.S. Remington, 2008. Management of *Toxoplasma gondii* infection during pregnancy. Clin. Infect. Dis., 47: 554-566.

Montoya, J.G., O. Liesenfeld, S. Kinney, C. Press and J.S. Remington, 2002. VIDAS test for avidity of *Toxoplasma*-specific immunoglobulin G for confirmatory testing of pregnant women. J. Clin. Microbiol., 40: 2504-2508.

Nimri, L., H. Pelloux and L. Elkhatib, 2004. Detection of *Toxoplasma gondii* DNA and specific antibodies in high-risk pregnant women. Am. J. Trop. Med. Hyg., 71: 831-835.

Pappas, G., N. Roussos and M.E. Falagas, 2009. Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int. J. Parasitol., 39: 1385-1394.

Pinon, J.M., H. Thoannes and N. Gruson, 1985. An enzyme-linked immune-filtration assay used to compare infant and maternal antibody profiles in toxoplasmosis. J. Immunol. Methods, 77: 15-23.

Remington, J.S., P. Thulliez and J.G. Montoya, 2004. Recent developments for diagnosis of toxoplasmosis. J. Clin. Microbiol., 42: 941-946.

Remington, J.S., R. McLeod and G. Desmonts, 1995. Toxoplasmosis. In: Infectious Diseases of the Fetus and Newborn Infant, Remington, J.S. and J.O. Klein (Eds.). 4th Edn., W.B. Saunders Co., Philadelphia, PA., USA., pp: 140-267.

Rodrigues, I.M., A.M. Castro, M.B. Gomes, W.N. Amaral and M.M. Avelino, 2009. Congenital toxoplasmosis: Evaluation of serological methods for the detection of anti-*Toxoplasma gondii* IgM and IgA antibodies. Mem. Inst. Oswaldo Cruz, 104: 434-440.

Tonkal, A.M., 2008. PCR versus ELISA in diagnosis of human toxoplasmosis in Jeddah, Saudi Arabia. J. Egypt Soc. Parasitol., 38: 707-714.

Varella, I.S., I.C. Canti, B.R. Santos, A.Z. Coppini, L. Argondizzo, C. Tonin and M.B. Wagner, 2009. Prevalence of acute toxoplasmosis infection among 41,112 pregnant women and the mother-to-child transmission rate in a public hospital in South Brazil. Mem. Inst. Oswaldo Cruz, 104: 383-388.

Wong, S.Y. and J.S. Remington, 1994. Toxoplasmosis in pregnancy. Clin. Infect. Dis., 18: 853-861.