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Seroprevalence of *Toxoplasma gondii* in sheep and buffalo of District Charsadda, Khyber Pakhtunkhwa, Pakistan

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Abstract

Toxoplasma gondii is a globally distributed protozoan parasite. This study was performed to find out the seroprevalence of *Toxoplasma gondii* in *sheep* and *buffalo* in District Charsadda. A total of 270 samples 127 from *buffalo* and 143 from *sheep* were collected and examined by latex agglutination test. Out of 127 *buffalo* 22(17.32%) were detected seropositive for Toxoplasmosis and out of 143 *sheep* 58(40.55%) were found seropositive. In *sheep*, a high seroprevalence rate of (46.42%) was obtained in age group of above 03 years while in *buffalo*; the highest seroprevalence rate of (19.04%) was detected in age group above 04 years. In *sheep* the seroprevalence rate was higher in female (49.23%) as compared to male (33.33%). In *buffalo* the seroprevalence rate was higher in female (19.45%) as compared to male (11.11%). The present study shows that the prevalence in *sheep* and *buffalo* is higher in District Charsadda, which is a risk factor for human infection. Therefore, proper control measure should be taken to avoid infection of Toxoplasmosis.

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Introduction

Toxoplasmosis, caused by *Toxoplasma gondii*, is one of the most important zoonotic diseases with a worldwide geographical distribution (Amin *et al.,* 2013). *Toxoplasma gondii*, an obligate intracellular protozoan parasite, is one of the common parasitic infections of man and other warm-blooded animals (Zhou *et al.,* 2011).

The definitive hosts of *T. gondii* cats belong to felids family while other warm blooded animals are the intermediate hosts (Carrada-Bravo. 2005). *Sheep* toxoplasmosis is one of the most important parasitic diseases which cause serious economic losses among *sheep* industry all over the world, especially at lambing time (Shaapan *et al.,* 2008; Barakat *et al.,* 2009). A primary infection in pregnant *sheep* and *buffalo* may lead to a placental and foetal infection which may result in foetal death and resorption, abortion or stillbirth. There is a good knowledge about the role of *T. gondii* as a serious cause of foetal mortality in *sheep* and *buffalo* (Buxton. 1998). In humans, the congenital toxoplasmosis causes a variety of clinical syndromes as abortion, mental retardation, blindness, or hydrocephalus in addition to congenital anomalies in children (Aghwan. 2005; Razzak *et al., 2005*). Toxoplasmosis a zoonotic protozoan disease is horizontally transmitted to humans by the accidental ingestion of oocyst in cat faces or by eating raw or undercooked meat containing cysts, while vertical transmission from an acutely infected pregnant woman can cause a serious disease in the foetus (Roberts and Janovy. 2000). One third of the human population is chronically infected with *T. gondii* (Schares *et al.*, 2008). Among livestock, *sheep* and *buffalo* are more widely infected with *T. gondii* than cattle and chicken. This parasite causes abortion and neonatal death with significant economic losses to *sheep*, *buffalo* and pig farmers (Tenter *et al.,* 2000; Ghazaei. 2006).

This is more serious especially when primary infection occurs during pregnancy (Radostits *et al.*, 2006). The infection does not usually cause clinical symptoms in cattle (Ghazaei. 2006). Toxoplasmosis causes abortion, stillbirths and neonatal mortality in *sheep* and it is manifested by encephalitis and pneumonia (Radostits *et al.*, 2006). Toxoplasmosis is worldwide in distribution and cats have a major influence in the epidemiology of the disease. Islands with geographical isolation and absence of cats have been found to be free of toxoplasmosis (Acha & Szyfres. 2003). This fact is explained by the preying habits of this group and their diet that includes wild birds, rodents and Toxoplasma infected placentas and stillborn foetuses in some cases (Steven *et al.,* 2000). Historically, cats have been associated with domestic animals as an aid to rodent control (Radostits *et al.,* 2006).

The aim of the current study was to determine the seroprevalence of toxoplasmosis infection in Sheep and Buffalo in Charsadda, KPK Pakistan.

Materail and methods

Study area

Area of the study is conducted in district Charsadda. Charsadda is a town and headquarters of Charsadda District, in the Khyber Pakhtunkwa province of Pakistan.

Sample size

A total of 270 samples, 127 samples of *buffalo* and 143 samples of *sheep* were collected from different parts of district Charsadda and examined for seroprevalence of *T.gondii.*

Collection of blood

3-5ml of blood was collected from jugular vein by using 5ml of clean syringe. Blood was then centrifuge at 3500 rpm and for 10 minutes to collect the serum.

Test procedure

The test procedure was performed according to standard protocol and manufacturer of the company *Toxo* latex agglutination test is performed using "*toxo* latex kit" of "Spin react" company "Spain". After obtaining serum, 20 micro liter serum is added to the glass slide, and then 20 micro liter of *toxo* latex reagent is added to the slide and mix with the help of stirrer for three minutes. After mixing it is studied for positive and negative.

Results

Overall Seroprevalence of Toxoplasmosis in District Charsadda

A total of 270 animals including *buffalo* and *sheep* from different localities (Tehsil Charsadda, Tehsil Tangi, Tehsil shabqadar) of District Charsadda, Pakistan were examined for the presence of T. gondii antibodies. Out of 270 animals 22 (17.32%) were detected seropositive for T. gondii in *buffalo* and 58 (40.55%) were detected in *sheep* (Table 1).

Table 1. Overall Seroprevalence of Toxoplasmosis in District Charsadda.

Animals	Total sample	Positive sample	Negative sample	Positive %
Buffalo	127	22	105	17.32%
Sheep	143	58	85	40.55%

Total Number of Sample of three Tehsils of Buffalo

As this study was conducted in different Tehsils of district Charsadda (Tehsil Charsadda, Tehsil Tangi and Tehsil Shabqadar). A total of 57, 42 and 28 samples of *buffalo* is collected from Tehsil Charsadda.

Tehsil Tangi and Tehsil shabqadar in which 12(21.05%), 6(14.28%) and 4(14.28%) were seropositive respectively. A total of 66, 42 and 35 samples of *sheep* is collected from the three Tehsils of district Charsadda in which 25 (37.87%), 19 (45.23%) and 14 (40.00%) were seropositive in Tehsil.

Table 2. Tehsil wise Seroprevalence of Toxoplasmosis in Buffalo of District Charsadda.

Name	Total sample	Positive sample	Negative sample	Positive %
Charsadda	57	12	45	21.05%
Tangi	42	6	36	14.28%
Shabqadar	28	4	24	14.28%
Total samples	127	22	105	17.32%

Table 3. Tehsil wise Seroprevalence Toxoplasmosis in Sheeps of District Charsadda.

Name	Total sample	Positive sample	Negative sample	Positive %
Charsadda	66	25	41	37.87%
Tangi	42	19	23	45.23%
Shabqadar	35	14	21	40.00%
Total samples	143	58	85	40.55%

Number of Male and Female samples of Buffalo and Sheep of three Tehsils

Out of 40 male *buffalo*, 5 (11.11%)were detected seropositive. In 87 examined female *buffalo*, 17 (19.14%) were detected seropositive for *T. gondii* infection. Out of 78 examined male *sheeps* 26 (33.33%) were detected seropositive for *T. gondii* antibodies while 32 (49.23%) out of 65 female *sheep* were detected seropositive for *T. gondii* antibodies. High seroprevalence of toxoplasmosis was seen in female *buffalo* as compared to male *buffalo*. A significant difference was found in the male of *buffalo* and *sheep*. Similar results were observed for male and female of *buffalo* and *sheep* (Table 4 and Table 5).

Table 4. Sex wise Seroprevalence of Toxoplasmosis in *buffalo* of three Tehsils.

Name	Total Sample	Female Sample			Male Sample			Positive %	
Charsadda	57	+ive	-ive	Total	+ive	-ive	Total	Male	Female
		10	29	39	2	16	18	11.11%	25.62%
Tangi	42	4	24	28	2	12	14	14.28%	14.28%
Shabqadar	28	3	17	20	1	7	8	12.5%	15.00%
Total	127	17	70	87	5	35	40	11.11%	19.57%

Table 5. Sex wise Seroprevalence of Toxoplasmosis in *Sheep* of three Tehsils.

Name	Total Sample	Female Sample			Male Sample			Positive %	
Charsadda	57	+ive	-ive	Total	+ive	-ive	Total	Male	Female
		15	15	30	10	26	36	27.77%	50.00%
Tangi	42	8	12	20	11	11	22	50.00%	60.00%
Shabqadar	28	9	6	15	5	15	20	25.00%	60.00%
Total	127	32	33	65	26	52	78	33.33%	49.23%

Table 6. Age wise Seroprevalence of Toxoplasmosis in *Buffalo* of Charsadda.

Age of animals	Total sample	Positive sample	Negative sample	Positive %
Up to one year	23	3	20	13.04%
One to two years	27	4	23	14.81%
Two to three year	33	7	26	21.21%
Three to four years	23	4	19	17.39%
Above four years	21	4	17	19.04%
Total	127	22	105	17.32%

Age wise Results of Buffalo

Toxoplasma gondii infection was also examined in different age groups of *buffalo*. Out of 23 examined *buffalo* whose age was up to one year 3 (13.04%) were detected positive while 4 (14.81%) *buffalo* were infected in age group of 1 to 2year in 27 examined samples. In age group of 2 to 3 year 7 (21.21%) were seropositive in 33 examined samples. A seroprevalence of 4 (17.39%) was found in age group of 3 to 4 year in 23 examined samples. In age group of above 4 years 4 (19.04%) positive out of 21 examined samples were detected (Table 6).

Age wise Results of Sheep

The prevalence also varied in different age groups of *sheep* ranging from 33.33% to46.42%. Out of 27 examined *sheep* 9 (33.33%) were seropositive in age group of up to 1 year. The *T.gondii* infection was found in 17 (39.53%) out of 43 samples in age group of 1 to 2 year. A high prevalence 19 (42.22%) was found in age group of 2 to 3 year out of 45 examined *sheep*. Out of 28 examined sheep 13 (46.42%) were seropositive in age group of above three years (Table 7).

Table 7. Age wise Seroprevalence of Toxoplasmosis in *Sheep* of Charsadd.

Age of animals	Total sample	Positive sample	Negative sample	Positive %
Up to one year	27	9	18	33.33%
One to two years	43	17	26	39.53%
Two to three year	45	19	26	42.22%
Above three year	28	13	15	46.42%
Total	143	58	85	40.55%

Discussion

The present study was conducted in order to know the seroprevalence of *toxoplasma gondii* infection. A total of 127 samples of *buffalo* and 143 samples of *sheep* were collected and the result found were 17.32% and 40.55% respectively. Various studies carried out in other countries and other parts of Pakistan; have reported different contamination rates for *T. gondii* in *buffalo* and *sheep*. This may be due to difference in time and season of sampling and also differences of sensitivities and specificities of assays used. *T. gondii* infection is widely distributed at a worldwide scale, with incidences from zero to 100% in the different countries (Prelezov *et al*., 2008).

In present study, prevalence of toxoplasmosis in *sheep* is 40.55% which is less than that reported from Canada 57.6%, Greece 48.6% and Brazil 46.2%. The seropositivity rate of 40.55% found in *sheep* in present study is higher than 31% reported in Turkey and Northeastern China 4.4% (Yang *et al.,* 2013). In the present study prevalence of *T. gondii* infection in *sheep* 44.13% is higher than Pakistan 11.2 % Pakistan 2.5% and Iran 6.7% but is lower than reported from Brazil 60.8% (Amzan *et al.,* 2009).

The prevalence of *Toxoplasma gondii* in *buffalo* is 17.32%, which is less than reported from Lahore Pakistan 22% and southern Brazil 27.2% but is Higher than reported from Trinidad 7.8%, from the southern veitnam 3% from Bahia state Brazil 3.85%, from Punjab India 2.91%, from southwestern china 11.14% and from Khoozestan province Iran 8.8% (Gondim *et al,* 1999 Zhou *et al.* 2015%). In *sheep* the prevalence observed is higher in female (49.23%) than male (33.33%) which are similar to the previously conducted study in Pakistan (Shah *et al*., 2013). It is not possible to compare prevalence data of studies because of the use of different serological tests with variable specificity and sensitivity. Warm and humid environmental conditions are favorable for the spread of toxoplasmosis (Dubey. 2010).

The differences in the results of toxoplasmosis is due to various reasons, some factors depend on the climatic conditions, temperature, humidity and hygienic conditions hygienic condition is the major factor in the spread of toxoplasmosis. It is not possible to compare prevalence data of studies because of the use of different serological tests with variable specificity and sensitivity.

Warm and humid environmental conditions are favorable for the spread of toxoplasmosis. *Toxoplasma gondii* infection is high in regions where the people eat undercooked meat, unwashed vegetables and fruits and the people who have contact with cats and dogs or other domestic animals or have direct contact with the soil. Toxoplasmosis is more common in those areas where people drink municipal water.

Conclusion

The purpose of the present study is to know the seroprevalence rate of *Toxoplasma gondii* in District Charsadda, Pakistan. This study demonstrates that Toxoplasmosis is prevalent in District Charsadda. The percentage is greater in *sheep* (40.55%) as compared to *buffalo* (17.31%). In *sheep* the prevalence of Toxoplasmosis is 40.55% in 143 examined *sheep*. In *buffalo* the prevalence of Toxoplasmosis is 17.31% in 127 examined *buffalo*.

In *sheep* the prevalence recorded is 37.87%, 45.23% and 40.00% in Tehsil Charsadda, Tehsil Tangi and Tehsil Shabqadar respectively. In *buffalo* the prevalence recorded is 21.05%, 14.28% and 14.28% in Tehsil Charsadda, Tehsil Tangi and Tehsil shabqadar respectively. In *buffalo* the prevalence recorded is greater in female *buffalo* (19.54%) as compared to male *buffalo* (11.11%). In *sheep* the prevalence recorded is greater in female *sheep* (60.00%) as compared to male *sheep* (25.00%). In *buffalo*, in age group of above 2 years the prevalence rate of toxoplasmosis is greater from below two years. In *sheep* the prevalence is also greater in age group of above two years. This study demonstrates that toxoplasmosis is prevalent in female and older *sheep* and *buffalo*, therefore proper measure should be taken to control toxoplasmosis.

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