# SEROEPIDEMIOLOGICAL STUDY OF *TOXOCARA* INFECTION IN NEPAL

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**Abstract.** Seroepidemiological study of *Toxocara* infection in Nepalese people aged more than fourteen years was carried out by enzyme linked immunosorbent assay (ELISA) using *T. canis* soluble embryonated egg antigen. Of the total 200 subjects included in this study, 162 (81%) were found to possess antibodies to *Toxocara* spp. Males showed higher (85%) antibody positive rates than females (77%). Inhabitants of Kathmandu valley showed higher antibody positive rates (84%) compared with those living outside of the valley (78%). These differences, however, were not significant statistically ($p > 0.05$). Ethnically, Newar, Rai/Limbu, Tamang/Sherpa, Brahmin/Chhetri, Jha/Yadavs, and others (Kami, Damai) showed *Toxocara* antibody positive rates of 85, 85, 83, 70, 77 and 100%, respectively. Half of the *Toxocara* antibody positive subjects (49%) showed an elevated level (two plus) of antibody.

## INTRODUCTION

*Toxocara canis* and *T. cati* are roundworms of dogs and cats, respectively and are distributed all over the world. Three to 81% of dogs and cats elsewhere in the world are reportedly infected by *Toxocara* spp. The infected dogs and cats contaminate the environment (garden, fields, play ground, public park and sandpits) with *Toxocara* eggs, where they develop into an infective form. In public gardens, rest grounds of drive-in restaurants, playgrounds of kindergartens and sandpits in public parks the contamination rate by *Toxocara* eggs in different parts of the world ranges from 13% to as high as 92% (Uga, 1993). These nematodes can infect various animals including man. *Toxocara* in animals other than the dogs and cats, however, cannot develop into the adult worm and remains restricted to the larval form. The migrating larvae cause extensive damage in the organs involved and the condition is known as larva migrans, characterized by various clinical manifestations.

The nematode endophthalmitis described by Wilder in 1950 constitutes the first reported case of human toxocariasis. The nematode observed by Wilder (1950) in histopathological section of the enucleated eye was, later on, identified as *Toxocara canis* by Nichols (1956). Since then numerous reports of human toxocariasis have been occurred from elsewhere in the world and have been divided into visceral larva migrans (VLM) and ocular larva migrans (OLM). The migrating larvae both in visceral organs and the eye cause extensive damage that gives rise to a syndrome with various clinical manifestations including death due to encephalitis with seizure (Moore, 1962; Mikhael *et al*, 1974; Hill *et al*, 1985).

*Toxocara* infection in man takes place by ingestion of embryonated eggs of *T. canis* and *T. cati*. Children with the habit of pica or geophagy, dog breeders (Glickman and Schantz, 1981; Worley *et al*, 1984; Thompson *et al*, 1986) and hydatid control workers (Clemett *et al*, 1985) are more likely to be infected. *Toxocara* infection is more prevalent in less developed tropical countries (Thompson *et al*, 1986; Schantz, 1989; Uga *et al*, 1996) and is attributed to poor sanitary facilities and lack of public health awareness. The seroprevalence rates of *Toxocara* infection elsewhere in the world vary from less than 10% (Herrmann *et al*, 1985; Schantz, 1989; Uga *et al*, 1990) to more than 80% in certain developing countries (Thompson *et al*, 1986). Pollard *et al* (1979) have reported a seropositive rate of 90% in patients suspected of having clinical toxocariasis. No such data are available from Nepal to

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date. However, *Ascaris lumbricoides* and *Trichuris trichiura*, which share a common mode of infection with *Toxocara* spp are the most prevalent geohelminths in Nepal (Rai and Gurung, 1986; Rai *et al*, 1994). There is therefore, likely to be a high rate of *Toxocara* infection in Nepal. This study examines the seroprevalence rate of *Toxocara* infection in Nepal.

## MATERIALS AND METHODS

### Sample collection

Blood samples were collected at the out patient sample collection table of Department of Pathology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Approximately 2.0 ml blood samples were collected from each of the subjects (having no clinical diagnosis of fever, anemia, hepatitis or jaundice) in a separate disposable tube while collecting their blood samples for other routine examination and were allowed to clot. The serum samples separated by centrifugation were transferred into the polypropylene serum tubes and were stored at -70°C. Sera were then transported to the Department of Medical Zoology, Kobe University School of Medicine, Kobe, Japan in a cool box and were examined for *Toxocara* antibodies.

### Preparation of *Toxocara* antigens

*Toxocara* ova were obtained by dissecting the gravid female *T. canis* taken from the intestines of dogs. The ova were suspended in 1% formalin solution and incubated at an ambient temperature for three weeks for embryonation. The embryonated eggs were then sonicated using an ultrasonicator (Ultrasonic Generator, Model US-50, Nihonseiki Kaisa Ltd (Japan) for 5 minutes at 4°C. The protein concentration was measured by Lowry's method (Lowry *et al*, 1951).

### Measurement of *Toxocara* antibody:

Antibodies to *Toxocara* were measured by enzyme linked immunosorbent assay (ELISA). The flat bottom microtiter plates (Dynatech Laboratories, USA) were sensitized with 10 μg/ml of soluble sonicated embryonated egg *(T. canis)* antigen. This was first allowed to react with the test sera along with the positive and negative controls (Uga *et al*, 1990) at a dilution of 1 : 100 followed by peroxidase conjugated anti-human rabbit IgG (DAKOPATTS, Denmark) at a dilution of 1 : 1,000. Subsequently, the enzyme activity in the ELISA plate was measured with 2, 2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma, USA). The absorbance at 405 nm was measured in an ELISA reader (Model-2550, Bio-Rad, USA). Each of the test sera was tested in duplicate and the mean absorbance (A) was taken. The absorbance was expressed as the ratio of $A_{405}$ of test sera/mean $A_{405}$ of negative control sera (T/N) (Inoue and Tsuji, 1989). The negative controls were prepared by treating the pooled sera with excretory-secretary *Toxocara* antigen (Uga *et al*, 1990). T/N values of less than 3.3 were regarded as negative, those of 3.3 and more than 3.3 to 11.4 as positive (one plus) and those of more than 11.4 as strongly positive (two plus).

### Data analysis

The results were stratified against the sex, inhabiting area and ethnicity of the study population. Significant difference was calculated using chi-square test.

## RESULTS

### Study population

Of the total 200 subjects above the age of 14 years attending the Out Patient Department of TUTH, 95 were males and 105 females. Ninety subjects were inhabitants of Kathmandu valley while the remaining 110 were from outside of Kathmandu valley. Ethnically, Newar, Rai/Limbu, Tamang/Sherpa, Brahmin/Chhetri, Jha/Yadav and others (Kami, Damai) were 40, 40, 40, 40, 31 and 9, respectively.

The overall *Toxocara* antibody positivity was found to be 81% (162/200) (Table 1). Males showed a higher seropositive rate (85%) than females (77%) ($p > 0.05$). A higher seropositive rate was observed in the inhabitants of Kathmandu valley (84%) compared to those living outside of Kathmandu valley (78%) ($p > 0.05$) (Table 2). Ethnically, Newar, Rai/Limbu, Tamang/Sherpa, Brahmin/Chhetri, Jha/

Table 1

Seroprevalence of *Toxocara* infection in Nepal.

| Sex | Total no. | Positive no. | (%) |
|---|---|---|---|
| Male | 95 | 81 | 85 |
| Female | 105 | 81 | 77 |
| Total | 200 | 162 | 81 |

Table 2

Seroprevalence of *Toxocara* infection in two different geographical areas in Nepal.

| Study area | Total no. | Positive no. | (%) |
|---|---|---|---|
| Kathmandu valley | 90 | 76 | 84 |
| Outside of Kathmandu valley | 110 | 86 | 78 |
| Total | 200 | 162 | 81 |

Yadav and others (Kami/Damai) showed a *Toxocara* antibody positive rate of 85, 85, 83, 70, 77 and 100%, respectively ($p > 0.05$) (Table 3). Half of the *Toxocara* antibody positive subjects (49%) showed an elevated level (two plus) of antibody.

## DISCUSSION

In this study, a very high *Toxocara* antibody positive rate (81%) was observed. This clearly showed the extent of *Toxocara* infection among Nepalese people. Furthermore, half of the *Toxocara* antibody positive study population showed an elevated (two plus) antibody level. This finding correlated well with the high prevalence of geohelminths, particularly the *A. lumbricoides* (Rai and Gurung, 1986; Rai *et al*, 1994) which share the similar mode of infection with *Toxocara* spp. High seroprevalences of *Toxocara* infection have also been reported from other developing countries elsewhere in the world. The *Toxocara* seroprevalence rates in Taiwan (Schantz, 1989), Puerto Rico (Schantz, 1989), Indonesia (Uga *et al*, 1995), Colombia (Schantz, 1989) and St Lucia (Thompson *et al*, 1986) were 51%, 54%, 63% 68% and 86%,

Table 3

Seroprevalence of *Toxocara* infection in different ethnic groups in Nepal.

| Ethnic group | Total no. | Positive no. | (%) |
|---|---|---|---|
| Newar | 40 | 34 | 85 |
| Rai/Limbu | 40 | 34 | 85 |
| Tamang/Sherpa | 40 | 33 | 83 |
| Brahmin/Chhetri | 40 | 28 | 70 |
| Jha/Yadav | 31 | 24 | 77 |
| Others* | 9 | 9 | 100 |
| Total | 200 | 162 | 81 |

* Kami and Damai

respectively. All of these investigators have correlated the high seroprevalence rate with poor sanitary conditions, lack of health education, presence of large number of stray dogs and cats and heavy contamination of environment by *Toxocara* eggs. Similar conditions exist in Nepal except that the environmental contamination rate by *Toxocara* eggs has not been investigated yet.

In the present study, though not significantly, males showed higher seroprevalence rate (85%) compared with their female counterparts (77%), the reason for which is not clearly understood. Havasiova *et al* (1993) from Slovak Republic have also reported a higher seroprevalence rate in adult males than in females. The overall seropositive rate in their study, however, was only 14%. Hakim *et al* (1992) also observed no significant relationship between *T. cani* antibody positive rates and sex among Orang Asli aborigines in Peninsular Malaysia. Our present study revealed a wide distribution of *Toxocara* infection both in and outside Kathmandu valley. *Toxocara* infection is reportedly high among inhabitants of rural areas with low educational and socio-economic status (Lynch *et al*, 1988; Havasiova *et al*, 1993). In contrast, inhabitants of Kathmandu valley, where the capital city is located, showed higher prevalence rate (84%) than those living outside Kathmandu valley (78%). The higher *Toxocara* seroprevalence in Kathmandu valley appears to be attributed to the presence of large number of stray dogs and cats, a poor sanitary disposal system, ever increasing population density and environmental pollution, and other suitable environmental conditions including

topography of the valley for the survival and embryonation of *Toxocara* eggs. Moreover, the raw buffalo meat dish *(Kachila)* popular among the Newar community in Kathmandu valley also might have contributed to the higher *Toxocara* antibody positive rate.

*Toxocara* infections have also been reported to vary with ethnicity of the study population. Blacks in United States of America (Herramann *et al*, 1985) and Amazon Indians in Venezuela (Lynch *et al*, 1988) reportedly have higher seroprevalence rates. Keeping in view the multi-ethnicity of the Nepalese population we analyzed the antibody positive rate for different ethnic groups included in this study. We did not find any significant differences in the seropositive rates in various ethnic groups included in this study. However, a slightly higher positive rate was observed in ethnic groups belonging to Tibeto-Burman race and lower caste people such as Kami and Damai compared with the Indo-Aryans.

Our present findings have clearly shown a very high rate of *Toxocara* infection among adult populations and its public health importance in Nepal. Considering the present environmental pollution in Nepal, particularly in Kathmandu valley, the infection rate in children must be much higher, as has been reported in Caribbean children of less than 6 years (86%) (Thompson *et al*, 1986). Furthermore, as VLM has been implicated as one of the cause of human morbidity, as well as mortality, particularly in children (Moore, 1962; Mikhael *et al*, 1974; Hill *et al*, 1985), *Toxocara* infection in Nepal, in part, might be contributing to the high child morbidity and mortality rate (Thapa and Rutherford, 1982). However, this remains to be elucidated. Nevertheless, the eradication of stray dogs and cats, education of dog and/or cat owners and the application of health education and hygienic practice appears to be the most urgent need in combating toxocariasis and other dog and cat associated zoonotic diseases in Nepal.

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