SEROEPIDEMIOLOGICAL STUDY OF TOXOPLASMOSIS IN TWO DIFFERENT GEOGRAPHICAL AREAS IN NEPAL

Shiba Kumar Rai 1 , Hiroshi Shibata 1 , Katsumi Sumi 1 , Koji Kubota 1 , Kazuko Hirai 2 , Akira Matsuoka 1 , Takashi Kubo 3 , Toshihide Tamura 4 , Shital Raj Basnet 5 , Hari Govinda Shrestha 3 and Ramesh Chander Mahajan 6

1Department of Clinical Laboratory, Hyogo College of Medicine Hospital, Nishinomiya-shi, Hyogo, Japan; 2Department of Nutritional Biochemistry, Osaka City University, Osaka, Japan; 3Department of Pathology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal; 4Department of Bacteriology, Hyogo College of Medicine, Nishinomiya-shi, Hyogo, Japan; 5Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal; 6Department of Parasitology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

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 western hemisphere (Luft et al., 1983). It has been estimated that 25 - 50% of AIDS patients ultimately develop toxoplasmic encephalitis in western hemisphere (Luft et al., 1983). 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
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.

### Materials and Methods

**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°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>
<thead>
<tr>
<th>Study areas</th>
<th>Sex</th>
<th>n</th>
<th>+ve n (%)</th>
<th>Ethnic group</th>
<th>n</th>
<th>+ve n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitawan</td>
<td>M</td>
<td>79</td>
<td>45/79 (56.9)</td>
<td>Tibeto-Burman</td>
<td>133</td>
<td>84/133 (63.1)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>80</td>
<td>57/80 (71.2)</td>
<td>Indo-Aryan</td>
<td>26</td>
<td>18/26 (69.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>159</td>
<td>102/159 (64.1)</td>
<td>Total</td>
<td>159</td>
<td>102/159 (64.1)</td>
</tr>
<tr>
<td>Mustang</td>
<td>M</td>
<td>76</td>
<td>44/76 (57.8)</td>
<td>Tibeto-Burman</td>
<td>117</td>
<td>63/117 (53.8)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>67</td>
<td>29/67 (43.3)</td>
<td>Indo-Aryan</td>
<td>26</td>
<td>10/26 (38.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>143</td>
<td>73/143 (51.0)</td>
<td>Total</td>
<td>143</td>
<td>73/143 (51.0)</td>
</tr>
<tr>
<td>Grand total</td>
<td></td>
<td>302</td>
<td>175/302 (57.9)</td>
<td>Grand total</td>
<td>302</td>
<td>175/302 (57.9)</td>
</tr>
</tbody>
</table>
Fig 2—Incidence rate of human toxoplasmosis 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 α-lactalbumin (14 kDa) (Pharmacia

Fig 3—Incidence rate of human toxoplasmosis among various age-groups 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.

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 JNSEB-15 (Jooko 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
alitude (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.

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.

ACKNOWLEDGEMENTS

Prof Takeo Matsumura (Head of the Department) and Dr Shoji Uga, Department of Medical Zoology, Kobe University School of Medicine, Kobe, Japan kindly provided T. gondii (RH) and permitted us to use necessary facilities during the preparation of toxoplasmic antigen used in this study.

REFERENCES


Van der Veen J, Polack MF. Prevalence of Toxoplasma antibodies according to age with comments on the risk of prenatal infection. J Hyg Camb 1980; 85 : 165-74.