

# SEROEPIDEMIOLOGICAL STUDY OF TOXOPLASMOSIS IN TWO DIFFERENT GEOGRAPHICAL AREAS IN NEPAL

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**Abstract.** A total of 302 serum samples collected from Chitawan (159) and Mustang (143) districts of Nepal were included in this study. Anti-toxoplasma antibody was detected using micro-latex agglutination (MLA) and ELISA methods. An overall positive rate was found to be 57.9%. The positive rate in Chitawan was significantly higher (64.1%) (less than 1,000 m altitude) compared to that in Mustang (51.0%) (more than 3,000 m altitude) ( $p < 0.05$ ). Females in Chitawan showed significantly higher positive rate (71.2%) compared to males (56.9%) ( $p < 0.05$ ). On the contrary, though insignificantly, males showed higher positive rate (57.9%) compared to that of females (43.3%) in Mustang. Almost equal positive rate was observed among males in both study area. Females in Chitawan showed significantly higher (71.2%) positive rate compared to their counterparts in Mustang (43.3%) ( $p < 0.001$ ). A slight increase in positive rate with age was observed in Chitawan while in Mustang a decreasing trend was noticed. Ethnically though statistically not significant, Indo-Aryans showed a higher positive rate (69.2%) compared to the positive rate shown by Tibeto-Burmans (63.1%) in Chitawan while the reverse was true in Mustang (Tibeto-Burmans: 53.8% and Indo-Aryans: 38.4%). Interestingly, 2.9% and 1.3% of MLA positive samples showed toxoplasma IgM antibody. None of the IgM positive samples were positive for toxoplasmic antigens.

## INTRODUCTION

*Toxoplasma gondii*, a coccidian parasite is estimated to infect about half of the population in the world. Most of the human infections, however, are asymptomatic. Severe and fatal infections occur among immunocompromised individuals. During recent years, *T. gondii* has been implicated as one of the most important opportunistic pathogens, particularly among patients with acquired immunodeficiency syndrome (AIDS) (Levy *et al.*, 1985; Luft and Remington, 1988). Recently there has been an epidemic of severe and fatal toxoplasmic encephalitis in western hemisphere (Luft *et al.*, 1983). It has been estimated that 25 - 50% of AIDS patients ultimately develop toxoplasmic encephalitis in areas where there is high seroprevalence of human toxoplasmosis (Luft and Remington, 1992). Diagnosis of toxoplasmosis alone has been reported to lead, in turn, to the diagnosis of

approximately 5.0% of new cases of AIDS (Fauci *et al.*, 1985). In addition, *T. gondii* associated clinical manifestations such as abortion, still birth, congenital abnormalities and vision impairment also constitute an important medico-social problem. Very recently, Murrell (1993) reported an estimate of toxoplasmosis associated annual economic/public health burden amounting to more than 400 million US dollars in United States of America.

The highest seroprevalence rate of 72.0% has been reported among Brazilians and none in Eskimos and Taiwanese Aborigines (Feldman; 1982). In certain specific group of study population, the positive rate has been found to be as high as 90.0% (Desmonts *et al.*, 1965; Sousa *et al.*, 1988). For the first time in Nepal, we reported a positive rate of 48.6% in a community in eastern Terai (plain area) (Rai *et al.*, 1989). The positive rate has been found to be 54.8% among pregnant women (Rai *et al.*, 1993). Among medical students, the

## MATERIALS AND METHODS

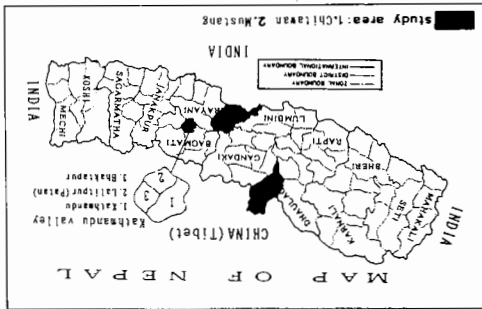


Fig 1—Study areas in Nepal (Chitawan district: less than 1,000 m altitude and Mustang district: more than 3,000 m altitude).

positive rate has been reported to be 30.6% (Upadhyay *et al*, 1989). The positive rate is reported to vary from place to place (Tizard *et al*, 1977; Feldman, 1982; Suzuki *et al*, 1987). Nepal is a country of vast diversification in geotopography. Infection is therefore likely to differ in positive rate accordingly. We therefore, conducted a seroepidemiological study of human toxoplasmosis in two geographical areas of less than 1,000m altitude (Chitawan district) and more than 3,000m altitude (Mustang district) (Fig 1) in Nepal.

**Sample collection:** A total of 302 serum samples were collected from Chitawan (less than 1,000m altitude) (159) and Mustang (more than 3,000m altitude) (143) districts in Nepal (Fig 1). Blood samples were collected from apparently healthy individuals (by a clean venipuncture) by field visit to have an actual representation of study area. Age, sex and ethnicity of each subject were noted. At first, serum samples were transported to Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal and subsequently to Hyogo College of Medicine, Hyogo, Japan under cold conditions. Samples were then stored at  $-70^{\circ}\text{C}$  until tested.

**Toxoplasma antibody detection:** Anti-toxoplasma antibody was detected by micro-latex agglutination test (MLAT) using commercially available test reagents (Eiken Chemicals Co, Tokyo, Japan). We used MLAT because of its simplicity and qualitative agreement with dye test (DT) (Kobayashi *et al*, 1977; Hirai and Nagai, 1978) and good correlation with ELISA (Lappin and Powel, 1991). All positive samples were subjected to IgM antibody detection using Toxo-IgM ELISA system (Labsystem, Helsinki, Finland). Serum samples were pre-treated to remove the non-specific IgM antibodies such as rheumatoid factor as described by the manufacturer. MLAT titers of 1 : 16 and more than

Table 1

Seroprevalence of human toxoplasmosis in two different geographical areas in Nepal (Chitawan district, less than 1,000m and Mustang district more than 3,000m).

Study areas	Sex	n	+ve n (%)	Ethnic group	n	+ve n (%)
Chitawan	M	79	45/79 (56.9)	Tibeto-Burman	133	84/133 (63.1)
	F	80	57/80 (71.2)	Indo-Aryan	26	18/26 (69.2)
	Total	159	102/159 (64.1)	Total	159	102/159 (64.1)
Mustang	M	76	44/76 (57.8)	Tibeto-Burman	117	63/117 (53.8)
	F	67	29/67 (43.3)	Indo-Aryan	26	10/26 (38.4)
	Total	143	73/143 (51.0)	Total	143	73/143 (51.0)
Grand total		302	175/302 (57.9)	Grand total	302	175/302 (57.9)

TOXOPLASMOSIS IN NEPAL

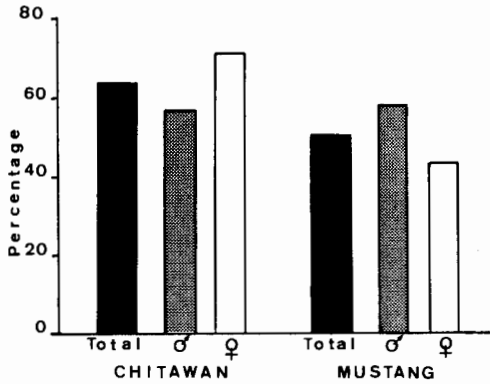


Fig 2—Incidence rate of human toxoplasmosis in Chitawan and Mustang districts in Nepal.

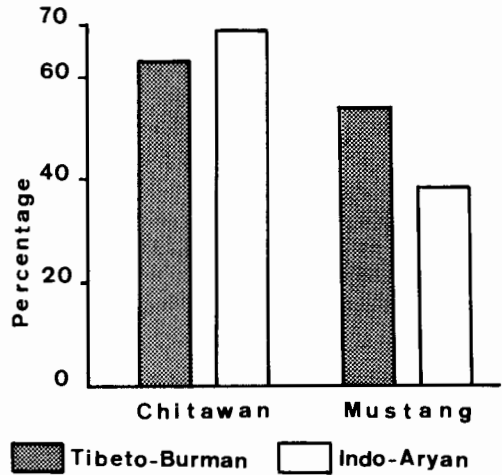


Fig 4—Incidence rate of human toxoplasmosis among two ethnic groups in Chitawan and Mustang districts in Nepal.

1 : 16 were considered to be positive. Samples showing titers of 1 : 16 were re-tested for confirmation and only samples showing a consistent titer were regarded as positive. ELISA tests were performed manually and the results were read in ELISA reader (Sanko Junyaku Co Ltd, Japan: Model ER-8000). The ELISA values were expressed in terms of enzyme immunoassay unit (EIU) and values of more than 20 EIU were regarded as positive for IgM toxoplasma antibodies.

**SDS-PAGE and Western blotting:** All IgM positive samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970) using five molecular weight standards namely, phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), trypsin inhibitor (20 kDa), and  $\alpha$ -lactalbumin (14 kDa) (Pharmacia

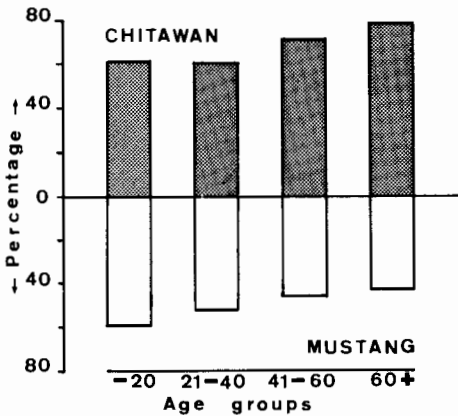


Fig 3—Incidence rate of human toxoplasmosis among various age-groups in Chitawan and Mustang districts in Nepal.

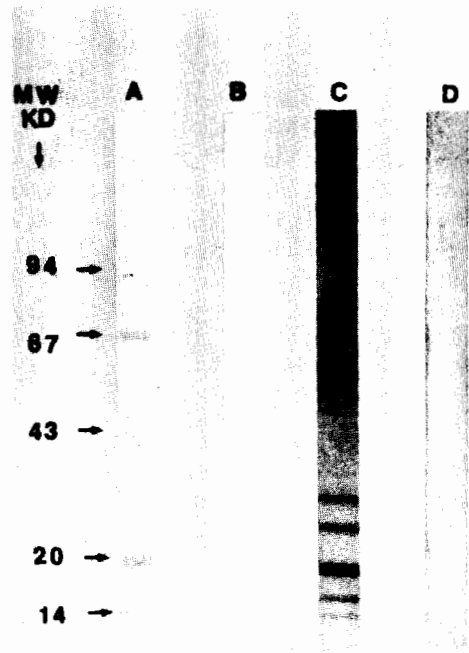


Fig 5—Result of Western blotting. A: Molecular markers, B: Sonicated *T. gondii* (RH) tachyzoite antigen (Both A and B stained with Coomassie Brilliant Blue), C: *T. gondii* (RH) tachyzoite antigens recognized by toxoplasma antibody positive human serum (MLAT titer-1 : 512) and D: IgM positive human serum - No *T. gondii* antigens detected on Western blotting.

AB, Uppsala, Sweden) and sonicated toxoplasma antigen prepared from *T. gondii* (RH) tachyzoite harvested from ICR mice infected three days earlier, and were blotted on polyvinylidene difluoride (PVDF) microporous membrane (Millipore Corporation, Bedford, MA, USA) using electro-blotter JN5EB-15 (Joko Corporation, Japan). Western blotting was performed as described by Huskinson *et al* (1989) using biotin conjugated anti-human goat IgG and streptavidin-peroxidase (Zymed Laboratories Inc, San Francisco, USA).

**Data analysis:** The findings were stratified against the study area, and age, sex and ethnicity of study population. Chi-square test was applied to check the statistical differences.

## RESULTS

In the present study, and overall positive rate of toxoplasmosis was found to be 57.9% (175/302). The positive rate in Chitawan and Mustang districts were observed to be 64.1% (102/159) and 51.0% (73/143), respectively, and the difference was statistically significant ( $p < 0.05$ ) (Table 1; Fig 2). In Chitawan, females showed higher positive rates (71.2%) compared to males (56.9%) but reverse was true in Mustang (Male: 57.8% and female: 43.3%) (Table 1; Fig 2). However, these differences were not significant ( $p > 0.05$ ). The positive rate was observed to be almost in equal rate among the males in both Chitawan (56.9%) and Mustang (57.8%) districts (Table 1; Fig 2). The positive rate among females in Chitawan was significantly higher (71.2%) compared to their counterparts in Mustang (43.3%) ( $p < 0.001$ ) (Table 1; Fig 2).

In both the study areas, the age-group of less than 20 years showed high positive rate (Chitawan: 61.4%; Mustang: 59.2%). The positive rate further increased with age in Chitawan reaching a peak level of 78.5% in the over 60 years age-group while a decreasing trend was observed among the inhabitants of Mustang district (Fig 3). In Chitawan district, Indo-Aryan ethnic-group showed higher positive rate (69.2%) compared to Tibeto-Burmans (63.1%). On the contrary, the positive rate was observed to be higher among Tibeto-Burmans (53.8%) compared to Indo-Aryans (38.4%) in Mustang. These differences however, were not significant ( $p > 0.05$ ). Subjects of the same ethnic group in two different geographical areas showed different positive rates (Fig 4). Tibeto-Burmans in Chitawan showed a higher positive rate (63.1%) compared to that

in Mustang (53.8%) but the difference was not significant. The difference in positive rate among Indo-Aryans in two study area (Chitawan: 69.2%; Mustang: 38.4%) was significant ( $p < 0.05$ ). Interestingly, 2.9% and 1.3% of positive samples from Chitawan and Mustang respectively showed toxoplasma IgM antibody. IgM positive samples however, did not show toxoplasmic antigen on SDS-PAGE and Western blotting (Fig 5).

## DISCUSSION

In the present study, the overall positive rate was found to be 57.9%. Study area-wise, the positive rate was found to be significantly higher (64.1%) in Chitawan district (a plain area of less than 1,000m altitude) compared to that in Mustang district (51.0%) (a remote mountain district located at more than 3,000m altitude). Both of these findings were higher than that of reported earlier (48.6%) from a community in eastern Terai in Nepal (Rai *et al*, 1989). In our previous study, we collected samples from parts of Sunsari and Morang districts. Both the previous study area and Chitawan district lies in the Terai plain region of less than 1,000m altitude and have good access road links. However, Chitawan is a relatively newly inhabited area where people from all 75 districts of the country have migrated and settled. The relatively recent migration of inhabitants might have partially contributed to the high positive rate in Chitawan as has been reported earlier elsewhere in the world (Gille *et al*, 1992; Nishri *et al*, 1993). In addition, the Indo-Aryans in Chitawan district are not orthodox in their traditional culture of being either vegetarian or eating only mutton (Rai *et al*, 1989). Therefore, in addition to mutton most of them take chicken, beef as well as pork. Also they take meat as frequently as Tibeto-Burmans (Rai *et al*, 1989). However, this trend is now being seen in other parts of the country as well. Of these four types of meat, mutton and pork are considered to be good sources of *T. gondii* infection (Feldman, 1982). In part, this also might have contributed to the overall high positive rate found in Chitawan district.

On the other hand, the positive rate in Mustang was significantly low (51.0%) compared to Chitawan (64.1%). Mustang district is one of the remote districts having no access to road links and is inhabited mainly by Thakalis (who originally settled near a small river Thank khola; and are Tibeto-Burmans). The high

altitude (more than 3,000m) and mountainous rural setting might have partially contributed to the low positive rate. However, this along with other contributing factors remains to be elucidated. About 60% subjects of less than 20 years in both study areas showed antibody positivity indicating that the infection in these areas was taking place at an early stage of life. The positivity was seen to be increased with age in Chitawan with a highest rate of 78.5% in the more than 60 years age-group. This finding is in agreement with our previous finding in eastern Terai, except the low positive rate in the less than 20 years age-group (Rai *et al*, 1989). In contrast to these findings, the positive rate in Mustang was observed to be decreased with the increase of age. This finding was not in agreement with previous reports from Nepal (Rai *et al*, 1989) and elsewhere in the world (Tizard *et al*, 1977; Van der Veen and Polack, 1980; Sousa *et al*, 1988). The various factors contributing in this regard remain to be studied. However, our present findings revealed that the toxoplasmosis in these two study areas is widely spread. Keeping in view the opportunistic nature of the parasite, the increasing number of cases of malignancy, use of corticosteroids and cytotoxic drugs, radiation therapy, and more importantly, spread of HIV infection, it is likely to result in a serious health/economic problem in Nepal in days to come.

Nepal is well-known for its multi-ethnic inhabitants. Broadly, they can be grouped in to two groups: Tibeto-Burman (characterized by round face, small eyes, blunt nose and no facial hairs) and Indo-Aryan (characterized by oval face, big eyes, pointed nose and beard). Although insignificantly, Tibeto-Burmans in Mustang district showed a higher positive rate (53.8%) compared to Indo-Aryans (38.5%). This finding was in agreement with our previous report (Rai *et al*, 1989) and is attributed to the socio-cultural factors of these two ethnic groups. However, it was interesting to observe a higher positive rate among Indo-Aryans (69.2%) compared to Tibeto-Burmans (53.8%) in Chitawan district. This might be attributed to their changing meat eating habits and relatively recent migration to this place. The Tibeto-Burmans in Chitawan district showed a higher positive rate (63.1%) compared to their counterparts in Mustang (53.8%). However, the difference was not significant. On the contrary, the higher positive rate among Indo-Aryans (69.2%) in Chitawan district was significantly higher compared to the same ethnic group living in Mustang (38.4%). This also can be explained on the basis of their relatively recent migration and changing food habits of Indo-Aryans inhabiting in Chitawan district.

Interestingly enough, 2.9% and 1.3% of positive samples from Chitawan and Mustang districts respectively showed toxoplasma IgM antibodies at significant level (more than 20 EIU). In cases of acute toxoplasmosis, *T. gondii* antigen can be detected in blood (Van Knapen *et al*, 1977) as well as in urine samples from patients with AIDS and experimentally infected mice (Huskinson *et al*, 1989). However, all IgM positive sera may not be positive for toxoplasmic antigen. Van Knapen *et al* (1977) detected toxoplasmic antigen only in 5.7% of serum samples collected from suspected cases of acute toxoplasmosis, while Araujo and Remington (1980) found 63.6% positivity in samples collected from recently acquired cases of acute toxoplasmosis. Low detection rate of toxoplasmic antigen however, could be attributed to its intermittent circulation in the blood (Araujo and Remington, 1980). Since our study population were apparently healthy, the findings indicated that a small portion of apparently healthy subjects in the community normally possess toxoplasma IgM antibodies which can be naturally occurring IgM antibodies as described by Konishi (1991). Keeping in view present findings we are presently undertaking further study in this regard, taking samples from various other parts of Nepal.

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