



## RESEARCH PAPER

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## Seroprevalence of *Toxoplasma gondii* Infection in Domestic Animals of District Charsadda, Khyber Pakhtunkhwa, Pakistan

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### Abstract

This study was conducted in to find out the seroprevalence of toxoplasmosis in domestic animals of District Charsadda. Toxoplasmosis is an infection caused by a unicellular parasite *Toxoplasma gondii* which is cosmopolitan in distribution among the animals including domestic animals and human beings. Very little information is available about the infection rate of toxoplasmosis in domestic animals of District Charsadda, Pakistan. A total of 374 blood samples were collected from cows, buffaloes, sheep and goats and were tested by Latex agglutination test, out of which (79.7%) were found seropositive. Out of 260 females, (82.69%) were found infected while in 114 males (78.80%) were found infective. A high prevalence of (81.65%) was detected in age group above one year. Individual sex wise prevalence also revealed that females were more positive as compared to males. In male buffaloes, (76.92%) were positive while in females, (75.67%) were detected positive. In male goats (72.41%) were positive while in case of females, (84.78%) were found infective. The infection rate was higher in female sheep (91.42%) as compared to male sheep (84.78%). In male cows (69.23%) were detected seropositive while in females cows the infection rate was (73.77%). This increased rate of infection may be due to free access of cat to drinking water and other food sources as well as warm and humid conditions of the area.

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## Introduction

*Toxoplasma gondii* which is an intracellular parasite distributed worldwide (Shah *et al.*, 2013a) and is responsible for toxoplasmosis disease (Aldebret *et al.*, 2011). The discovery of cats as definitive host for *T. gondii* was done in 1960 (Innes, 2010). Life cycle of *T. gondii* is completed in two stages, sexual stage starts in definitive host, which include cats and asexual stage is completed in warm blooded animals (Afonso, 2008). Intermediate hosts are infected by ingesting oocysts, which change into tachyzoites through asexual reproduction and then change into bradyzoites. Once again the felines are infected by eating bradyzoites-infected meat (Webster, 2007). About (33-60%) of the human worldwide population have been infected by this parasite (Holliman, 1997; Shah *et al.*, 2014), but this rate changes according to the geographical, climatic and nutritional factors, socio-cultural habits and routes of transmission (Shah *et al.*, 2013b). Prevalence rate of *T. gondii* varies in different countries.

The infection rate of (28.44%) was recorded in Mohmand Agency, Pakistan (shah *et al.*, 2014). The seroprevalence of *T. gondii* was 63% in Punjab, 48% in Azad Kashmir and 38% in Khyber Pukhtunkhwa (Tenter *et al.*, 2000). Prevalence rate of (17.4%) was found in young school children in Islamabad, Pakistan (Sadaruddin *et al.*, 1991). The prevalence rate in Dera Ghazi Khan, Pakistan was detected (29.5%) (Tasawar *et al.*, 2011), Infection rate has declined recently due to awareness in people by not using under cooked meat and advancement in animal husbandry work (Shah *et al.*, 2017). The seroprevalence of *T. gondii* in domestic animals and meat producing animals, such as goats, and sheep, has found the same infection all the time. In District Mardan (Pakistan) the prevalence rate of toxoplasmosis in goats was recorded 42.28% whereas in sheep the infection rate was 44.13% (Shah *et al.*, 2013b). In Mohmand Agency, Pakistan, the prevalence rate of toxoplasmosis in goats was recorded 53.84% while in sheep it was 36.00% (Shah *et al.*, 2013a). In farmed sheep, the prevalence in Europe is related with age, increasing from lambs (17-

22%) to adult (65 -89%) (Halos, 2010). Viable *T. gondii* have been detected in about 67% of sheep samples. In Southern European countries infected meat of Sheep is the main source of infection. Seropositivity for goats varies from 4 to 77% (Dubey, 2011). The seroprevalence of this infection in sheep in Newzeland was reported to be 30-90% and in UK 77% prevalence was reported in goats while 29% was reported in sheep (De Bhur, 2008). In Mohmand Agency, Pakistan, the seroprevalence of toxoplasmosis reported in cows was 20% whereas 16% was recorded in buffaloes (Shah *et al.*, 2013a).

*T. gondii* Infections show mild symptoms or mostly without symptoms (Sarkar *et al.*, 2012). Immunocompetent individuals in acute infection are generally asymptomatic or show some common specific symptoms (Selseleh, 2012), often give flu-like symptoms in the early acute stage (Menotti *et al.*, 2003). While in immunosuppressed patients *T. gondii* causes encephalitis and leading illness (Jones and Roberts, 2013) and infect brain, heart which results in myocarditis, and infection of liver, pancreas, bone marrow, bladder and lymph nodes, kidneys, spleen, and skin (Arnold *et al.*, 1997).

The placental barrier is very strong against the parasite during the first three months of pregnancy, but transmission rate increases as pregnancy proceeds which results in abortions (Dunn, 1999). Congenital toxoplasmosis also causes deafness, mental retardation, microcephalous, eye lesions, cataract, retinal necrosis (Delair, 2011). Abortion or still birth are common during first three months of pregnancy while Infection acquired in later pregnancy the newborn is usually without symptoms but may cause eye problems (shah *et al.*, 2014) Congenital toxoplasmosis leads to CNS damage, blindness epilepsy and mental growth is retarded with fetal death or spontaneous abortion in about 10% of cases [23]. *T. gondii* causes abortions, stillbirth and neonatal death in sheep and goats (Engeland *et al.*, 1998).

The distribution of *T. gondii* is related with weather condition of an area and environment where the oocysts survive (Dubey, 2004).

Prevalence of toxoplasmosis varies in different parts of the world and this variation related to life style, age, climatic conditions, nutritional habits and other socio-cultural factors (shah *et al.*, 2014). This study was carried out in the study area with the aim to determine the infection rate in domestic animals, minimize the economic loss in domestic animals and to aware the people about the adverse effects of toxoplasmosis.

### Materials and methods

A total of 374 blood samples were collected from domestic animals (cows, goats, sheep and buffaloes) from District Charsadda, Pakistan. About five ml of blood was collected from each animal. The samples were centrifuged for extraction of serum.

#### Serological examination

The latex reagent is a suspension of polystyrene particle which is sensitized with the antigens of *T. gondii*. The distant agglutination pattern when observed after mixing the serum reflects formation of antigen-antibody complexes. When the organism has no infection, then no agglutination is observed. A greater than 4 IU/ml value was considered as standard for positive result.

#### Test procedure

According to the manufacturer standard protocol (Toxocell Latex- Spain) the test procedure was performed. The samples were diluted in NaCl 0.9% saline solution. One drop or 50ul of diluted sera was mixed with one drop (25ul) of chemical and mixed well by sterile wood stick, then the slide was slowly rotated for 5 minutes and visible agglutination was observed. The result was classified into positive or negative on a slide, using pip stirrers provided. A negative reaction was the indication of the absence of toxoplasma antibodies. A clear positive reaction showed the presence of toxoplasma antibodies equal or greater than 4 IU/ml which showed either an evolving infection or a past infection.

#### Statistical analysis

For simplification all the results were expressed in percentages. The values between different sex and age groups were recorded and relevantly expressed in

percentages. Microsoft Excel (version-10) was utilized by windows-08, (Release 16.0 standard version).

### Results

A total of 374 blood samples were collected from domestic animals of three Tehsils (Shabqadar, Tangi and Charsadda) of District Charsadda and were tested for the presence of *T. gondii* infection by using Latex Agglutination Test. Out of these 374 blood samples, 298 (79.7%) were found positive for toxoplasmosis. A total of 100 blood samples were collected from cows in which 72 (72%) were found positive and 28 (28%) were negative, out of 121 goats, 99 (81.8%) were positive and 22 (18.2%) were negative. Similarly, 103 samples were collected from Sheep, out of which 89 (86.4%) were positive and 14(13.6%) were found negative whereas out of 50 samples from buffalo, 38(76%) were found positive and 12(24%) were detected negative (Table 1).

**Table 1.** Comparative seroprevalence of *T. gondii* infection in domestic animals.

Animals	Samples (N)	Positive N (%)	Negative N (%)
Cows	100	72 (72)	28 (28)
Goats	121	99 (81.8)	22 (18.2)
Sheep	103	89 (86.4)	14 (13.6)
Buffaloes	50	38 (76)	12 (24)
Total	374	298	76

A total of 260 females and 114 males were tested for *T. gondii* infection. Out of which 215 (82.69%) females 83 (78.80%) males were found positive. This also indicated that prevalence among the females was high among these animals. Prevalence of toxoplasmosis was also detected in different sex groups. The highest prevalence of toxoplasmosis was found in females as compared to males. Out of 13 male buffaloes, 10 (76.92%) were positive while out of 37 females, 28 (75.67%) were found positive. In goats out of 29 males, 21 (72.41%) were found infective whereas out of 92 females, 78 (84.78%) were positive. Out of 33 male sheep 25 (84.78%) were found seropositive and out of 70 female sheep (91.42%) were found infective. Out of 39 male cows, 27 (69.23%) were detected infective whereas out of 61 females, 45 (73.77%) were found seropositive (Table 2).

**Table 2.** Sex wise seroprevalence of toxoplasmosis among goats, sheep, cows and buffaloes.

Animals	Samples Male	Positive N (%)	Female	Positive N (%)
Buffaloes	50	13	10 (76.92)	37 28 (75.67)
Goats	121	29	21 (72.41)	92 78 (84.78)
Sheep	103	33	25 (84.78)	70 64 (91.42)
Cows	100	39	27 (69.23)	61 45 (73.77)
Total	374	114	87	260 215

Seroprevalence of toxoplasmosis was also detected in different age groups. A total of 178 (81.7%) of age above one year and 120 (76.9%) under one year were detected seropositive. Seroprevalence of toxoplasmosis was higher in age group above one year (Table 3).

**Table 3.** Age wise seroprevalence of toxoplasmosis among domestic animals.

Age	Sample (N)	Positive (%)	Negative (%)
> One year	218	178 (81.7)	40 (18.3)
<One year	156	120 (76.9)	36 (23.1)
Total	374	298	76

### Discussion

Toxoplasmosis is a zoonotic disease arising from close contact of human with felids (kravetz and federman, 2002). Domestic cats play a vital role in the spread of toxoplasmosis because they are the definitive hosts and play an important role in transmission of *T. gondii* infection by shedding oocysts in their faeces (Dubey, 1994).

It varies between, herds, countries and regions, methods of diagnoses and even at different times in the same herd. In our study, infection rate for *T. gondii* in cattle, goats and sheep was extremely high (79.7%) than 32.29% in domestic animals in Mohmand Agency in Pakistan ((shah *et al.*, 2013a)), 9.2% reported from Guangxi and 46.4% from Xinjiang, China (Lv and Cui, 1994).

In present study 81.81% prevalence rate of *T. gondii* was found in goats, which is higher than 51% in goat's population recorded in Saudi Arabia (Sanad and Alghabban, 2007), in Brazil 28.9% (Bisson *et al.*, 2000), Thailand 27.9% (Jittapalapong *et al.*, 2005), 25.4% in Pakistan (Ramzan *et al.*, 2009). Toxoplasmosis was also detected in sheep population of Charsadda. The present study, revealed that in sheep toxoplasmosis is very high (86.40%) than reported from Brazil 46.2% (Silva *et al.*, 2013) and

Greece 48.6% (tzanidakis *et al.*, 2012), 31% reported in Turkey (oncel and Vural, 2006) and Northeastern China 4.4% (Yang *et al.*, 2013), 44.13% from Mardan.

Toxoplasma infection in cattle shows a high degree of variation across the globe, ranging virtually from 0% to 99% (Ivana *et al.*, 2006). Seropositivity rate recorded for *T. gondii* in the present study in cows is extremely high (66%) as compared to 9% in Indonesia (Matsuo and Husin, 1996), while seroprevalence for *T. gondii* in Mohmand Agency, Pakistan was found 20% in cows and 16% in buffaloes (shah *et al.*, 2013a), which is lower than reported in the present study but seroprevalence in present study (66%) is lower than (76.3%) in cattle in Serbia (Ivana *et al.*, 2006).

The differences in prevalence reported by all these studies could be accounted on host, age, breed, sex, environmental conditions, farm size and number of cats and management practices (Van Der *et al.*, 2000). During the present study, a high prevalence of 82.7% was reported in females as compared to males (78%), indicating high prevalence among the females as compared to 35.8% in female and 21.1% in male sheep and goats (Ramzan *et al.*, 2009; Van Der *et al.*, 2000). Different studies indicated that females are more easily infected by protozoan parasites as compared to males (Alexander and Stinson, 1998).

In female immunity can be broken due to various factors e.g., nutrition, age, pregnancy and environmental factors. Toxoplasmosis and age relationship in the present study revealed that seroprevalence is high in age group above one year (81.65%) as compared to age group less than one year (76.92%) which are in support with 77.7% in age group of 60 to 75 months and lowest prevalence 39.3% in age group of 12 to 27 months (Jittapalapong *et al.*, 2005).

Older goats were more seropositive as compared to younger ones under one year old (Olivier *et al.*, 2007).

The present work was an attempt to find out the prevalence rate of toxoplasmosis in the study area. The high prevalence rate of toxoplasmosis in the study area may be due to warm and humid climatic conditions of this area. The increased infection rate in study area may also be due to unhygienic conditions because *T. gondii* prevalence ranged from 0-100% in different areas of the world and this variation is due to the life styles of the inhabitant's customs, traditions, weather conditions, age of the animals and husbandry practices (Olivier *et al.*, 2007).

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