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Seroprevalence of *Toxoplasma gondii* in goats and sheep of district Mardan, Pakistan

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Abstract

This study was carried out in order to investigate the prevalence of *Toxoplasma gondii* infection in goats and sheep of District Mardan, Pakistan. Indirect Haemagglutination Test (IHA) was used for detection of *T. gondii* antibodies in sera. Out of 350 goats 148 (42.28%) were detected positive for *T.gondii* antibodies. The prevalence in male and female goats were 39 (26%) and 109 (54.5%) respectively. Goats of age ≥ 2 years had the highest seroprevalence (54.44%) followed by those of 1-2 years old (33.33%) and those ≤ 1 year old (20%). *Toxoplasma gondii* antibodies were detected in 128 out of 290 examined sheep (44.13%). A total of 55 (45.83%) out of 10 male sheep were detected seropositive for *T. gondii* infection and 73 (42.94%) out of 170 female sheep were detected seropositive. High prevalence of *T. gondii* was seen in female as compared to male sheep. Among the examined sheep, those which were ≥ 2 year old had the highest infection (66.66%) followed by 1-2 year old (36.36%) and ≤ 1 year old (13.33%). The seroprevalence of *T. gondii* antibodies was higher in all goats and sheep with titer ranging from 1:80 to 1:160. The infection rate in sheep was higher as compared to goats. The results of the present study indicate that *T. gondii* infection is very common in goats and sheep of District Mardan, Pakistan, which may be a risk factor for public health in this area because goats and sheep are the intermediate hosts of *T. gondii*. Proper control strategies and suitable measures should be carried out in this region, in order to minimize the risk of exposure of human population to *T. gondii* infection.

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Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite which causes toxoplasmosis. This disease is zoonotic which is worldwide in distribution (Tenter *et al.*, 2000). The definitive hosts of *T. gondii* are felids family including cats while warm blooded animals are the intermediate hosts (Carrada-Bravo, 2005).

About 33% of world human population is infected by *T. gondii* but the frequency differs depending on geographical areas. Seroprevalence of toxoplasmosis of 20%-30% was found in USA, 25% Japan, 60% Netherland and Italy, 50% Finland and 50%-60% Poland. Some countries have incidence of more than 80% (Montoya and Liesenfeld, 2004; Condolfi and Cesbron-Delauw, 2006). Seroprevalence of *T. gondii* infection was found to be 17.4% in young school children in Islamabad, Pakistan (Sadaruddin *et al.,* 1991). The prevalence rate in Dera Ghazi Khan, Pakistan was observed to be 29.5% (Tasawar *et al.,* 2011).

Cats and its faeces are the main sources which transfer the infection or it may also be transferred by eating tissue cysts in undercooked or uncooked meat (Schlundt *et al.*, 2004). The symptoms of the disease are mild flu-like illness which is characterized by fever, headache, bodyache or show no illness but the people having weak immune system (HIV infected persons or pregnant women) may suffer serious illness such as weight loss, diarrhoea, pneumonia, liver diseases and infection of central nervous system and in such cases infection can result deaths (Lindstrom *et al.*, 2006; Negash *et al.*, 2008). Cats have the potential to shed the infected oocysts and transmit infection to intermediate host. *T. gondii* oocysts become infective when these oocysts are sporrulated up to three days after being shed in the faeces. Animals get infection by ingestion of faeces of cats, eating infected meat or by transmission from mother to foetus. Contaminated drinking water with cat can also transmit infection to people. Cats are the vectors of this transmission. The sexual reproduction of the *T. gondii* occurs only in cat (Montoya and Liesenfeild, 2004).

It is commonly recommended to avoid cats to uninfected pregnant women but the contribution of this risk factor in the spread of disease is controversial. It has been identified from some studies that cat ownership or contact with cat is a minor source of risk in the spread of *T. gondii* infection while several other studies have failed to identify exposure to cats as a significant risk factor for toxoplasmosis (Cook *et al.*, 2000).

Acute stage toxoplasmosis infections can be asymptomatic, but often give flu-like symptoms in the early acute stage. The acute stage fades in a few days to months, leading to the latent stage of the disease. Latent infection can reattack in persons who are immunocompromised (HIV infected persons or transplant receipts) (Menotti *et al.*, 2003). This was the first study on toxoplasmosis in sheep and goats in this area. Infected goats and sheep may be a risk factor for public health in this area because goats and sheep are the intermediate hosts of *T. gondii*. This study was carried out with the aim to aware the general population of the area about the dangerous of toxplasmosis. Proper measures are needed in order to control and prevent toxoplasmosis in goats and sheep in the region.

Materials and methods

This study was carried out in order to find out the prevalence of *T. gondii* in goats and sheep of District Mardan, Pakistan. Goats and sheep were sampled by a simple random sampling method to find out the prevalence of anti-*Toxoplasma gondii* antibodies. A total of 640 samples were collected including 350 goats and 290 sheep from different localities (Takht bhai, Mehtar Ghundi, Takkar, Sher Garh, Lundkhawar, Seri Behlol, Gujar Gari, Mardan city, Sheikh Multon etc) of District Mardan. 5 ml blood was collected through disposable syringe from jugular vein. The blood was transferred to the blood collecting tubes containing EDTA and these tubes were then placed in ice box. The blood samples were

then transported to the laboratory within 24 hours. The blood was centrifuged at 3500 rpm for five minutes for the extraction of serum. The serum was separated and transferred to eppendorff tubes by using micropipette. The serum was stored at -20 °C for further analysis.

Serological examination

The commercial Indirect Hemagglutination antibody test (IHA) Kits were used for detection of antibodies against the *T. gondii* in the serum according to manufacturer protocol (SERFIB, France). The results were obtained in 2 hours for the detection of *T. gondii* antibodies in serum.

Procedure

The test procedure was carried out according to the standard protocol given by the manufacturer (SERFIB, France). Reagents and samples were allowed to return to room temperature. A stock dilution of test serum of 1:40 was prepared. For the preparation of 1:40 stock dilution of test serum, 1.95 ml (1995 µL) of phosphate buffer solution was delivered into haemolysis tube. Then 50 µL of test serum was delivered into haemolysis tube and was mixed, 50µL of phosphate buffer solution was delivered in 8 wells of the microplate, 50 µL of serum stock dilution was added in the 1st well of the microplate. It was mixed with phosphate buffer and transferred, preferably by means of a microdilutor (tulip), 50µL from the 1st well into the 2nd well, from 2nd into the 3rd well, and so on until the 6th well. 50µL from the 6th well was discarded. The different dilutions were obtained.

Red blood cells suspensions were carefully shaked. A drop of sensitized red blood cells was distributed in the first 6 wells. One drop of un-sensitized red blood cells was distributed in the 7th well (positive serum control). One drop of sensitized red blood cells was distributed in the 8th well (reagent control). The wells content were homogenized very carefully by lateral thrumming on the edges of the microplate, placed flat wise. The plate was allowed to remain motionless, protected from vibrations. The reaction was read 2 hours later and was observed for positive and negative results. All sera reactivated at ≥1:80 were considered as positive.

Statistical analysis

The results were expressed in percentages. The values between different sex and age groups of goats and sheep were analysed by using Chi Square test for Windows (Release 16.0 standard version). The P value < 0.05 was considered as statistically significant.

Results

A total of 640 animals including goats and sheep from different localities (Takht bhai, Mehtar Ghundi, Takkar, Sher Garh, Lundkhawar, Seri Behlol, Gujar Gari, Mardan city, Sheikh Multon) of District Mardan, Pakistan were examined for the presence of T. gondii antibodies by using IHA. Out of 640 animals 276 (43.12%) were detected positive for T. gondii at dilution ≥1:80. Toxoplasma gondii antibodies were detected in 148 (42.28%) out of 350 examined goats while T. gondii antibodies were detected in 128 (44.13%) out of 290 examined sheep. A high percentage of infection was found in sheep as compared to goats but the difference was not significant (Table 1).

Table 1. Seroprevalence of *T. gondii* infection in goats and sheep of District Mardan.

Species	Number of examined	Number of positive	Percentage of positive (%)
Goats	350	148	42.28
Sheep	290	128	44.13
Total	640	276	43.12

Out of 150 male goats, 39 (26%) were detected seropositive. In 200 examined female goats, 109 (54.5%) were detected seropositive for T. gondii infection. The prevalence of toxoplasmosis was higher in females as compared to male goats. 55 (45.83%) out of 120 examined male sheep were detected seropositive for T. gondii antibodies while 73 (42.94%) out of 170 female sheep were detected seropositive for T. gondii antibodies. High seroprevalence of toxoplasmosis was seen in female sheep as compared to male sheep. A significant difference was found in the male of goats and sheep. Similar results were observed for male and female of goats and sheep. Although the percentage of infection in male sheep was higher (45.83%) as compared to female sheep (42.94%) but the difference was non-significant (Table 2).

Table 2. Sex wise distribution of goats and sheep with *T.gondii* infection.

Sex	Number of examined	Number of positive	Percentage of positive (%)
Male Goats	150	39	26
Female	200	109	54.5
Male Sheep	120	55	45.83
Female	170	73	42.94

Table 3. Age wise distribution of goats and sheep with *T.gondii* infection.

	Age	Number of examined	Number of positive	Percentage of positive
Goats	≤1	50	10	20
	1-2	120	40	33.33
	≥2	180	98	54.44
Sheep	≤1	60	8	13.33
	1-2	110	40	36.36
	≥2	120	80	66.66

The prevalence also varied in different age groups of goats ranging from 20% to 54.44%. Out of 50 examined goats whose age was less than one year, 10 (20%) were detected seropositive for *T. gondii* infection. The *T. gondii* infection was found in 40 (33.33%) out of 120 examined goats of age 1-2 years. The highest prevalence was observed in goats of age more than 2 years whose prevalence was 98 (54.44%) out of 180 examined goats. *Toxoplasma gondii* infection was also examined in different age groups of sheep. Out of 60 examined sheep whose age was less than 1 year 8 (13.33%) were detected positive for *T. gondii* infection while (36.36%) sheep were infected between age group 1-2 years. High percentage (66.66%) of toxoplasmosis was observed in age group of more than 2 years. Statistical analysis showed that seroprevalence of *T. gondii* infection was significantly higher in all goats and sheep of age more than 2 years (Table 3).

Discussion

The present study exhibited a higher seroprevalence of toxoplasmosis among goats and sheep in the region of District Mardan, Pakistan, 42.28 % and 44.13 % respectively. The prevalence of *T. gondii* infection varies among countries, depending on customs and traditions of the people living there (Smith, 1991). Out of 640 examined animals (goats and sheep) 43.12% were detected positive for toxoplasmosis by IHA, which is higher than that reported from Guangxi 9.2% (Lv and Cui, 1994) but is lower than that reported from Xinjiang 46.4% (Mi *et al.*, 2007).

In present study, prevalence of toxoplasmosis in sheep is 44.13% which is less than that reported from Canada 57.6% (Waltner-Toews *et al.,* 1991), Greece 48.6 % (Tzanidakis *et al.,* 2012) and Brazil 46.2 % (Silva *et al.,* 2013). The seropositivity rate of 44.13% found in sheep in present study is higher than 31% reported in Turkey (Oncel *et al.,* 2006) and Northeastern China 4.4 % (Yang *et al.,* 2013). *Toxoplasma gondii* infection in sheep is worldwide in distribution (Tenter *et al.*, 2000). Prevalence of toxoplasmosis is widespread in goats and sheep in the present study, which show resemblance with other work (Mirdha *et al.,* 1999; Chandrawathani *et al.*, 2008).

Prevalence of *T. gondii* infection of 42.28% found in sera of goats in present study is higher than 35.5% from Malaysia (Chandrawathani *et al.*, 2008), Greece 30.7 % (Tzanidakis *et al.,* 2012), Brazil 30.6 % (Neto *et al.,* 2008), Mexico 31 % (Alvarado-Esquival *et al.,* 2011), Thailand 27.9 % (Jittapalapong *et al.,* 2005), but is lower than that reported from Romania 52.8 % (Iovu *et al.,* 2012).

In the present study prevalence of *T. gondii* infection in sheep (44.13%) and goats (42.28%) is higher than Pakistan (11.2 % sheep, 25.4 % goats; Ramazan *et al.,* 2009), Pakistan (2.5 % sheep, 0 % goats; Zaki, 1995) and Iran (6.7 % sheep, 4.6 % goats; Kamani *et al.,* 2010) but is lower than reported from Brazil (60.8 % sheep, 81.8 % goats; Costa *et al.,* 2012) while a similar results of 44.1 % sheep and 42.8 % goats were reported from West Indies (Chikweto *et al.,* 2011).

A higher seropositivity rate of 43.12% was detected in our study in animals (goats and sheep) as compared to previous studies (Singh *et al.*, 1967; Rajamanickam *et al.*, 1990) and (Normaznah *et al.*, 2004). The age of the goats and sheep were analysed for the association with *T. gondii* infection (Table 3). The prevalence of *T. gondii* infection varied in different age groups of goats and sheep, ranging from 20 %- 54.44 % and 13.33 %- 66.66 % respectively with the highest prevalence of 54.44 % in goats and 66.66 % in sheep for the age of ≥ 2 years old. A positive correlation between age and toxoplasmosis was observed as previously reported (Ramazan *et al.,* 2009 and Kamani *et al.,* 2010).

A high prevalence of *T. gondii* infection was observed in females as compared to male goats and sheep which are similar to the previously conducted studies (Ramazan *et al.,* 2009 and Swai and Kaaya, 2012). The infection rates were different from different regions because of different exposure to the disease. 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 favourable for the spread of toxoplasmosis (Dubey, 2010). *Toxoplasma gondii* infection is high in regions where the people eat undercooked meat, unwashed vegetables and fruits (De Moura *et al.*, 2006) and the people who have contact with cats and dogs or other domestic animals or have direct contact with the soil (Etheredge *et al.*, 2004). Toxoplasmosis is more common in those areas where people drink municipal water (Ertug *et al.,* 2005).

Conclusions

This study demonstrated that toxoplasmosis is prevalent in both the sexes (male and female) and all age groups of sheep and goats in District Mardan, Pakistan. The present study revealed that the frequency of toxoplasmosis is more in females and older goats and sheep. The goats and sheep of age more than 2 years were more likely to be seropositive with toxoplasmosis than the younger goats and sheep.

It means that older and female goats and sheep possess low immunity to toxoplasmosis. This study demonstrates that infected sheep and goats may be a potential risk for human toxoplasmosis. Therefore, proper measures should be taken to control and prevent toxoplasmosis in goats and sheep in the region.

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