# *TOXOPLASMA* ANTIBODY PREVALENCE IN NEPALESE PREGNANT WOMEN AND WOMEN WITH BAD OBSTETRIC HISTORY

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**Abstract.** Sera from randomly selected 345 pregnant Nepalese women aged 16 - 36 years and 13 women with bad obstetric history (BOH) were tested for the presence of *Toxoplasma* antibodies using microlatex agglutination (MLA) and ELISA methods. The overall prevalence was 55.4% (191/345). Prevalence was slightly higher (59.0%) in older age-group (27-36 years) compared with younger age-group (16-26 years) (52.2%). No significant difference in antibody prevalence in women belonging to two different ethnic-groups (*Tibeto-Burmans* 57.8%, *Indo-Aryans* 52.7%) was observed ($p>0.05$). MLA antibody titer ranged from 1:16 to 1:2,048. Over three-fourth of the women showed either high (1:510 or over) or low (1:16 or 1:32) antibody titer. Three percent (6/191) of MLA antibody positive subjects had *Toxoplasma* IgM antibodies by IgM-ELISA. All six IgM antibody positive pregnant women had MLA antibody titer of over 1:510. Of the total 13 women with BOH, 5 (38.5%) had *Toxoplasma* antibodies of which 2 (40.0%) were positive for *Toxoplasma*-IgM antibodies.

## INTRODUCTION

*Toxoplasma gondii* infection in pregnant women may give rise to intrauterine infection of the fetus. Outcome of infections such as abortion, stillbirth or various congenital anomalies depends on the gestational age at the time of infection. In France, it is mandatory for pregnant women to undergo *Toxoplasma* antibody screening test and the fate of fetus is decided by a two-member team of physicians, one of them being "surveyor" (Desmonts *et al,* 1985). However, it is under debate in many countries (Eskild *et al,* 1996).

*Toxoplasma* antibody prevalence in pregnant women elsewhere in the world vary from less than 12% (Samad *et al,* 1997; Jenum *et al,* 1998) to over 80% (Lelong *et al,* 1995, Onadeko *et al,* 1996). In the neighboring country Bangladesh, *Toxoplasma* antibody prevalence in pregnant women is reported to be 11.1% (Samad *et al,* 1997). *Toxoplasma* antibody prevalence in women with BOH in India reportedly vary from 2.9 to 43.8% (Mittal *et al,* 1990; Gogate *et al,* 1994). Sharma *et al,* (1997) from India have also reported an increasing seropositivity in women with BOH. Previously, we reported the *Toxoplasma* antibody prevalence ranging from 30.6% in medical students, hospital staffs and blood donors in Kathmandu (Upadhyay *et al,* 1989) to 67.1% in apparently healthy subjects living in some parts of Nepal (Rai *et al,* 1989, 1994a, 1996a, 1998). Also, we reported a seropre- valence of toxoplasmosis ranging from 41.1 - 79.6% in four different common meat animals in Nepal (Rai *et al,* 1996b). In this paper, as a part of our ongoing study on *Toxoplasma* infection in Nepal, we report the *Toxoplasma* antibody prevalence in pregnant women with BOH.

## MATERIALS AND METHODS

A total of 345 randomly selected pregnant women aged 16-36 years and 13 women with BOH were included in this study. Blood samples were col-

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lected at antenatal clinics in Kathmandu valley during the year 1995 and 1996. Serum samples thus separated and transferred into polypropylene tubes were stored at - 20°C. Age and ethnicity of pregnant women included in the study were noted. Total *Toxoplasma* antibodies were detected by microlatex agglutination (MLA) method using commercial test reagents (Eiken Chemicals Co Ltd Japan) and U type microplates (Dynatec Laboratories, USA), whereas *Toxoplasma*-IgM antibodies were determined by ELISA (Labsystem, Finland). MLA titers of 1:16 or more than 1:16 were considered as positive. Samples showing a titer of 1:16 were subjected to repeat test and only those with consistent results were considered. All MLA positive samples were subjected to *Toxoplasma*-IgM antibodies detection by ELISA method performed manually. ELISA values were read at 405 nm (ELISA reader, ER2000, Sanko Junyaku Co Ltd, Japan) and were expressed as enzyme immunoassay unit (EIU) with a cut off value of 20 EIU. The results were stratified against age and ethnicity of subjects. Chi-square test was applied to see significant differences.

## RESULTS

Sera collected from 345 randomly selected pregnant Nepalese women aged 16 to 36 years were included in this study. Of the total 345, 191 (55.4%) showed *Toxoplasma* antibodies. No significant difference in antibody prevalence in women belonging to two different ethnic-groups namely, *Tibeto-Burmans* (57.8%) and *Indo-Aryans* (52.7%) was observed ($p>0.05$) (Table 1). Over all *Toxoplasma* antibody prevalence was slightly higher (59.0%) in older age-group (27-36 years) compared with younger age-group (16-26 years) (52.2%) ($p>0.05$) (Table 2). MLA antibody titer ranged from 1:16 to 1:2,048. Over one-third (42.4%) of women showed high antibody titer (1:510 or over) whereas another one-third (34.6%) exhibited low titer (1:16 or 1:32) (Fig 1 ). *Toxoplasma* specific IgM antibodies were detected in 3.1% of antibody positive subjects (n=191). All of the IgM positive pregnant women had MLA antibody titers over 1:510. Approximately half of the women in the age-group of 16-26 years were in their first pregnancy whereas a majority of the women in the age-group of 26-36 years were in their third and fourth pregnancies. Of the total 13 women with BOH, 5 (38.5%) had *Toxoplasma* antibodies of which 2 (40.0%) were positive for *Toxoplasma*-IgM antibodies.

Table 1

*Toxoplasma* seroprevalence in pregnant Nepalese women of two different ethnic-groups aged 16-36 years

| Ethnic-group | Total no. | Positive no. (%) | p | Age-group | Total no. | Positive no. (%) | p |
|---|---|---|---|---|---|---|---|
| *Tibeto-Burman* | 178 | 103 (57.8) | > 0.05 | 16-26 | 184 | 96 (52.2) | > 0.05 |
| *Indo-Aryan* | 167 | 88 (52.7) | | 27-36 | 161 | 95 (59.0) | |
| Total | 345 | 191 (55.4) | | Total | 345 | 191 (55.4) | |

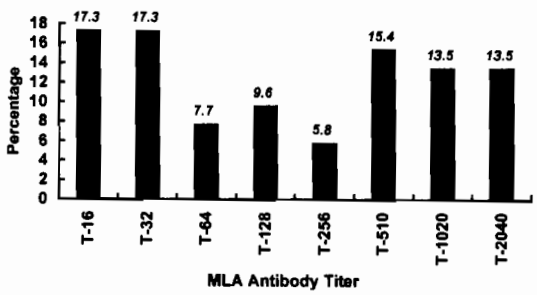


Fig 1–Distribution pattern of *Toxoplasma* antibody titer in Nepalese pregnant women aged 16-36 years.

## DISCUSSION

Prevalence of *Toxoplasma* antibody in Nepalese pregnant women and the incidence of congenital

toxoplasmosis in Nepal have not been studied yet. Also no single case report on congenital toxoplasmosis available. This was not because of absence of *Toxopalsma* parasites in Nepal but due primarily to lack of diagnostic facilities. In part, this could also be due to the immunity developed before pregnancy because of an early exposure. On an average, over 40.0% of Nepalese acquire *Toxoplasma* infection by the age of twenty (Rai *et al,* 1989, 1994a, 1996a, 1998), a phenomenon that occurs in many developing countries (Stagno-Thiermann, 1973). Lelong *et al* (1995) reported a high *Toxoplasma* seroprevalence (75%) in young pregnant women in Madagascar indicating that most of the infections occur during an early life. Reactivation of maternal toxoplasmosis in immunocom- promised pregnant women with immunity to *Toxoplasma* and congenital infection, however, can occur (D'Ercole *et al,* 1995).

We have been using commercial MLA and IgM-ELISA test reagents for the detection of total and IgM *Toxoplasma* antibodies, respectively. We choose MLA test for total *Toxoplasma* antibodies detection because of its qualitative agreement with dye test (DT) (Kobayashi *et al,* 1977), ELISA and direct agglutination test (DAT) (Rai *et al,* 1994b) as well as its simplicity and cost-effectiveness. *Toxoplasma* antibody prevalence observed in this study was higher than the mean *Toxoplasma* antibody prevalence in apparently healthy Nepalese women (49.7%) reported earlier but with no significant difference ($p>0.05$). This, however, was significantly higher compared with the overall mean antibody prevalence in apparently healthy subjects (both males and females) (46.4%) ($p<0.01$) which was attributed to a significantly low prevalence in male subjects (Rai *et al,* 1989, 1994a, 1996a, 1998). Elsewhere in the world, prevalence of *Toxoplasma* antibody in pregnant women vary from less than 12% (Samad *et al,* 1997; Jenum *et al,* 1998) to over 80% (Lelong *et al,* 1995; Onadeko *et al,* 1996) and appears to be attributed to geopraphical differences, meat eating habit and the type of meat the people eat. In France, where pregnant women are required to undergo *Toxoplasma* antibody screening test during pregnancy mandatorily (Desmonts *et al,* 1985), standardized prevalence rates of 71% in pregnant French women and 51.4% among immigrant women in Paris area have been reported (Jeannel *et al,* 1988). Although, the strict rule for *Toxoplasma* screening during pregnancy in France has reportedly reduced the incidence of congenital toxoplasmosis (Desmonts *et al,* 1985); mandatory *Toxoplasma* antibody screening is under debate in many other countries (Eskild *et al,* 1996).

In this study, MLA titers ranged from 1:16 to 1:2,040. Over three-fourths of the women showed either high (1:510 or over) or low (1:16 or 1:32) antibody titers for which we do not have an explanation. Three percent of MLA positive pregnant women had *Toxoplasma* IgM antibodies. This was 1.7% of the total subjects included in this study. All IgM positive subjects had MLA titers of over 1:510. Previously, we reported prevalence of *Toxoplasma* IgM in 0.4 to 2.9% of antibody-positive, apparently healthy Nepalese living in different parts of country (Rai *et al,* 1994a, 1996a, 1998). These findings were in support of the postulate that most of the *Toxoplasma* infections in Nepal occur in early life as have been reported elsewhere in the world (Stagno and Thiermann, 1973; Lelong *et al,* 1995). Jacquier *et al* (1995) from Switzerland have also reported the *Toxoplasma* IgM prevalence in 1.7% of pregnant women. In Henan province of China, it has been reported to be 3.3% (Jhang *et al,* 1996). As indicated by the presence of IgM antibodies very few pregnant women in Nepal acquire primary *Toxoplasma* infection during pregnancy. However, presence of IgM antibody alone demands a careful interpretation. We, therefore, are now in the process of studying *Toxoplasma* antibodies in both maternal and cord blood collected at the time of delivery.

No significant difference was found in *Toxoplasma* antibody prevalence in women belonging to two different ethnic-groups namely *Tibeto-Burman* and *Indo-Aryans* in spite of a significantly higher mean seroprevalence in *Tibeto-Burmans* among apparently healthy groups ($p<0.05$) (Rai *et al,* 1989, 1994a, 1996a, 1998). Though we do not have a concrete explanation for this discrepancy, in part, it could be due to the difference in study populations. Over 90% of subjects included in this study were inhabitants of Kathmandu valley (mainly from urban and sub-urban areas) where *Indo-Aryans* are liberal with regard to consumption of meat including pork. Jenum *et al* (1998) in Norway found a significantly higher seroprevalence in immigrants compared with native Norwegian pregnant women. However, Lelong *et al* (1995) in Madagascar observed no influence of ethnicity in *Toxoplasma* seroprevalence. On the contrary, Jeannel *et al* (1988) in France have shown lower standardized prevalence rate (51.4%) among immi-

grant pregnant women compared with French women (71%) in the Paris area.

This study revealed that over one third of women with BOH (38.5%) in Nepal possess *Toxoplasma* antibodies. However, this does not mean that 38.5% of BOH is caused by *Toxoplasma* beacuse, over one third of *Toxoplasma* infections occur during early age (Rai *et al,* 1989, 1996a, 1998) and might have been the cause of positive antibody. This subject, therefore, demands a systematic study with bigger sample size. Our present finding (38.5%), however, was much higher compared with the prevalence in women with BOH in Delhi (2.9%) (Mittal *et al,* 1990) but lower than that reported by Gogate *et al* (1994) (43.8%) from Bombay, both in India. Galvan-Ramirez *et al* (1995) from Mexico have also reported a prevalence of 44.9% in a group of women with habitual abortions. Sharma *et al* (1997) in Chandigarh area (north-west part of India), however, found an increasing *Toxoplasma* seropositivity in women with BOH and in newborns. In one series of study in India, toxoplasmosis was found to be associated with 38% of abortions, 6% of stillbirths, 16% of premature delivery and 6% of congenital anomalies (Mookherjee *et al,* 1995). However, such data from Nepal are not available yet.

Our finding showed that nearly half (44.6%) of the pregnant women in Nepal are at the risk of primary *Toxoplasma* infection during pregnancy. The present finding, therefore, suggests the need for informing the pregnant women about preventive measures of *Toxoplasma* infection during pregnancy so as to prevent the *Toxoplasma*-associated fetal loss and/or congenital anomalies in Nepal. Such an activity appears to be significant also in the pretext of rapid spread of HIV infection in Indian sub-continent and the opportunistic nature of *Toxoplasma* parasite.

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