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Relationship of Sero-Positivity against *Toxoplasma Gondii* Antibodies with Age, Body Weight, Sex, Abortion Rate and Breeds of Goat (Caprine)

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Abstract

Background: Toxoplasma has worldwide distribution and every 3rd animal in the world is affected with *toxoplasma gondii*. *Toxoplasma* is mainly transmitted by cat, and also can be transmitted by some warm-blooded intermediate hosts.

Objective: To determine the relationship of Sero-positivity against *Toxoplasma gondii* antibodies with age, body weight, sex, abortion rate and breeds of goat (Caprine)

Methodology: Study samples were collected from all three tehsils of district D.G.Khan, Punjab for a period of six months. A total of 410 goats were included in our study. A dichotomous and multiple-choice questionnaire was designed to quantify and identify different determinants of variation among prevalence. 5ml of blood was collected from jugular vein of all the selected goats and transported to University of Agriculture, Faisalabad (UAF). latex agglutination test was used to confirm the presence of antibodies to T.Gondii.

Results: Overall prevalence of toxoplasma gondii in goat is 9.75%. Our results showed that prevalence increased with age, goat 3 year or above were found 38.7% positive and 2-2.5-year-old were 8.79% and 0 to 1.5-year-old were found 0% positive. By breed, prevalence in teddy breed was 11.55% as compared to Nachi breed 6.92%. Prevalence in female was 9.94% (higher) as compared to 8.62% in male. Higher body weight goats 47kg or above and 37-47 body weight were found positive as compared to 26-36 kg and 15-25 kg body weight. The goat samples with a toxoplasmosis abortion in history had also higher prevalence

Conclusion: Our study concludes that prevalence of toxoplasma gondii is high (9.75%) in goats of Dera Ghazi Khan and it increases with increased age, teddy breed, female gender, high body weight and abortion.

Key words: Sero-positivity; *Toxoplasma gondii*; Antibodies; Goat

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Introduction

Toxoplasma gondii is an obligatory intracellular parasite which falls in Phylum Apicomplexa. Toxoplasma is mainly transmitted by cat, and also can be transmitted by some warm-blooded intermediate hosts (1). Many host species are involved in spread of toxoplasma infection (2). Nicolle and Manceaux and Splendor in 1908 discovered the toxoplasma gondii from rodents (3). Both birds and animals are infected by toxoplasma (4). The life cycle is divided into two stages sexual stage is in feline (final host) and non-sexual stage is in non-feline member (intermediate host (3). The predilection site of parasite is intestinal mucosa (5). In Schizont stage multiplication take place in intestinal epithelial cells (6).

After infestation into intestine, infection may spread to other tissue either for a month or for entire life of animal (7). Intermediate host is naturally infected by ingesting the contaminated meat, food and water with cat feces. After infection toxoplasma is spread through blood lymphatic system but it can affect any cell in the body (8).

Toxoplasma has worldwide distribution and every 3rd animal in the world is affected with toxoplasma gondii (9). Toxoplasma is pandemic in nature and seroprevalence varies among different species 9%, 15%, 30% in cattle, goat and sheep respectively (10). It is also evident that vertical transmission of toxoplasma cyst is possible through infected milk and eggs (4). The toxoplasma infected patient show sign such as myositis, lethargy, abortion, emesis, jaundice, fever, difficult respiration, diarrhea, encephalitis and discharge from eyes. Due to zoonotic potential of toxoplasma gondii, its important increase for early detection (11). When mother is infected with T.Gondii congenital abnormalities in neonate may be seen mental abnormality, hydrocephaly, defenses, chorioretinitis, epilepsy, microcephaly, calcification (12). In immune-compromised patients of AIDS and cancers it causes neuro-pathologic infection (13).

Toxoplasma Vaccine Toxovax is only effective against sheep (14) . An antigen SAGI, expressed by tachyzoite helps in diagnostic and vaccine preparation purpose. It regulate both humoral and cellular immune response (15) . Goats are mostly raised for milk and meat purpose and some peoples used unpasteurized milk which leads to toxoplasma infection (16). *T.Gondii* infection causes fetal mummification, still birth EED (early embryonic death) and abortion (4). Toxoplasma sero-prevalence in domestic cattle and sheep was 25% and 2.5% respectively in south western Pakistan (17). In Rahim Yar Khan 19% seroprevalence was found in urban areas (18). A previous study showed that Sero-prevalence of T. Gondii was 29.6% in leprosy patients (19). Toxoplasmosis can be diagnosed by different techniques including serological (IHA, LAT, ELISA and PCR) fecal test, histological and by inoculation of lab animals (cat and mice) (20). Different animals were used worldwide for serological study (21) but in Punjab, Pakistan only very few studies have been conducted on sheep and goats to detect prevalence of toxoplasma (18). This study was conducted in district Dera Ghazi Khan on goats to find sero-prevalence of T.Gondii and their relationship for seropositivity against toxoplasma gondii with respect to sex, abortion rate, body weight, age and their breeds.

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Materials and methods

Study samples were collected from three tehsils of district D.G.Khan, punjab., differ geographically and demographically. Samples were collected randomly over a period of 6 months. The sample size was 410 goats, calculated according to the previous study (22).

The study objective was to find the prevalence of toxoplasmosis and variation among different determinants. A dichotomous and multiple-choice questionnaire was designed to quantify and identify different determinants of variation among prevalence (22). After proper selection of animal (goat) 5ml of blood was collected from jugular vein by sterile syringe and blood was later shifted to gel vacutainers (Xinle). Vacutainer was labelled with a printed label and placed in ice box (4C°) and transported to University of Agriculture, Faisalabad (UAF). To collect serum from samples, vacutainer were centrifuged at 5000 rounds per minutes for 7 minutes, after centrifugation serum were collected from vacutainer with help of micropipette and transferred into cryovials (1.5ml, imec) and preserved at -20C°. Collected blood sera were tested in laboratory with latex agglutination test kits for detection of antibodies to T.Gondii. The test kits used are commercially available with the trade name Antec Diagnostic Products, UK. The samples with cut of value of 1.32 (agglutination reaction) or above was considered positive (23). In 1.5 ml vial 20ul of latex bead was taken and after that 40ul of glycine saline buffer and 60ul of antigen was added in it and incubated for 3 hours at 37C°. after incubation solution is centrifuged at 5000 round per minutes for 10mint and supernatant was collected with the help of micro pipit, solution is re-suspend in blocking buffer (1ml) and again centrifuged at 5000 rounds per minute. Washed once again and blocking buffer (90ul) was added and thoroughly mixed before overnight incubation (4C°). Semi-quantitative method was used to perform the test on diluted serum 1:16 samples. Suspected particle (white clumps) presence shows positive result and absence of clumps shows sample is negative. All of the data was analyzed by using the SPSS version 23 software. Mean and standard deviation were documented for continuous variables, while categorical variables were calculated as percentages and proportions. A chi-square test was used for categorical variables. Logistic regression was used to determine the relationship of Sero-positivity against *Toxoplasma gondii* antibodies with age, body weight, sex, abortion rate and breeds of goat. <0.05 p value considered as significant statistically. All the data was presented in figure and tabulated form.

Results

In this study a total of 410 **b**lood samples were collected. According to the distribution of samples 200 samples were collected from D.G.Khan, 110 from Tounsa Sharif and 100 from Kot Chutta. There were 352 (85.85%) female goat samples and 58 (14.15%) samples were from male goat. Out of 410 collected samples 40 samples were found to be positive at cut of value of 1:32. Overall prevalence of toxoplasma gondii in goat was 9.75% in our study (Figure 1). Positive samples from all three tehsil were found but among them highest no of positive samples (10%) were from tehsil Tounsa Sharif and Kot Chutta (Table 1). Our results showed that prevalence increased with age, goat 3 year or above were found 38.7% positive and 2-2.5-year-old were

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8.79% and 0 to 1.5-year-old were found 0% positive (Figure 2). By breed, prevalence in teddy breed was 11.55% as compared to Nachi breed 6.92% (Figure 3). Prevalence in female was 9.94% (higher) as compared to 8.62% in male (Figure 4). Higher body weight goats 47kg or above and 37-47 body weight were found positive as compared to 26-36 kg and 15-25 kg body weight (Figure 5). The goat samples with a toxoplasmosis abortion in history had also higher prevalence (Figure 6).

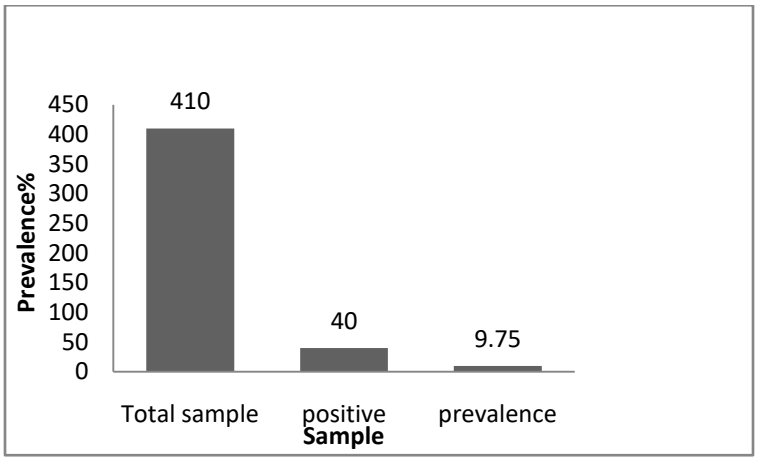

Figure 1: **Total toxoplasma gondii prevalence in goats of district D.G. Khan**

Table 1: Statistical analyses of antibodies against *Toxoplasma gondii* in goats belonging to district Dera Ghazi Khan, Punjab, Pakistan

Variable name	Category	Negative	Positive	Total	Prevalence (95%CI)	Odds Ratio	95%CI	P value
Sex	Female	317	35	352	9.94 (-)	1.17	0.44-3.12	χ^2 =0.099
	Male	53	5	58	8.62 (-)	1.00	-	P = 0.753
Age	1	33	0	33	0.00 (0.00-2.22)	-	-	χ^2 =410.0
	1-1.5	133	0	133	0.00 (0.00-8.67)	-	-	P = 0.000
	2-2.5	166	16	182	8.79 (5.11-13.88)	1.00	-	
	3	38	24	62	38.71 (26.60-51.93)	6.55	3.18-13.52	
body weight	15-25	52	0	52	0.00 (0.00-5.60)	-		χ^2 =43.092
	26-36	187	9	196	4.59 (2.12-8.54)	0.093	0.037-0.232	P = 0.000
	37-47	102	16	118	13.56 (7.95-21.08)	0.303	0.134-0.686	
	47	29	15	44	34.09 (20.49-	1.000	-	

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					49.92)			
Abortion	Yes	35	5	40	14.28 (4.8-30.26)	26.28	4.92-140.49	χ^2 =30.77
	No	368	2	370	0.54 (0.06-1.95)			P = 0.000
Area	D.G. Khan	181	19	200	9.50 (5.82-14.44)	0.95	0.43-2.07	χ^2 =0.029
	Kot Chutta	90	10	100	10.00 (4.90-17.62)	1.00	0.41-2.47	P = 0.986
	Tounsa Sharif	99	11	110	10.00 (5.10-17.19)	1.00	-	
Breed	Teddy	222	29	251	11.55 (-)	1.76	0.85-3.63	χ^2 =2.376
	Nachi	148	11	159	6.92 (-)			P = 0.123

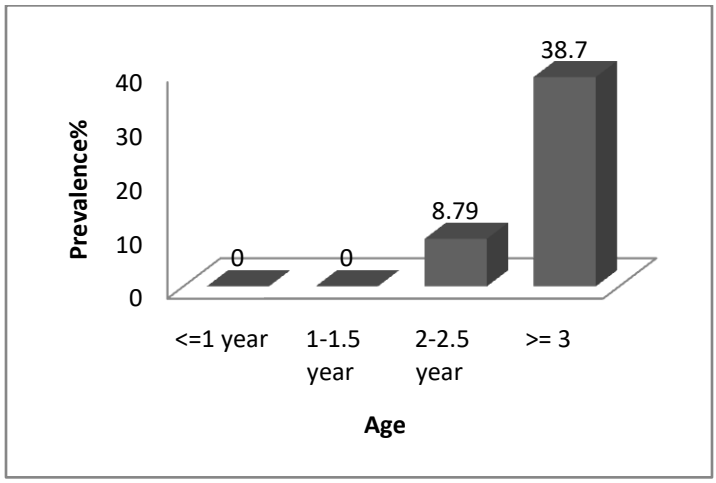

Figure 2: Toxoplasma gondii sero-prevalence related to age in D.G. Khan

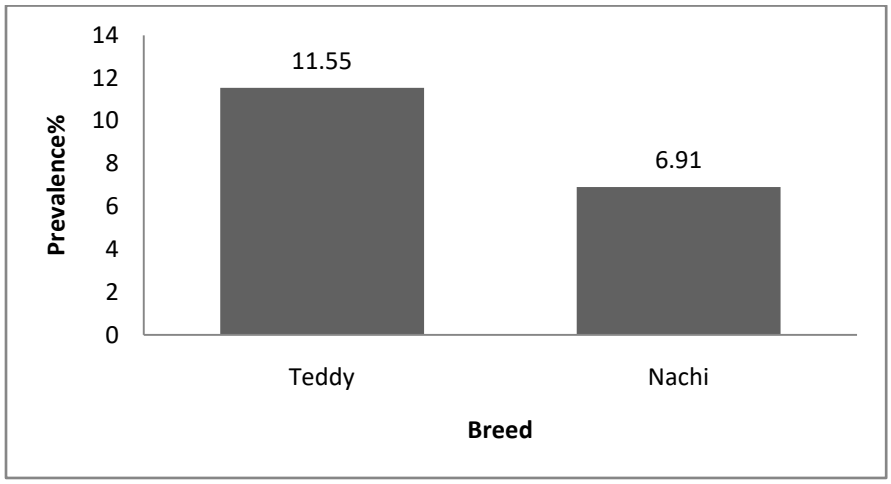

Figure 3: Toxoplasma gondii sero-prevalence related to breed in D.G. Khan

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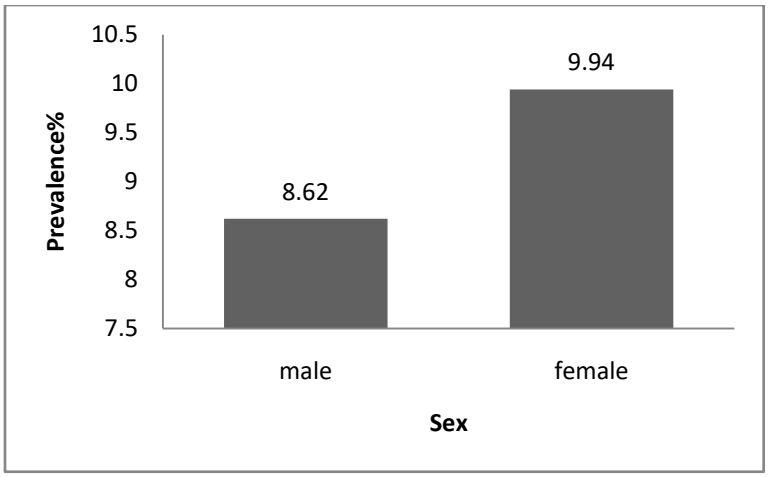

Figure 4: Toxoplasma gondii sero-prevalence related to sex in D.G. Khan

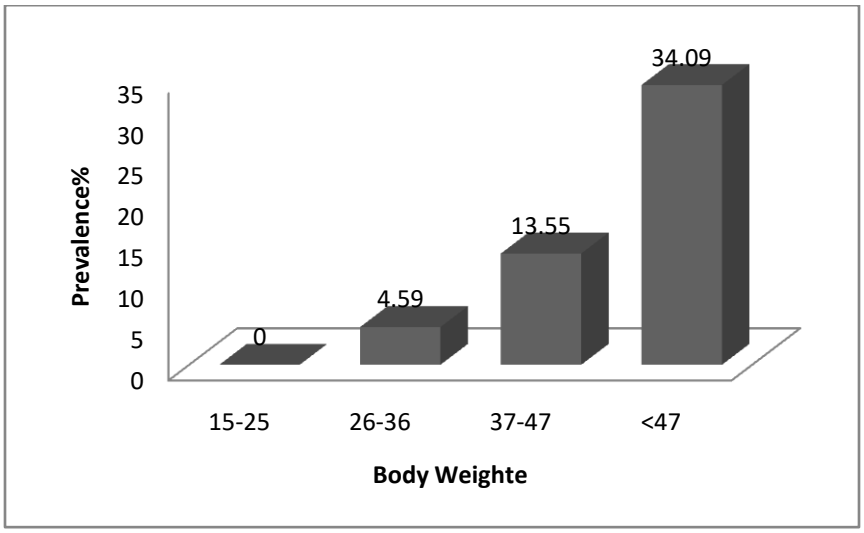

Figure 5: Toxoplasma gondii sero-prevalence related to Body weight in D.G. Khan

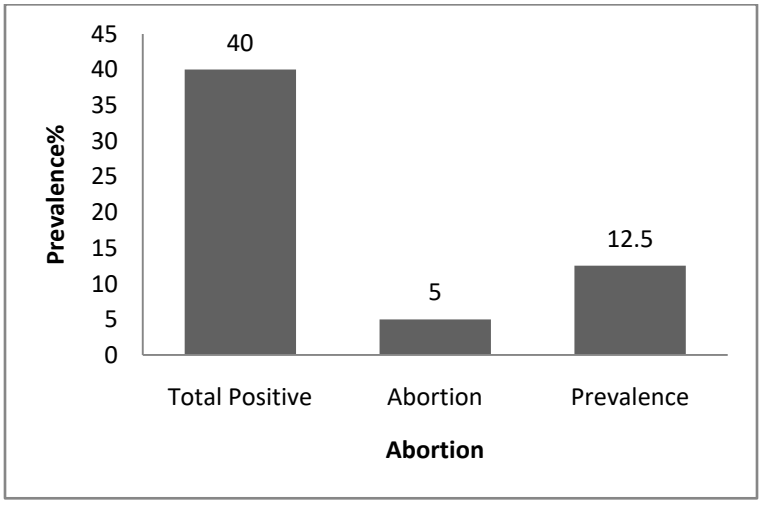

Figure 6: Toxoplasma gondii sero-prevalence related to abortion in D.G. Khan

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Discussion

Toxoplasma gondii is an important disease to be controlled as it causes financial losses by infertility, abortion, still birth and fetal death. Prevalence estimate vary among different countries and no exact estimate of toxoplasmosis in goat population is available in Pakistan. The study was piloted to find the exact prevalence of the toxoplasma gondii in goats of Punjab Pakistan.

Our study results showed us that prevalence of toxoplasma gondii at a cut of value of 1:32 was 9.75%. Our findings are in line with other studies where prevalence was difference in different sampled areas (24, 25). Sero-prevalence of Toxoplasma gondii in three tehsils of D.G.Khan was 10% in Tounsa Sharif, 10% in Kot Chutta and 9.5% in tehsil D.G.Khan. The prevalence was significantly higher in tehsil D.G.Khan then other two tehsils (Tounsa Sharif and Kot chutta) of Dera ghazi khan district. The higher prevalence may be due to large no of stray cat (definitive host) in these tehsils which help in disease dissemination or it may be due the cyst survival in environment due to favorite environmental condition. 40 goats were found positive among 410 goats' blood samples tested for toxoplasma. Depending upon the climate zone, livestock practices, customs, living life style, animal age and tradition of different countries prevalence rate vary from 0 to 100% (26). In another study in Goat population of Saudi Arabia 12% prevalence were found which is like our findings (27). Our study result showed us that prevalence was 9.75% in goats which is lower than other researches by different authors in different parts of the world. In southern Punjab, Pakistan, a previous study (18) showed 25.4% prevalence. Two studies conducted in Brazil and Thailand showed 28.9% and 27.9% prevalence respectively (11, 21). In Egypt 59.4% and Zimbabwe 67.9% prevalence rate was found which is very high from our findings (24, 28). This difference from our results may be because that these researches might be done in different climatic zones, no of animal in farm, different management practices adopted, cats population, breed difference, host age and sex (22).

On the basis of sex, males showed significantly ($P<0.05$) lower prevalence of 8.62% than females which showed higher prevalence of 9.94%. A previous study was conducted to find the exact prevalence of anti- toxoplasma antibodies in Ghanaian sheep and goats' population and our study results agree with this study. Anti-toxoplasma gondii antibodies prevalence in females was significantly higher than in males (18, 22). Due to sex difference, hormones vary among male and female and due to hormonal difference; there is also difference in infection susceptibility. Now it's proven by researches that the immunes system directly influenced by different hormones (29). Antibody production increased by estrogen hormone and suppressed by androgen i.e. both B-cell and T-cell immune response, female immune response is also affected by different factors such as environmental factors, age, nutrition, and gestation period (30).

In teddy and Nachi breeds prevalence was 11.55% & 6.92% respectively but in teddy breed goat's infection rate was higher than Nachi breed goats which shows that difference was non-significant ($P>0.05$). Our finding is in line with the findings of previous study (31). Due to breed and genetic difference of animal's prevalence of toxoplasma varies (32).

By age, the toxoplasma gondii prevalence in goats' group with the age of 3 years and above had highest prevalence of 38.71% and the goats of 1-year age or below had a prevalence of 0%. This

shows that parasitic infection increases as animal become older and older. In earlier studies it is reported that animal continuous and longtime exposure to toxoplasma in environment results high prevalence in older animals (11, 33).

In Satun province of Thailand a research study was carried out in goats to find the prevalence of T.Gondii by using commercial latex kit (11). Teshale et al., 2007 reports that a goat older than 1 year was more seropositive than younger goats (34). Our study result showed that older goats are more significant than younger goats which agree with the result reported by previous study (32). Roberts et al., 2001 reported that more susceptible of the older animal to the toxoplasma might be due to their weak immunity. The goats with weight of 47kg or more had prevalence of 34.09% and the goats with the body weight of 15 to 25kg had 0% toxoplasma prevalence. It shows that T. gondii prevalence increases with the increase in body weights of animal. In our study, highest prevalence in heavy weight of animals is may be due to lower resistance to toxoplasma infection which agree with study conducted by (35).

Conclusions:

Our study concludes that prevalence of toxoplasma gondii is high (9.75%) in goats of Dera Ghazi Khan and it increases with increased age, teddy breed, female gender, high body weight and abortion. To deal with this extremely significant problem, it is recommended that some efficient local and worldwide Toxoplasma control programs and strategies be devised. It will assist in taking steps ahead to guarantee and improve animal health care systems.

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