SEROEPIDEMIOLOGICAL STUDY OF *TOXOPLASMA* INFECTION IN CENTRAL AND WESTERN REGIONS IN NEPAL

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Abstract. The present study was carried out to ascertain the seroprevalence rate in different geographical areas in Central and Western Regions in Nepal. A total of 1,237 serum samples collected from Nuwakot (217), Kathmandu valley (402) and Chitawan (159) districts in Central Region, and Mustang (143), Surkhet (64) and Banke (252) districts in Western Region in Nepal were included in this study. *Toxoplasma* antibodies were detected by micro-latex agglutination (MLA) and enzyme linked immunosorbent assay (IgM-ELISA) methods. The seropositive rate in Central and Western Regions were found to be 48% and 49%, respectively; with an overall positive rate of 48 percent. Districtwise, the seropositive rate in Nuwakot, Kathmandu valley, Chitawan, Mustang, Surkhet and Banke districts were 38, 46, 64, 51, 67 and 44%, respectively. Interestingly, the relatively newly inhabitated Surkhet district in Western Region and Chitawan district in Central Region showed significantly higher seropositive rate compared with those of two other districts in the respective Regions ($p<0.05$). Ethnically, Tibeto-Burmans showed higher seropositive rates in Central Region ($p>0.05$). In contrast, Indo-Aryans showed higher seropositive rate in Western Region ($p>0.05$). Age related increase in seropositivity was observed only in Central Region. One percent of *Toxoplasma* antibody positive samples also showed *Toxoplasma* IgM antibody positivity.

INTRODUCTION

Toxoplasma gondii is an ubiquitous coccidian parasite infecting about half of the world population. The infection rate, however, vary greatly with the geographical area, study population and their customs, cat population and climate. The reported prevalence of *T. gondii* infection among general population elsewhere in the world varies from none in Eskimos and Taiwanese aborigines (Feldman, 1982) to as high as 94% in Costarican and Guatemalan people (Gibson and Coleman, 1958). Similarly, the clinical manifestations vary from mild flu-like symptoms to severe and fatal toxoplasmic encephalitis (Luft *et al*, 1983; Levey *et al*, 1985; Luft and Remington, 1988; Luft and Remington, 1992) including abortion, still birth and congenital abnormalities. Severe and fatal infections occur mainly in immunocompromised hosts. *Toxoplasma* parasites thus remained in the obscurity in the minds of most physicians, veterinarians, research scientists and economists for many decades. During recent years, however, it has emerged as one of the important human pathogen, particularly due to the pandemic spread of human immunodeficiency virus (HIV) infections. Epidemics of severe and fatal toxoplasmic encephalitis in HIV infected patients in Europe and North America has also been reported (Luft *et al*, 1983). According to Luft and Remington (1992) about 25-50% of acquired immunodeficiency syndrome (AIDS) patients in areas having high seroprevalence rate develop toxoplasmic encephalitis. Therefore, diagnosis of toxoplasmosis alone leads, in turn, to the diagnosis

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of approximately 5% of new cases of AIDS (Fauci *et al*, 1985). In United States of America, the *Toxoplasma* associated annual economic public health burden reportedly cost more than 400 million US dollars (Murrell, 1993).

Few reports on seroprevalence of *T. gondii* infection in Nepal are available (Rai *et al*, 1989; 1993; 1994a,b; 1995; Upadhyay *et al*, 1989). Previously, we reported a seroprevalence rate of 51% and 64% in Mustang district in Western Region and Chitawan district in Central Region, respectively (Rai *et al*, 1994). The significantly higher prevalence rate in relatively newly inhabitated Chitawan district encouraged us to study further with the inclusion of more other districts of same Region including yet another relatively newly inhabitated Surkhet district in Western Region to confirm the higher positive rate among immigrants as have been reported from elsewhere in the world (Gille *et al*, 1992; Nishri *et al*, 1993). In the present study, we have included three districts from each of Central (having relatively more educational and other facilities), and Western (having relatively less educational and other facilities) Regions (Fig1) along with the previously reported Mustang and Chitawan districts in Nepal.

MATERIALS AND METHODS

Sample collection

A total of 1,237 serum samples were collected from six districts belonging to Central and Western Regions in Nepal (Fig 1). Blood samples were collected from healthy subjects by venipuncture using disposable syringe (Terumo Co, Japan) by field visit except in Kathmandu valley. In Kathmandu valley, blood samples were collected from blood donors. Age,sex and ethnicity of each subject included in this study were noted. Nuwakot district in Central Region and Banke district in Western Region were represented by subjects of less than twenty years of age. Serum samples in the field were separated as previously described by us (Rai *et al*, 1994a). At first, sera were transported to Tribhuvan University Teaching Hospital and stored at-70°C till they were transported to Department of Clinical Laboratory, Hyogo College of Medicine, Nishinomiya, Japan for analysis.

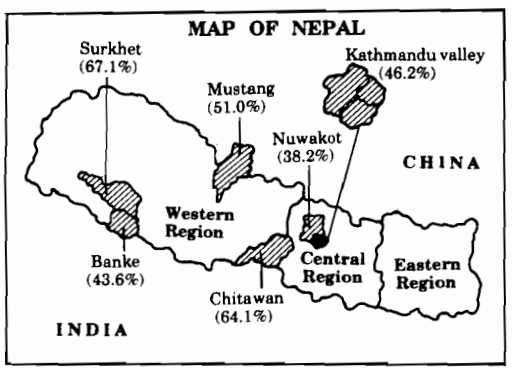

Fig 1 – Study areas with seroprevalence rate in Nepal.

Antibody detection

Toxoplasma antibodies were detected by microlatex agglutination (MLA) test using commecial reagents (Eiken Chemicals Co Ltd, Japan). A twofold dilution of serum samples was made in the serum diluent using U type microtiter plates (Dynatech Laboratories, USA) as described by the manufacturer. Titer of 1:16 or more than 1:16 were regarded as positive. All MLA positive samples were subjected to *Toxoplasma* IgM antibody detection by Toxo-IgM ELISA (Labsystem, Finland). Serum samples were pre-treated as described by the manufacturer. ELISA was performed manually and the absorbance was read at 405 nm (Model ER-2000, Sanko Junyaku Co Ltd, Japan). The ELISA values were expressed as enzyme immunoassay unit (EIU). Samples showing 20 or more than 20 EIU were regarded as positive for *Toxoplasma* IgM antibody.

Data analysis

The results were stratified against the study areas and age, sex and ethnicity of the study populations. Chi-square test was applied to check the significant differences.

RESULTS

Of the total 1,237 samples studied, 597 (48%) were positive for *Toxoplasma* antibody by MLA test. No difference in positive rate was observed in Central and Western Region (Table 1). However, a significant difference was found in districts within the Regions ($p<0.05$) (Table 2). In both the Re-

Table 1

Toxoplasma seroprevalence in central and Western Regions in Nepal.

Region	Sex	Total No.	Pos No. (%)	Ethnic group	Total No.	Pos No. (%)
Central Region	M	619	288 (47)	Tibeto-Burman	464	230 (46)
	F	159	83 (52)	Indo-Aryan	314	141 (45)
	Total	778	371 (48)	Total	778	371 (48)
Western Region	M	274	133 (49)	Tibeto-Burman	195	92 (47)
	F	185	93 (50)	Indo-Aryan	264	134 (51)
	Total	450	226 (49)	Total	450	226 (49)
Grand total		1,237	597 (48)	Grand total	1,237	597 (48)

Table 2

Toxoplasma seroprevalence in different districts in Central and Western Regions in Nepal.

Central Region			Western Region		
Study area	Total No.	Pos No. (%)	Study area	Total No.	Pos No. (%)
Nuwakot	217	83 (38)	Mustang	143	73 (51)
Kathmandu #	402	186 (46)	Surkhet	64	43 (67)
Chitawan	159	102 (64)	Banke	252	110 (44)
Total	778	371 (48)	Total	459	226 (49)

Kathmandu valley

gions, the relatively newly inhabitated districts namely, Chitawan (in Central Region) and Surkhet (in Western Region) showed significantly higher seropositive rate (64 and 67%, respectively) compared with other districts in respective regions ($p<0.05$). A higher positive rate was observed among females compared with their counterpart males (Table 1). Ethnically, Tibeto-Burmans showed higher positive rate (50%) than in Indo-Aryans (45%) in Central Region (Table 1). In contrast, the Indo-Aryans showed higher positive rate (51%) in Western Region compared with their Tibeto-Burman counterparts (47%). However, these differences were not significant ($p>0.05$). In both study areas, more than 40% of subjects of less than 20 years of age were *Toxoplasma* antibody positive. A steady increase in seroprevalence rate was observed in Central Region but the same was not true for Western Region (Table 3). Of the total 597 MLA test positive sera, 8 (1%) were positive for *Toxoplasma* IgM antibody. All of those *Toxoplasma* IgM positive sera had MLA titer of 1:512 or higher than 1:512.

DISCUSSION

In the present study, we used MLA test because of its simplicity and qualitative agreement with dye test (DT) (Kobayashi *et al*, 1977), and good corre-

Table 3

Toxoplasma seroprevalence in different age-groups in Central and Western Regions in Nepal.

Study area	-20 %	21-40 %	41-60 %	60+ %
Central Region	43	53	70	79
Western Region	46	60	52	43
Total	44	55	58	61

lation with ELISA (Lappin and Powel, 1991; Rai *et al*, 1994b) and direct agglutination (DA) test (Rai *et al*, 1994b). Moreover, MLA test reagents manufactured by Eiken Chemicals Co, Japan detects both *Toxoplasma* IgM and IgG antibodies (Rai *et al*, 1994b).

Almost a half of the populations studies showed *Toxoplasma* antibodies and correlated well with that of Eastern Region (Rai *et al*, 1989; 1995). Our present observations in Central and Western Regions in Nepal were not influenced by educational and other facilities, revealed a wide distribution of *T. gondii* in Nepal irrespective of its vast geo-topographic and climatic differences. In Nepal, females perform both indoor and outdoor work during which they are more likely to be exposed to the *Toxoplasma* oocysts as the cats defecate both inside the house (for example in grain storage) as well as in the field. However, no significant difference in seroprevalence was observed in males and females. This observation was in agreement with our previous finding in Eastern Region (Rai *et al*, 1989; 1995).

Toxoplasma infections reportedly vary with ethnicity (Jacobs and Mason, 1977; Saleha, 1984) and is attributed to their traditional culture and food habits. The two major ethnic groups namely the Tibeto-Burmans and Indo-Aryans (Woolley *et al*, 1984) in Nepal have differences in their tradition, culture and food habits. Tibeto-Burmans consume various types of meat including the pork, and also more frequently than those Indo-Aryans. On the other hand, Indo-Aryans are either vegetarian or consume only mutton. Therefore, the high *Toxoplasma* seropositive rate in Tibeto-Burmans, in part, appeared to be atributed to the very high *Toxoplasma* seropositive rate in pigs (unpublished data). However, a relatively high seropositive rate in Tibeto-Burmans was true only in Central Region. This finding correlated well with the findings reported earlier (Rai *et al*, 1989; 1993; 1995). On the other hand, Indo-Aryans in Western Region had higher positive rate compared with their Tibeto-Burman counterparts. This finding indicated that there were some other factors other than the type of meat consumed in Western Region in Nepal.

The seroprevalence rate in Chitawan district (64%) in Central Region and Surkhet district (67%) in Western Nepal were significantly higher compared with those of other two districts in respective Regions. Both of these districts are relatively newly inhabitated by immigrants from other parts of country. It is noteworthy that the Chitawan district is inhabitated by immigrants from all 75 districts of the country. In addition, few immigrants from Darjeeling district of India have also been settled. Therefore, the hight seroprevalence of *Toxoplasma* infection in these two districts appeared to be attributed to the migration and change in meat eating habits of inhabitants. Indo-Aryans in these two districts are not orthodox in their traditional culture of being either vegetarian or eating only mutton (Rai *et al*, 1989), and most of them take chicken, buff (buffalo meat) as well as pork. Furthermore, they take meat as frequently as the Tibeto-Burmans. This correlated well with the high *Toxoplasma* seroprevalence rate in meat animals in Nepal (unpublished data). Gille *et al* (1992) and Nishri *et al* (1993) have also reported high seropositive rate in immigrants. The significantly low positive rate in Nuwakot district in Central Region and Banke district in Western Region could be due to the inclusion of subjects only of less than 20 years of age.

Toxoplasma seroprevalence increased with age in Central Region as have been reported by us from Nepal (Rai *et al*, 1989; 1995) and from elsewhere in the world (Tizard *et al*, 1979; Sousa *et al*, 1988). This was, however, not true in Western Region for which we do not have an explanation. However, a considerably high seroprevalence rate (44%) observed in the age-group of less than 20 years indicated that the majority of *T. gondii* infections in these areas occurred during early age. A high seroprevalence rate in children has also been reported from elsewhere in the world by other investigators (Stagno and Thiermann, 1973).

One percent of *Toxoplasma* antibodies positive sera also showed IgM antibodies indicating recently acquired *Toxoplasma* infection. All of these IgM positive sera had MLA titer of 1:512 or higher than 1:512. On the other hand, it can also be a naturally occurring *Toxoplasma* IgM antibodies as has been reported by Konishi (1991).

Present study revealed that *Toxplasma* infections were widely spreaded in Nepal irrespective of its vast differences in geo-topography, ehtnicity and climatic conditions. This study also revealed that most of the *Toxoplasma* infections in Nepal occur during early age. Keeping in view of the opportunistic nature of *Toxoplasma* and the increasing number of immunocompromised patients particularly due to HIV infections, appropriate measures to combat the *Toxoplasma*-associated morbidity, mortality and economic burden in Nepal in future are indicated.

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