# SEROLOGICAL DIAGNOSIS OF *TOXOPLASMA GONDII* INFECTION IN WOMEN ASSOCIATED WITH GYNECO-OBSTETRIC PROBLEMS

Muhammad Abdus Samad[1], Nasima Begum[2], Shamsunahar[2] and Muzahed Uddin Ahmed

[1]Department of Medicine, Bangladesh Agricultural University, Mymensingh-2202, [2]Department of Obstetrics and Gynaecology, Mymensingh Medical College, Mymensingh, Bangladesh

**Abstract.** The latex agglutination test (Toxoreagent) was used to detect the sero-prevalence of *Toxoplasma gondii* in 302 Bangladeshi women who were under treatment for various gyneco-obstetric problems at the Mymensingh Medical College during January to June 1991. The over-all sero-prevalence rate was 15.89%, of which 6.25% reacted at 1:32, 33.33% at 1:64, 16.67% at 1:128, 22.92% at 1:256, 6.25% at 1:512 and 14.58% at 1:1024. The positivity rate of 18.60% recorded in women between 31 and 40 years was insignificantly ($p > 0.05$) higher than that of 15.44% in women between 17 and 30 years of age. Over-all 26.49% of infections resulted in abortion, 6.62 in stillbirths and 30.79% in dystocia, of which 20.0%, 30.0% and 7.53% women had positive titers to *T. gondii*, respectively, whereas 17.43% women with normal live births had also positive titers to *T. gondii*. The over-all prevalence rate of abortion in association with *T. gondii* infection was 5.30% which was higher than the rates of stillbirths (1.99%) and perinatal death (0.66%). It is concluded that toxoplasmosis can cause abortion, stillbirths and perinatal death in Bangladeshi women.

## INTRODUCTION

Toxoplasmosis is a world-wide zoonotic infection, caused by the protozoan parasite, *Toxoplasma gondii*. Judging from antibody data in the world literature, about 38.5% of the human population have been infected with *T. gondii* (Samad and Begum, 1990). Serologic evidence of *T. gondii* was found in Bangladesh in 17.0% of 194 cattle (Samad *et al*, 1982) but there is no report, so far, on the prevalence of *T. gondii* in humans in Bangladesh. This disease has been recognized as an important cause of abortion, stillbirths and perinatal death both in man and animals elsewhere (Samad and Begum, 1990) but it has not been incriminated with reproductive abnormalities in Bangladesh. This study was therefore undertaken to detect the sero-prevalence of *T. gondii* in Bangladeshi women and to determine whether an association exists between adverse reproductive outcome and the serologic status of the women to *T. gondii*.

## MATERIALS AND METHODS

This study was conducted on 302 pregnant women who were under treatment for various gyneco-obstetric problems at the Department of Obstetrics and Gynaecology, Mymensingh Medical College during the period from January to June 1991. Venous blood collected from each of these patients was allowed to clot and serum was separated according to standard methods. The sera were stored without preservatives at -20°C until tested.

Assessment of reproductive outcome of these 302 pregnant women was made by determining the fate of fetus of each women. Each serum was tested for *T. gondii* antibodies by using the commercial latex agglutination test kit (Toxoreagent, Eiken Chemical Co Ltd, Japan) at a dilution of 1:32 in U-well microtiter plates (Nunc, Inter Med, Denmark) and those which gave positive reactions at that dilution were titrated to the end point reaction by doubling serial dilutions. Appropriate positive controls were included in the test. Standard chi-square test was used for significance (Gupta, 1982).

## RESULTS

The results were based on a single collection of blood from each woman during the detection of gyneco-obstetric problems or normal delivery. Sera that gave reaction at 1:32 dilution were considered sero-positive to *T. gondii* (according to the manufacturer). Of 302 pregnant women tested, 48

Table 1

Prevalence of *T. gondii* antibodies in different age groups and gestation numbers in Bangladeshi pregnant women.

| Gestation number | 17 to 30 years | | 31 to 40 years | | Total | |
|---|---|---|---|---|---|---|
| | No. tested | Positive No. (%) | No. tested | Positive No. (%) | No. tested | Positive No. (%) |
| 1st | 106 | 16 (15.09) | 2 | 0 (0) | 108 | 16 (14.81) |
| 2nd | 53 | 5 (9.43) | 6 | 3 (50.00) | 59 | 8 (13.56) |
| 3rd | 41 | 10 (24.39) | 6 | 0 (0) | 47 | 10 (21.28) |
| 4th | 27 | 4 (14.81) | 4 | 1 (25.00) | 31 | 5 (16.13) |
| 5th | 16 | 3 (18.75) | 7 | 1 (14.29) | 23 | 4 (17.39) |
| 6th | 6 | 1 (16.67) | 4 | 0 (0) | 10 | 1 (10.00) |
| 7th | 3 | 0 (0) | 7 | 3 (42.86) | 10 | 3 (30.00) |
| 8th | 3 | 0 (0) | 4 | 0 (0) | 7 | 0 (0) |
| 9th | 2 | 0 (0) | 1 | 0 (0) | 3 | 0 (0) |
| 10th | 2 | 1 (50.00) | 1 | 0 (0) | 3 | 1 (33.33) |
| 11th | 0 | 0 (0) | 1 | 0 (0) | 1 | 0 (0) |
| Over-all | 259 | 40 (15.44) | 43 | 8 (18.60) | 302 | 48 (15.89) |

(15.89%) had *T. gondii* antibodies, of which 3 (6.25%) reacted at 1:32, 16 (33.33%) at 1:64, 8 (16.67%) at 1:128, 11 (22.92%) at 1:256, 3 (6.25%) at 1:512 and the highest titre of 1:1024 was recorded from 7 (14.58%) cases (Table 2). All the tested pregnant women were between 17 and 40 years old. The results were analysed on the basis of two age groups and gestation number of pregnant women. Statistical analysis showed that the over-all sero-prevalence rates were insignificantly ($p > 0.05$) higher in 31 to 40 years (18.60%) as compared to 17 to 30 years (15.44%) age groups (Table 1). Though an increasing tendency of sero-prevalence was observed with gestation number, the results were inconsistent (Table 1).

Of 48 *T. gondii* sero-positive patients, 29 (60.42%) resulted in reproductive wastage and the remaining 19 (39.58%) had normal live births (Table 2). Of 254 *T. gondii* sero-negative patients, 64.57% had abnormal reproductive outcome and 35.43% had normal live births (Table 2). Overall 63.91% of pregnancies resulted in reproductive wastage due to abortion (26.49%), stillbirths (6.62%) and dystocia (30.79%) of which only 20.0, 30.0 and 7.53% were sero-positive to *T. gondii*, respectively, whereas of 109 (36.09%) patients with normal live births, 17.43% had positive titers to *T. gondii* (Table 3). Spontaneous abortion was recorded between 6 and 32 weeks with a mean of 14.63 weeks of gestation age but a similar gestation length was observed between the *T. gondii* sero-positive ($\bar{x} = 15.88$ weeks) and sero-negative ($\bar{x} = 14.31$ weeks) aborted women. No marked differences were observed in the gestation length in women with normal live births ($\bar{x} = 37.59$ weeks), dystocia ($\bar{x} = 38.1$ weeks) and stillbirths ($\bar{x} = 36.9$ weeks). The status of domestic and/feral house cat populations were analysed on the basis of histories collected from each patient. Of 48 *T. gondii* sero-positive cases, 41 (85.42%) patients had an association with cats but the remaining 7 (14.58%) cases did not have any history of such association.

## DISCUSSION

The over-all sero-prevalence of *T. gondii* in Bangladeshi pregnant women was 15.89%, which is much lower than 62.6% reported from Germany (Gringmuth and Muller, 1977) 53.3% from Italy (Campello *et al*, 1979), 23.36% from UK (Broadbent *et al*, 1981), 46.03% from Austria (Mayer *et*

Table 2

Serologic status of *T. gondii* and reproductive outcome in Bangladeshi pregnant women.

| Antibody titer | Women with reproductive outcome | | |
|---|---|---|---|
| | Normal | Abnormal | Total |
| | No. (%) | No. (%) | No. (%) |
| 1:32 | 1 (33.33) | 2 (66.67) | 3 (6.25) |
| 1:64 | 5 (31.25) | 11 (68.75) | 16 (33.33) |
| 1:128 | 2 (25.00) | 6 (75.00) | 8 (16.67) |
| 1:256 | 5 (45.45) | 6 (54.55) | 11 (22.92) |
| 1:512 | 5 (100.00) | 0 (0) | 3 (6.25) |
| 1:1024 | 3 (42.86) | 4 (57.14) | 7 (14.58) |
| Total +ve | 19 (39.58) | 29 (60.42) | 48 (15.89) |
| Total −ve | 90 (35.43) | 164 (64.57) | 254 (48.11) |
| Over-all | 109 (36.09) | 193 (63.91) | 302 |

Table 3

Sero-positivity to *T. gondii* and nature of reproductive outcome in pregnant women.

| Outcome | No. (%) of patients examined | No. of positive at titer | | | | | | | Over-all incidence rate, % |
|---|---|---|---|---|---|---|---|---|---|
| | | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 | 1:1024 | Total No. (%) | |
| Abortion | 80 (26.49) | 1 | 6 | 3 | 3 | 0 | 3 | 16 (20.00) | 5.30 |
| Stillbirth | 20 (6.62) | 1 | 2 | 0 | 2 | 0 | 1 | 6 (30.00) | 1.99 |
| Dystocia | 93 (30.79) | 0 | 3 | 3 | 1 | 0 | 0 | *7 (7.53) | 2.32 |
| Sub-total | 193 (63.91) | 2 | 11 | 6 | 6 | 0 | 4 | 29 (15.03) | 9.60 |
| Normal | 109 (36.09) | 1 | 5 | 2 | 5 | 3 | 3 | 19 (17.43) | 6.29 |
| Total | 302 | 3 | 16 | 8 | 11 | 3 | 7 | 48 (15.89) | 15.89 |

* Perinatal death recorded of two children (0.66%)

*al,* 1983), 48.5% from France (Marty *et al,* 1985) and 60.0% from Mexico (Fernandez-Torrano, 1987). However, a similar sero-prevalence rate of 16.3% has been reported from Japan (Konishi and Takahashi, 1987) and 13.0% from Thailand (Bunnag *et al,* 1988). These variations of sero-prevalence rate could probably be due to difference of food habbits, association with cats, environmental conditions and socio-economic status.

The slightly higher sero-positivity rate with age, 15.44% at age 17 to 30 years and 18.60% at 31 to 40 years is in conformity with earlier reports of Castagnar *et al* (1980) and Cuadrado-Mendez *et al* (1981) but the difference was not statistically significant.

Toxoplasmosis was only evaluated in this study to determine its association with the reproductive outcome in pregnant women. This disease is well recognized as an important cause of abortion, stillbirths and perinatal death but the severity of reproductive wastage is related to the period of gestation when infection takes place (Desmonts and Couvreur, 1974; Sever *et al,* 1988). The reproductive wastage due to toxoplasmosis is mainly

associated with infection occurring during gestation and those who have *T. gondii* infection before conception will not have an infant with congenital toxoplasmosis (Johnson *et al,* 1979). The sero-results of this study revealed that 9.60% of sero-positive pregnant women might have become infected during the gestation period which resulted in reproductive wastage and 6.29% women who had normal live births might have had old infection (Leke *et al,* 1983, Guessous-Idrissi *et al,* 1984). However, chronic *T. gondii* infection could be dangerous not only in first pregnancy but also in subsequent ones in women (Jezyna and Zajac, 1983).

Of 80 aborted women, 20.0% had *T. gondii* positive titers, which is markedly lower than the reported values of 58.9% from Colombia (Restrepo *et al,* 1976), 40.8% from Greece (Lolis *et al,* 1978) and 60.0% from Nigeria (Megafu and Ugwuegbulam, 1981). Analysis of the sero-results of 20 mothers who had stillbirths revealed that 30.0% such mothers had *T. gondii* antibodies. This result could be compared well with the occurrence of 20.0% stillbirths in *T. gondii* positive women in Saudia Arabia (Kandil *et al,* 1980). However, the low sero-positivity to *T. gondii* in association with abortion in Bangladeshi women can probably be attributed to the preference of the people to eat well cooked meat, while the question of cats as a source of infection is difficult to assess because the prevalence of toxoplasmosis in the local cats is virtually unknown.

Antibody titers for *T. gondii* of 1:128 reflect a recent infection and higher titers suggest an active infection, a titer of 1:64 suggests recent exposure (Mackie *et al,* 1971), whereas, titers > 1:256 are considered as the cause of abortion (Lolis *et al,* 1978). It is concluded from the results of this study that toxoplasmosis can cause abortion, stillbirths and perinatal death in Bangladeshi women.

Studies are currently in progress on the sero-epidemiology and parasitological aspects on toxoplasmosis in man and animals which would provide more information. Endemic infection with *T. gondii,* rubella and other agents have been shown to co-exist in women elsewhere (Guessous-Idrissi *et al,* 1984, Hunter *et al,* 1983). Thus, additional studies are needed to determine the relative importance of these agents and others in reducing reproductive wastage in Bangladesh.

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