

## Serodiagnosis of Toxoplasmosis using Lateral Flow Chromatographic Immunoassay among Animals and Humans in Sunsari District of Nepal

R. P. Sah<sup>1\*</sup>, Md. H. Talukder<sup>2</sup> and A. K. M. A. Rahman<sup>3</sup>

<sup>1</sup>Agricultural Research Station, Pakhribas, Nepal Agricultural Research Council, Nepal

<sup>2</sup>Department of Parasitology, Bangladesh Agricultural University, Bangladesh

<sup>3</sup>Department of Medicine, Bangladesh Agricultural University, Bangladesh

Corresponding author: rpsnarc@yahoo.com

### ABSTRACT

*Toxoplasmosis is a cosmopolitan parasitic zoonosis, infecting human and other warm-blooded animals worldwide. This disease has economic importance in regard to animal reproduction, and it leads to abortions and neonatal complications in humans. This study was carried out to determine the seroprevalence of Toxoplasma gondii in sheep, cattle, cats and human in Inaruwa and surrounding areas of Sunsari district, Nepal. Altogether 336 blood samples, of which 50 from sheep, 92 from cattle, 44 from cats and 150 from human were collected and tested immediately using lateral flow chromatographic immunoassay (Toxo IgG/IgM Combo Rapid test®). Associated biometric information such as age, sex, pregnancy status, occupation, association with cat was recorded and analyzed to determine the association of risk factors with the disease. Data were analyzed using R 3.2.2 (The R foundation for Statistical Computing, 2015). Seroprevalence of toxoplasmosis was detected 12.00% (95% CI: 4.53- 24.31%) in sheep, 8.70% (95% CI: 3.83- 16.42%) in cattle, 36.36% (95%CI: 22.41- 52.23%) in cats and 12.67% (95% CI: 7.80- 19.07%) in human. In case of human, 31 to 45 years age group were found more susceptible to toxoplasmosis (21.74%, OR: 6.4) in comparison to 21 to 30 years (10.0%) and up to 20 years (4.17%) age groups. Toxoplasmosis was found highly significantly associated with abortion (58.33%, OR= 15.4, P=0.0001) in human in the tested individuals. Regarding occupation, 20.83% butchers were seropositive followed by farmers (15.52%), housewives (10.0%) and diagnostic lab technicians (8.0%). Female and higher age group showed high prevalence of toxoplasmosis in all studied species. Therefore, this assay is a useful method for the serological screening of toxoplasmosis in different animals and humans.*

**Keywords:** Animals, Human, Nepal, Seroprevalence, Toxoplasmosis

## INTRODUCTION

Toxoplasmosis is a global zoonosis occurs in almost all warm-blooded animals including human beings and birds, is caused by *Toxoplasma gondii*. Based on serological investigations, it is reported that upto one third of the world's human population has been infected to this parasite (Innes, 2010; Pereira *et al.*, 2010; Montoya and Liesenfeld, 2004). The parasite is known to cause congenital disease and abortion both in humans and livestock species (Dubey and Beattie, 1988; Remington *et al.*, 2001). In most countries, toxoplasmosis comes as the second cause of abortion in prevalence after chlamydial abortion (Dubey *et al.*, 2013). Therefore, the infection has an economic and clinical significance in many sheep and goat producing countries.

A broad spectrum of animals can be infected by ingestion of raw or undercooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats (Dubey, 2004; Lake *et al.*, 2002). People usually acquire infection via ingestion of tissue cysts in undercooked meat, consuming food and water that has been contaminated with sporulated oocysts, or by accidentally ingesting oocysts from the environment, or vertically by transplacental transmission of tachyzoites. Once infected, humans may remain infected for the entire life. Clinically, patients may have headache, disorientation, drowsiness, hemiparesis, reflex changes, and convulsions and may become comatose (Dubey, 2010). Infection with *T. gondii* during pregnancy can result in fetal death, neonatal death or various congenital defects, such as hydrocephalus, central nervous system abnormalities and chorioretinitis (Zewdu *et al.*, 2013).

The majority of infections are asymptomatic and unapparent or latent, but in sheep and goat clinical toxoplasmosis most often reported. Infections during pregnancy can cause abortions, stillbirths, mummification or resorption of the fetus (Hill and Dubey, 2002). There are numerous serological procedures available for the detection of IgG and IgM antibodies; these include the Sabin–Feldman dye test (DT), indirect hemagglutination assay (IHA), indirect fluorescent antibody assay (IFA), modified agglutination test (MAT), latex agglutination test (LAT), enzyme-linked immunosorbent assay (ELISA) and complement fixation test (CFT) (Pal, 2007). For the diagnosis of *T. gondii* infection, detection of the organism itself is confirmative but very difficult.

Seroprevalence in different populations may vary according to different environments, social customs and habits. In Nepal, seropositivity rates were 57.9% (Rai *et al.*, 1994); 30.6% (Upadhyay *et al.*, 1989) and 50.6% (Jaiswal *et al.*, 2014) in human using different serological tests. Nepal is a country of vast diversification in geotopography.

The positive rate is reported to vary from place to place (Tizard *et al.*, 1977; Feldman, 1982; Suzuki *et al.*, 1987). Cats are the most popular pet in the world and are now found in almost every place where humans live (Driscoll Carlos, 2009; Sah *et al.*, 2016). No doubt Nepal has unknown number of domestic, stray and wild cats. Usually cats do not show the clinical signs even during shedding of oocysts. So, cats have a key and crucial role in the epidemiology of toxoplasmosis. Therefore, expanding the basic knowledge about *T. gondii* infection in both humans and animals in Nepal is a matter of importance. Therefore, lateral flow chromatographic immunoassay (LFCIA) (Toxo IgG/IgM Combo Rapid test<sup>®</sup>) was used in sheep, cattle, cats and humans. Prevalence of toxoplasmosis investigation is very important in Nepal for surveillance and monitoring for future planning control strategy. By considering these points, the present research work has been aimed with the following objectives: (1) To determine the seroprevalence of toxoplasmosis in sheep, cattle, cat and human in Sunsari District and (2) To identify risk factors of *T. gondii*.

## **MATERIALS AND METHODS**

### **Study Area**

Study areas lie in Sunsari district in Terai, plain region of eastern Nepal. This district is located 26.6276<sup>0</sup> N latitude and 87.1822<sup>0</sup> E longitude, having 1257 Km<sup>2</sup> area and consists of 763,487 human population with 610/Km<sup>2</sup> population density (Central Bureau of Statistics Nepal, 2014).

### **Study population and collection of samples**

Altogether 336 samples (50 sheep, 92 cattle, 44 cats and 150 human) were examined. The type of the study was cross-sectional. Random sampling method was used for sheep, cattle and cat whereas human sampling was done among risk group of people. Human blood samples were collected in Birat Diagnostic and Research Center, Inaruwa, Sunsari and few samples from home visit from fingertips for housewives and for butcher from Sunsari district. Similarly, blood samples of sheep, cattle and cats were collected from farmers' house in Inaruwa and surrounding areas and were examined at the spot.

Basic relevant history and data like age, sex, status of body condition- pregnant, aborted, non-pregnant, availability of cats, occupation of human and cooking method of meat etc were also taken from patient attendants both from human and animals during blood collection.

### Lateral flow chromatographic immunoassay (LFCIA)

For rapid test, Toxo IgG/IgM Combo Rapid Test®, CTK Biotech USA was used. This kit is a lateral flow chromatographic immunoassay and the test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *T. gondii* antigens conjugated with colloidal gold (*T. gondii* conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test lines (G and M lines) and a control line (C line). The M line is pre-coated with monoclonal anti human IgM for detection of IgM anti-*T. gondii* antibody, the G line is pre-coated with reagents for detection of IgG anti-*T. gondii* antibody, and the C line is pre-coated with a control line antibody. A drop of blood (35 µl) was kept into well of kit and same amount diluent supplied by kit was put into well and development of color line was observed within 10 minutes. Presence of a burgundy colored G /M line indicated a *T. gondii* IgG/IgM positive test result. Absence of any test lines (M and G) was considered a negative result.

### Data Analysis

All the collected data were compiled in Microsoft Excel. Data were analyzed using R 3.2.2 (The R foundation for Statistical Computing, 2015). Seroprevalence percentage, OR values and P value at 95 % significance level were calculated to show the association of risk factors with disease and results were tabulated.

## RESULTS

### Toxoplasmosis in different species

Humans as well as animals like sheep, cattle and cat were found to be infected with toxoplasmosis. The odds of toxoplasmosis was 6.0 times higher in cat than cattle (Table 1).

**Table 1:** Seropositivity of toxoplasmosis in human, sheep, cattle and cat

Species	Positive/ tested sample	Prevalence %	95% confidence interval (CI)	Odds Ratio (OR)	P value at <0.05
Human	19/150	12.67	7.80- 19.07		
Sheep	6/50	12.00	4.53- 24.31	1.4	0.5298
Cattle	8/92	8.70	3.83- 16.42	Reference	
Cat	16/44	36.36	22.41- 52.23	6.0	0.0002

### Toxoplasmosis in humans

Female had higher prevalence of toxoplasmosis than male. The odds of toxoplasmosis was 6.4 times higher in humans aged between 31-45 years in comparison to those below 20 years. About 58.33% aborted women were seropositive and the odds of toxoplasmosis was 15.4 times higher in aborted females than non-pregnant ( $p=0.0001$ , OR: 15.4) (Table 2).

The highest prevalence (20.83%) of toxoplasmosis was found in butcher while all students tested were seronegative (Table 3).

**Table 2:** Distribution of toxoplasmosis in humans

Variables	Positive/ tested person	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Sex					
Female	15/115	13.04	7.49- 20.60	1.2	0.8016
Male	4/35	11.43	3.20- 26.74	Reference	
Age					
Upto 20 years	1/24	4.17	0.11- 21.12	Reference	
21-30 years	8/80	10.00	4.42- 18.76	2.5	0.3882
31-45 years	10/46	21.74	10.95- 36.36	6.4	0.0866
History of individual					
Abortion	7/12	58.33	27.67- 84.83	15.4	0.0001
Non pregnant	8/96	8.33	3.67- 15.76	Reference	

**Table 3:** Distribution of toxoplasmosis in human based on their occupation and their association with cat

Variables	Positive/tested person	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Occupation					
Butcher	5/24	20.83	7.13- 42.15	3.0	0.2146
Housewife	3/30	10.00	2.11- 26.53	1.3	0.7976

Farmer	9/58	15.52	7.35- 27.42	2.1	0.3628
Diagnostic Lab technician	2/25	8.00	0.98- 26.03	Reference	
Student	0/13	0.00			
Contact with cat					
Yes	17/120	14.17	8.47- 21.71	2.3	0.2813
No	2/30	6.67	0.82-22.07	Reference	

### Toxoplasmosis in sheep

The distribution of toxoplasmosis in sheep is presented in Table 4. The prevalence of toxoplasmosis was higher in female (13.33%), sheep older than 18 months (20%) and in pregnant sheep (16.67%).

**Table 4:** Distribution of toxoplasmosis in sheep

Variables	Positive/tested animal	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Sex					
Female	4/30	13.33	3.76- 30.72	1.4	0.7232
Male	2/20	10.00	1.23- 31.70	Reference	
Age					
Below 6 months	0/8	0.00	0.00- 36.94		
6-18 months	2/22	9.09	1.12- 29.16	Reference	
Above 18 months	4/20	20.00	5.73- 43.66	2.5	0.3238
History of animal					
Pregnant	2/12	16.67	2.09- 48.41	1.8	0.5845
Non pregnant	2/18	11.11	1.38- 34.71	Reference	

## Toxoplasmosis in cattle

The distribution of toxoplasmosis in cattle is presented in Table 5. Female, cattle over 2.5 years old and pregnant cattle showed higher prevalence than respective another group.

**Table 5:** Distribution of toxoplasmosis in cattle

Variables	Positive/ tested animal	Prevalence %	95% CI	Odds Ratio	P value at <0.05
Sex					
Female	7/77	9.09	3.73-17.84	1.4	0.7615
Male	1/15	6.67	0.17-31.91	Reference	
Age					
2.5 years	0/5	0.00	0.0-52.18		
> 2.5 - 5 years	3/42	7.14	1.5-19.48	Reference	
> 5 years	5/45	11.11	3.71-24.04	1.6	0.5252
History of animal					
Pregnant	1/10	10.0	0.25-44.5	1.1	0.6115
Non pregnant	6/62	9.68	3.63-19.88	Reference	

## DISCUSSION

The study revealed variable rate of seroprevalences of toxoplasmosis among human, sheep, cattle and cats in the study area. Although toxoplasmosis is considered harmless for non-pregnant women, it is potentially harmful during pregnancy, especially at the first trimester (Giannoulis *et al.*, 2008). In this study 12.67% seropositive was found in human beings. Similar findings were reported by many other scientists in abroad. Slight lower prevalences were reported 10% in women in Egypt (Amany and Merwad, 2012); 10.66% in human in Bangladesh (Hossain, 2016) and 12.4% in women in Bangladesh (Samad *et al.*, 1997). High rates 15.89% in women with different gyno-obstetrical diseases (Samad *et al.*, 1993) and 38.5% in human (Ashrafunnessa *et al.*, 1998) were observed in Bangladesh using different serological tests. But this finding

seemed very low from previous reports in Nepal. In Nepal, high rates 54.8% (Rai *et al.*, 1994); 57.9% (Rai *et al.*, 1993) and 50.6% (Jaiswal *et al.*, 2014) were observed in human by using different serological tests. The positive rate is reported to vary from place to place (Suzuki *et al.*, 1987).

The present study demonstrated association between toxoplasmosis and different age groups of humans. The highest prevalence 21.74% found among age 31 to 45 year supported by findings of reports 44% among 36-40 years age group in Iran (Jafar *et al.*, 2014) and 27.9% in age group 31-45 year in Bangladesh (Margia, 2015).

Toxoplasmosis was significantly ( $P < 0.05$ ) associated with abortion in human. So, this parasite is harmful for pregnant women because it causes abortion. It may be due to ingestion of contaminated vegetables with oocysts or consumption of undercooked meat containing cysts.

According to occupation of people, butchers were found higher seropositive 20.83% compared to other occupational groups viz. farmer (15.52%), housewife (10%) and diagnostic lab technician (8%). It may be due to lack of awareness among slaughterhouse workers and there is no hygienic condition maintained at shop and also no trend to wear gloves during meat cutting and selling. Interestingly, this study found majority of the seropositive people had come in contact with cat (14.17%) by any means whereas people who were not in contact with cat found low (6.67%). The finding showed that 36.36% cats were seropositive. This finding is slightly higher than a report 33.33% (Samad *et al.*, 1997) and lower than 42% (Lamaj *et al.*, 2016). This finding is 2.5 times higher than a report made by Sah *et al.*, (2016) but they did work to detect oocysts in cat faeces in Bangladesh.

It was found that anti-*T. gondii* antibody is prevalent in sheep (12.0%) and cattle (8.7%). These findings are almost similar to 14% in sheep (Nunes *et al.*, 2015) and 8.57% in cattle (Azizi *et al.*, 2014). Surprisingly in all the studied species of animals, highly appreciable findings were that female, older age group (above 18 months in sheep and above 5 years in cattle) and pregnant animals were found more susceptible to *T. gondii*. The higher seroprevalence in female as compared to male might be attributed to the management system in those females are retained in the farm for longer periods for breeding purpose than males. Few males are retained for mating while the majority are culled and sold for cash purpose. The hormonal difference in relation to stress of lactation and pregnancy leading to immunosuppression may also increase susceptibility to toxoplasmosis in females (Dubey and Lappin, 1998). Higher seroprevalence in older age group compared to young is consistent with earlier studies and is the result of higher likelihood of ingestion of oocysts with increasing age (Dubey, 2010; Carneiro *et al.*, 2009; Ramzan *et al.*, 2009; Halos *et al.*, 2010).

## CONCLUSION

Based on LFCIA, the seroprevalences of toxoplasmosis were 12.67%, 12.00%, 8.70% and 36.36% in human, sheep, cattle and cats, respectively. With above results of the experiment, it is suggested that this assay is highly useful as a serodiagnostic tool in *T. gondii* infections. As this parasite has zoonotic importance, the knowledge should be disseminated to the people and animal raisers so that precautions can be taken in time.

## ACKNOWLEDGEMENTS

The authors acknowledge to staffs of Birat Diagnostic and Research Center, Inaruwa, Sunsari, Nepal for giving space to perform work in case of human.

## REFERENCES

- Amany, M. and Merwad, A.M. (2012). Epidemiology and molecular detection of zoonotic *Toxoplasma gondii* in cat feces and seroprevalence of anti-*Toxoplasma gondii* antibodies in pregnant women and sheep. *Life Science Journal*, **9**: 133-146.
- Ashrafunnessa, Khatun, S., Islam, M.N. and Huq, T (1998). Seroprevalence of toxoplasma antibodies among the antenatal population in Bangladesh. *Journal of Obstetrics and Gynaecology Research*, **24**(2): 115-9.
- Azizi, H., Shiran, B., Boroujeni, A.B. and Jafari, M. (2014). Molecular survey of *Toxoplasma gondii* in sheep, cattle and meat products in Chaharmahal va Bakhtiari Province, southwest of Iran. *Iranian Journal of Parasitology*, **9**(3): 429-434.
- Carneiro, A.C.A.V., Carneiro, M., Gouveia, A.M.G., Guimaraes, A.S., Marques, A.P.R., Vilas-Boas, L.S. and Vitor, R.W.A. (2009). Seroprevalence and risk factors of caprine toxoplasmosis in Minas Gerais, Brazil. *Veterinary Parasitology*, **160**: 225-229.
- Driscoll Carlos, A., Clutton Brock, J., Kitchener Andrew, C. and Brien, O.S.J. (2009). The Evolution of House Cats. *Scientific American* 2009. New York: Nature Pubg. Group. Retrieved 26 August 2009.
- Dubey, J.P. and Beattie, C.P. (1998). Toxoplasmosis of Animals and Man. Boca Raton, Florida: *CRC Press*; pp.220.
- Dubey, J.P., Hill, D.E., Remington, D.W., Kwox, O.C. and Sue, S. (2013). High

- prevalence and genotypes of *Toxoplasma gondii* isolated from organic pigs in northern USA. *Veterinary Parasitology*, **188** (1): 14-18.
- Dubey, J.P. and Lappin, M.R. (1998). Toxoplasmosis and neosporosis. In: Greene CE, editor. Infectious diseases of the dog and cat. 2<sup>nd</sup> edition. Philadelphia: *WB Saunders*; pp. 493–509.
- Dubey, J.P. (2004). Toxoplasmosis – a waterborne zoonosis. *Veterinary Parasitology*, **126**: 57–72.
- Dubey, J.P. (2010). Toxoplasmosis of Animals and Humans. 2<sup>nd</sup> edition. Boca Raton, Florida: *CRC Press*; pp.313.
- Feldman, H.A. (1982). Epidemiology of *Toxoplasma* infection. *Epidemiology Review*, **4**: 204-13.
- Giannoulis, C., Zournatzi, B., Giomisi, A., Diza, E. and Tzafettas, I. (2008). Toxoplasmosis during pregnancy: a case report and review of literature. *Hippokratia*, **12**(3):139–143.
- Halos, L., Thebault, A., Aubert, D., Thomas, M., Perret, C., Geers, R., Alliot, A., Escotte-Binet, S., Ajzenberg, D., Darde, M.L., Durand, B., Boireau, P. and Villena, I. (2010). An innovative survey underlining the significant level of contamination by *Toxoplasma gondii* of ovine meat consumed in France. *International Journal for Parasitology*, **40**: 193–200.
- Hill, D. and Dubey, J.P. (2002). *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clinical Microbiology and Infectious Diseases*, **8**: 634–640.
- Hossain, B. (2016). Serodiagnosis of toxoplasmosis in human and animals using lateral flow chromatographic assay. *MS Thesis*, Department of Parasitology, BAU, Bangladesh.
- Innes, E.A. (2010). A Brief History and Overview of *Toxoplasma gondii*. *Zoonoses and Public Health*, **57**: 1–7.
- Jafar, Y.Y., Nabi, J., Sameri, M.J. and Noorshahi, N. (2014). Seroprevalence of Anti-*Toxoplasma gondii* Antibodies Among pregnant woman in South Khuzestan, Iran. *Jundishapur Journal of Microbiology*, **7**(5): e9998.
- Jaiswal, S., Pokhrel, T., Sharma, S., Ranabhat, S.R., Yadav, D.K. and Koirala, B. (2014). Seropositivity Rates of Toxoplasmosis and Syphilis in Pregnant Women Visiting Western Regional Hospital, Nepal. *International Journal of*

*Health Sciences & Research*, 230(4): 9230-42.

- Lake, W.C., Khachaeramb, W., Koizumic, S. and Maruyama, S. (2002). Seroprevalence of *T. gondii* infection in domestic goats in Satun Province, Thailand. *Veterinary Parasitology*, 127: 17–22.
- Lamaj, S., Dhamo, G. and Dova, I. (2016). Epidemiology of *Toxoplasma gondii* infection of cats in southwest of Albania. *Journal of International Academic Research for Multidisciplinary*, 4(2): 76-81.
- Margia, U.H. (2015). Seroprevalence of toxoplasmosis in women of Mymensingh and Rangpur districts. *MS Thesis*, Department of Parasitology, BAU, Bangladesh.
- Montoya, J. and Liesenfeld, O. (2004). Toxoplasmosis. *Lancet*, 363: 1965-1976.
- Nunes, A.C.B.T., Silva, E.M.V., Oliveira, J.A., Yamasaki, E.M., Kim, P.C.P., Almeida, J.C., Nunes, K.B. and Mota, R.A. (2015). Application of different techniques to detect *Toxoplasma gondii* in slaughtered sheep for human consumption. *Brazil Journal of Veterinary Parasitology, Jaboticabal*, 24(4): 416-421.
- Pal, M. (2007). Zoonoses. 2<sup>nd</sup> edition. *Satyam Publishers*, Jaipur, India.
- Pereira, K.S., Franco, R.M. and Leal, D.A. (2010). Transmission of toxoplasmosis (*Toxoplasma gondii*) by foods. *Advances in Food and Nutrition Research*, 60: 1–19.
- Rai, S.K., Shibata, H., Sumi, K., Kubota, K., Hirai, K., Matsuoka, A., Kubo, T.T., Basnet, S.R., Shrestha, H.G. and Mahajan, R.C. (1994). Seroepidemiological study of toxoplasmosis in two different geographical areas in Nepal. *Southeast Asian Journal Tropical Medical Public Health*, 25(3): 479-84.
- Rai, S.K., Yano, K. and Kubo, T.T. (1993). Immuno-serological study of human toxoplasmosis in Nepal. Proceeding Third Congress Asia Pacific Societies of Pathologists and Eleventh National Convention, *Bangladesh Society of Pathologists*, pp. 98.
- Ramzan, M., Akhtar, M., Muhammad, F., Hussain, I., Hiszczynska Sawicka, E., Haq, A.U., Mahmood, M.S. and Hafeez, M.A. (2009). Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Tropical Animal Health and Production*, 41: 1225–1229.
- Remington, J.S., McLeod, R., Thulliez, P. and Desmonts, G. (2001). Toxoplasmosis. *Infectious diseases of the fetus and newborn infant*, 5<sup>th</sup> ed. W. B. Saunders,

Philadelphia, pp. 205-346.

- Sah, R.P., Talukder, M.H. and Hossain, M.B. (2016). Toxoplasmosis in cats and its zoonotic potential at Bangladesh Agricultural University campus. *3<sup>rd</sup> International Exhibition on Dairy, Aqua and Pet (IEDAP)* held at BICC Dhaka, Bangladesh, pp. 300-303.
- Samad, M.A., Dey, B.C., Chowdhury, N.S. and Akhtar, S. (1997). Sero-epidemiological studies on *Toxoplasma gondii* infection in man and animals in Bangladesh. *Southeast Asian Journal of Tropical Medicine and Public Health*, **28**(2): 339-43.
- Samad, M.A., Rahman, K.B. and Halder, A.K. (1993). Seroprevalence of *Toxoplasma gondii* in domestic ruminants in Bangladesh. *Veterinary Parasitology*, **47**(1-2): 157-9.
- Suzuki, H., Yamamoto, Y. and Matsumoto, K. (1987). A serological study of *Toxoplasma* infection in Nagasaki by ELISA. *Japanese Journal of Parasitology*, **36**: 118-24.
- Tizard, I.R., Chauhan, S.S. and Lai, C.H. (1977). The prevalence and epidemiology of toxoplasmosis in Ontario. *Journal of Hygiene Cambodia*, **78**:275-82.
- Upadhyay, M.P., Rai, S.K., Bhandari, R.K. and Bista, P. (1989). Serological study of *Toxoplasma gondii* infection in Kathmandu. Nepal. *Journal of Institute Medicine*, **2**: 211-5.
- Zewdu, E.G., Hailu, A., Tessema, T.S., Desta, K.T., Medhin, G., Vitale, M., Di Marco, V., Cox, E. and Dorny, P. (2013). Seroepidemiology of *Toxoplasma gondii* infection in women of child-bearing age in central Ethiopia. *BMC Infectious Diseases*, **13**: 101.