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# Detection of Toxoplasma Gondii Infection in Goats and Sheep using the Indirect Haemagglutination Test in Peshawar, Kyber Pakhtunkhwa-Pakistan

***Adnan Yousaf[1]*; Ali Gul Soomro[1]; Asghar Subhani[3]; Saqib Ali Fazilani[2]; Muhammad Naeem Jan[1]; Abdullah Babar[1]; Muhammad Bilawal Arain[1]; Loveson Lakhani[1]; Muhammad Ibrahim Panhwar[2]; Khush Hal[1]; Muhammad Mubashir Farooq[1]; Zainab Lanjar[2]; Abdul Latif Bhutto[1]; Sindhu Baloch[1]; Rehana Shahnawaz[1]***

*[1]Sindh Agriculture University, Tandojam, Pakistan.*

*[2]SBBUVAS, Sakrand, Pakistan.*

*[3]University of Agriculture Faisalabad, Pakistan.*

***Corresponding Author(s): Adnan Yousaf**

Faculty of Animals Husbandry and Veterinary Science, Sindh Agriculture University, Tandojam, Pakistan.

Email: dr.adnan011@gmail.com

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**Keywords:** Toxoplasma gondii; Prevalence; Goat and Sheep; Antibodies; Indirect Haemagglutination test.

**Abstract**

The goal of this study was to find out how common Toxoplasma gondii infection is in goats and sheep in Peshawar, Pakistan. T. Gondii antibodies were detected in serum using the Indirect Haemagglutination Test (IHA). T. Gondii antibodies were identified in 192 goats (45.71%) out of a total of 420 goats. Male goats had a prevalence of n=49 (28.82%) while female goats had a prevalence of n = 147 (58.80%). Prevalence was greatest in goats aged 3 years (56.90%), followed by those aged 1-2 years (45.59%), and those aged 1 year (28.18%). Antibodies to Toxoplasma gondii were found in 178 of the 360 sheep tested (49.44%). Out of 150 male sheep, n=79 (52.67%) were found to be seropositive for T. gondii infection, while n=95 (45.24%) were found to be seropositive out of 210 female sheep. Male sheep had a higher incidence of T. gondii than female sheep. Three-year-old sheep had the greatest infection rate (63.57%), followed by 1-2 year-old sheep (52.46%), and one-year-old sheep (25.51%). T. gondii antibodies were found in greater numbers in all goats and sheep, with titers ranging from 1:80 to 1:160. In comparison to goats, sheep had a greater infection rate. The findings of this study show that T. gondii infection is highly frequent in Peshawar goats and sheep, which might be a public health concern in this area because goats and sheep are intermediate hosts for T. gondii. In order to reduce the danger of human infection by T. gondii, proper control methods and appropriate measures should be implemented in this location.

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## Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite, which causes toxoplasmosis. This disease is zoonotic which is widespread in distribution [1]. The felid family, which includes cats, are the definitive hosts of T. gondii, while warm-blooded species are the intermediate hosts [2]. T. gondii infects around 33% of the world's human population, while the incidence varies by geographical area. Toxoplasmosis prevalence was reported to be 20%-30% in the United States, 25% in Japan, 60% in the Netherlands and Italy, 50% in Finland, and 50%-60% in Poland. In some nations, the prevalence is greater than 80% [4,5]. T. gondii infection was discovered to be present in 17.4% of early school pupils in Islamabad, Pakistan [7]. In Dera Ghazi Khan, Pakistan, the prevalence rate was found to be 29.5 % [9]. The virus is spread primarily by cats and their faeces, but it can also be spread by ingesting tissue cysts in undercooked or raw meat [10]. The symptoms of the disease are mild flu-like illness characterized by fever, headache, body ache, or no symptoms at all, but people with weakened immune systems (HIV infected people or pregnant women) may experience serious illness such as weight loss, diarrhea, pneumonia, liver diseases, and central nervous system infection, which can lead to death [1,3]. Cats are capable of shedding infected oocysts and transmitting infection to intermediate hosts. When T. gondii oocysts are sporrulated up to three days after being shed in the faeces, they become infective [7,21]. Animals become infected by ingesting cat faeces, consuming contaminated meat, or passing the virus from mother to fetus [11]. Cat-infected drinking water can also spread infection to humans [7]. Cats are the carriers of this disease. Only cats are capable of sexual reproduction with T. gondii [11]. Although it is widely recommended that uninfected pregnant women avoid cats, the role of this risk factor in the spread of disease is debatable [12]. Cat ownership or contact with cats has been reported as a modest source of risk in the transmission of T. gondii infection in certain research, whereas exposure to cats has not been identified as a substantial risk factor for toxoplasmosis in other studies [17]. Toxoplasmosis infections in the acute stage can be asymptomatic, but they frequently cause flu-like symptoms in the early stages [7]. The acute stage of the disease disappears in a few days to months, giving way to the latent stage. In immune compromised people (HIV infected people or transplant recipients), latent infection can resurface [14]. This was the area's first toxoplasmosis investigation in sheep and goats. Because goats and sheep are intermediate hosts of T. gondii, infected goats and sheep may pose a public health danger in this area. The purpose of this study was to educate the general public in the area about the dangers of toxoplasmosis. Toxoplasmosis in goats and sheep in the region must be controlled and prevented using proper procedures.

## Materials and methods

The purpose of this study was to determine the incidence of T. gondii in goats and sheep in the Peshawar. To determine the prevalence of anti-Toxoplasma gondii antibodies, goats and sheep were sampled using a simple random sampling procedure. A total of n = 780 samples were collected from various locations around Peshawar. A disposable syringe was used to collect 5 mL of blood from the jugular vein. The blood was transferred to EDTA-containing blood collection tubes, which were then put in a cold box. Within 24 hours, the blood samples were transferred to the laboratory. For serum extraction, the blood was centrifuged at 4000 rpm for five minutes. Using a micropipette, the serum was separated and transported to Eppendorf tubes. For subsequent examination, the serum was kept at -20 °C.

### Serological analysis

According to manufacturer procedure, commercial Indirect Hemagglutination Antibody Test (IHA) Kits were used to identify antibodies against T. gondii in serum (SERFIB, France). For the detection of T. gondii antibodies in serum, the findings were obtained in 2 hours.

### Procedure

The test was carried out in accordance with the manufacturer's specified protocol (SERFIB, France). The samples and reagents were allowed to cool to room temperature. A 1:40 stock dilution of test serum was made. 1.95 ml (1995 µl) of phosphate buffer solution was injected into a hemolysis tube to prepare a 1:40 stock dilution of test serum. Then 50 µl of test serum were provided into a hemolysis tube and mixed, 50 µl of phosphate buffer solution were given into 8 wells of the micro plate, and 50 µl of serum stock dilution were added to the microplate's first well. It was combined with phosphate buffer and transferred 50 µl from the 1st well to the 2nd well, 2nd well to 3rd well, and so on until the 6th well, preferably using a micro dilutor (tulip). The contents of the 6th well were thrown in the amount of 50 µl. The various dilutions have been obtained. The suspensions of red blood cells were properly shaken. In the first six wells, a drop of sensitized red blood cells was disseminated. In the seventh well, one drop of un-sensitized red blood cells was disseminated (positive serum control). In the eighth well, one drop of sensitized red blood cells was disseminated (reagent control). The contents of the wells were homogenized with care by lateral thrumming on the microplate's edges, which were put flatwise. Vibrations were prevented by allowing the plate to stay static. The reaction was read two hours later and the positive and negative results were noted. All sera reactivated at a concentration of 1:80 were declared positive.

### Statistics Analysis

The percentage were used to represent the results. The Chi Square test for Windows was used to compare the values of goats and sheep of different sexes and ages (Release 16.0 standard version). Statistical significance was defined as a $P<0.05$.

## Results

A total n=780 animals, including goats and sheep, were tested for T. gondii antibodies using IHA in different areas of Peshawar. At a dilution of 1:80, n=370 (47.44%) of the n=780 animals tested positive for T. gondii. Toxoplasma gondii antibodies were found in 192 (45.71 %) of the n=420 goats tested, and n=178 (49.44%) of the n=360 sheep tested. Sheep had a higher percentage of infection than goats, but the difference was not significant (Table 1).

**Table 1:** Prevalence of T. gondii infection in goats and sheep.

| Species | Animals examined | Number of Positive | Positive (%) |
|---|---|---|---|
| Goat | 420 | 192 | 45.71 |
| Sheep | 360 | 178 | 49.44 |
| Total | **780** | **370** | **47.44** |

An n=49 (28.82%) of n=170 male goats were found to be seropositive. T. gondii infection was detected in n=147 (58.80%) of the 250 female goats examined. Female goats had a higher prevalence of toxoplasmosis than male goats.

T. gondii antibodies were detected in n=79 (52.67%) of the n=150 male sheep examined, and n=95 (45.24%) of the 210 female sheep examined. Toxoplasmosis prevalence was found to be higher in female sheep than in male sheep. The males of goats and sheep showed a significant difference. Male and female goats and sheep both showed similar results. Although male sheep had a higher infection rate (52.67%) than female sheep (45.24%), the difference was not statistically significant (Table 2).

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

| Species | Sex | Animals examined | Number of Positive | Positive (%) |
|---|---|---|---|---|
| Goat | Male | 170 | 49 | 28.82 |
| | Females | 250 | 147 | 58.80 |
| Sheep | Male | 150 | 79 | 52.67 |
| | Females | 210 | 95 | 45. 24 |
| Total | | **780** | **370** | **47.44** |

The prevalence of the disease was also different in different age groups of goats, ranging from 28.18% to 63.57%. In a study of 110 goats under the age of one year 31 (28.18%) were found to be seropositive for T. gondii infection. T. gondii infection was found in 62 (45.59%) of the 136 goats examined between the ages of 1-2 years. The highest prevalence was observed in goats of age more than 3 years whose prevalence was 99 (56.90%) out of 174 examined goats. Toxoplasma gondii infection was also examined in different age groups of sheep. Out of 98 examined sheep whose age was less than 1 year 25 (25.81%) were detected positive for T. gondii infection while 64 (52.46 %) sheep were infected between age group 1-2 years. High percentage (63.57%) of toxoplasmosis was observed in age group of more than 3 years. Statistical analysis showed that prevalence of T. gondii infection was significantly higher in all goats and sheep of age more than 3 years (Table 3).

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

| Species | Sex | Animals examined | Number of Positive | Positive (%) |
|---|---|---|---|---|
| Goat | < 1 Years | 110 | 31 | 28. 18 |
| | 1-2 Years | 136 | 62 | 45.59 |
| | > 2 Years | 174 | 99 | 56.90 |
| Sheep | < 1 Years | 98 | 25 | 25.51 |
| | 1-2 Years | 122 | 64 | 52.46 |
| | > 2 Years | 140 | 89 | 63.57 |
| Total | | **780** | **370** | **47.44** |

## Discussion

The present study exhibited a higher prevalence of toxoplasmosis among goats and sheep in the region of Peshawar, 45.71% and 49.44% respectively. The prevalence of T. gondii infection varies among countries, depending on customs and traditions of the people living there [15]. Out of 780 examined animals (goats and sheep) 47.44% were detected positive for toxoplasmosis by IHA, which is higher than that reported from Guangxi 9.2% [17,18] but is lower than that reported from Xinjiang 49.49% [19]. In this study, prevalence of toxoplasmosis in sheep is 49.44% which is smaller than that reported from Canada 57.6% [20], Greece 49.79 % [22] and Brazil 49.67% [23]. The 49.44% positivity rate discovered in sheep in this study is higher than the 31% reported in Turkey [23] and the 4.4 % recorded in Northeastern China [28]. Toxoplasma gondii infection in sheep is seen all over the world [31]. Toxoplasmosis is prevalent in goats and sheep in the current study, which is consistent with previous research [32, 33]. The prevalence of T. gondii infection in goat sera in this study was 45.71%, which is higher than the 35.5% reported in Malaysia [34], Greece (30.7 %) [35], Brazil (30.6 %) [36], Mexico (31.3 %) [37], and Thailand (27.3 %) [38]. T. gondii infection was found in 49.44% of sheep and 45.71% of goats in the current study, which is higher than Pakistan (11.2 % sheep, 25.4 % goats; [25], Pakistan (2.5 % sheep, 0 % goats [26], and Iran (6.7 % sheep, 4.6 % goats; [8], but lower than Brazil (60.8 % sheep, 81.8% goats; [4]. In comparison to previous research [28, 29], we found a higher positivity rate of 47.44% in animals (goats and sheep) in our study. The age of the goats and sheep was looked at to see if it had anything to do with T. gondii infection (Table 3). T. gondii infection was found in goats and sheep of various ages, ranging from 28.18% to 56.90% and 25.51% to 63.57%, respectively, with the maximum incidence of 56.90% in goats and 63.57% in sheep for the age of 3 years old. As previously documented, there was a positive association between age and toxoplasmosis [30, 32]. Female goats and sheep had a higher frequency of T. gondii infection than male goats and sheep, which was similar to earlier investigations [6,9]. Because different places were exposed to the disease at different times, infection rates varied. Because different serological tests with varying specificity and sensitivity are used in different investigations, it is impossible to compare prevalence data. The spread of toxoplasmosis is aided by warm, humid circumstances [13]. Toxoplasma gondii infection is common in areas where people eat undercooked meat, unwashed vegetables and fruits, and humans who come into touch with cats, dogs, or other domestic animals, or who come into direct contact with the soil [27,28]. In locations where individuals drink municipal water, toxoplasmosis is more common [16].

## Conclusions

Toxoplasmosis is frequent in both sexes (male and female) and all age groups of sheep and goats in Peshawar, according to this study. Toxoplasmosis is more common in females and older goats and sheep, according to the current study. Positive toxoplasmosis was more common in goats and sheep beyond the age of three years than in younger goats and sheep. It means that toxoplasmosis immunity is low in older and female goats and sheep. Infected sheep and goats may provide a danger of human toxoplasmosis, according to this study. As a result, suitable measures should be adopted in the region to manage and prevent toxoplasmosis in goats and sheep.

## Conflicts of interest

The authors declare no conflict of interest.

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