

SEROPREVALENCE AND RISK FACTORS OF TOXOPLASMOSIS IN BEETAL GOATS IN DISTRICT FAISALABAD AND ITS ASSOCIATION WITH REPRODUCTIVE PROBLEMS

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Toxoplasmosis, due to its zoonotic importance, is a serious threat to human being and has severe consequences for livestock productivity due to abortion. In the current study, serological screening of caprine toxoplasmosis was carried out in a population of goats from five tehsils (Faisalabad, Tandlianwala, Samundri, Jaranwala and Jhumra) of Faisalabad district through random selection from October 2016 to March 2017. A total of 384 blood samples from goats were tested by using Latex Agglutination Test Kit with a cut-off value 1:32 dilution. Association of possible risk factors including age, sex, body condition scoring, lactation number, grazing management, water supply and housing management was also documented. A total of 129 (33.59%) goats were found positive serologically for *Toxoplasma (T.) gondii*. The highest seroprevalence (44.44%) was observed in Faisalabad Tehsil, followed by Jaranwala (38.04%), Jhumra (31.5%), Samundri (28.39%) and Tandlanwala (24.24%). Among the risk factors, the prevalence of Toxoplasmosis was significantly higher ($p < 0.05$) in different age groups (39.24%) water supply from canal (38.24%) and presence of definitive host (37.75%). The prevalence of *T. gondii* observed in goats was 41.55%, 38.77, 35.00% and 32.35% with history of abortion, repeat breeding, fetal mummification and still birth, respectively, the difference was non-significant ($p > 0.05$). Among the other factors, the prevalence observed was numerically high as 35.38%, 39.24%, 43.28%, 38.51% and 34.40% in goats having body condition score less than 2.5, age >5 years, lactation number >4, grazing and semi-intensive housing system, respectively. However, the difference was non-significant ($p > 0.05$). The results revealed a baseline data for the exposure of goat population in different tehsils of Faisalabad district. It is recommended that feral cats which are potential reservoirs should be investigated for exploration of risk associated with human population.

Keywords: Seroprevalence, Toxoplasmosis, Goats, Risk factors, Reproductive problems.

INTRODUCTION

Pakistan is a renowned agriculture country of the world. The agriculture sector contributes around 20.9% in the National Gross Domestic Product (GDP) and provides the livelihood to 43.5% of the rural population. In Pakistan, about 8 million families are raising livestock. During 2018-2019, Livestock shared 60.5% in agriculture and 11.2% in National GDP (GOP, 2020). Pakistan is the 4th largest goat producing country after India, Nigeria and China (Kumar *et al.*, 2018). In Pakistan, population of goats is 78.2 million heads and Punjab bear about 54% of the total goat population, which contributes 965000 tons of milk annually in Pakistan. The annual mutton production from goat in Pakistan is 748000 tones which is the main product of goat farming. Goat husbandry contributes 12% to the agriculture gross margin (GOP, 2020). Beetal is a large breed of goat and is said to be poor man cow. The average adult body weight of males and females is 65 and 45

kg, respectively (Devendra and Burns, 1983). The males of this breed are widely sold during Eid-ul-Azha festival to fetch prime price (Khan *et al.*, 2013).

Toxoplasmosis is a widely distributed parasitic disease caused by *Toxoplasma (T.) gondii*, which is an intracellular obligate apicomplexan protozoan parasite. It is widely distributed among the warm-blooded animals and considered as a zoonotic pathogen (Weiss and Dubey, 2009). The life cycle of this parasite comprises of two stages i.e. sexual and asexual. Sexual stage takes place inside the intestine of members of *Felidae* family like domestic cats (definitive host), which produces environment tolerated stage known as oocyst and the 2nd stage is asexual, which involves the encysted parasite that circulates between the prey and predator (Dubey, 2009). In sheep and goat, *T. gondii* is one of the major causes of still birth, abortion and neonatal mortality (Panadero *et al.*, 2010). Congenital transmission during

pregnancy occurs through placenta and may lead to abnormal fetal development (Dubey and Adams, 1990).

In different parts of the world, epidemiological studies have been conducted on *Toxoplasmosis* due to its economic importance in livestock sector. *Toxoplasmosis* is one of the main causes of low productivity and economic losses of goat farming which is an important source of income in terms of meat and milk production especially in Pakistan, where small holdings of goat is commonly a practice in villages. Few studies have been conducted in the past and confirmed the presence of toxoplasmosis in southern parts of the Punjab (Ramzan *et al.*, 2009).

Keeping in view the economic implication of *T. gondii* in production animals, current study was designed to investigate the prevalence of *T. gondii* in the goats of different Tehsils of District Faisalabad. Furthermore, relationship of different husbandry and reproductive factors associated with it were also studied in it. The results will deliver information to plan control strategies against *T. gondii* in goats which may minimise its infection in other species.

MATERIALS AND METHODS

Study area and management practices: The present study was carried out in district Faisalabad, Punjab, Pakistan. Faisalabad is the 3rd most populous city of Pakistan, located 31.4504° N, 73.1350° E and 176 meters above the sea level with average 784-millimeter annual rainfall (Raza *et al.*, 2014). Simple convenient random sampling method was used for selection of 454 goats from five different tehsils (Faisalabad, Tandlianwala, Samundri, Jaranwala and Jhumra) of district Faisalabad from October 2016 to March 2017 by proportional allocation according to estimated population in the study areas. These samples were randomized by using an Australian software, known as Survey Tool-Box. By using this software, we found the exact sample size of the current study i.e 384 while, considering the expected prevalence to be 50% with confidence interval limits 95% and desired absolute precision 5% to take maximum numbers of samples (Thrusfield, 2007). Goats were kept separately and in groups with sheep in different places. Mostly, these goats are grazed on unattended lands and scavenge on tree leaves present on side of roads and crop residues in the fields. Goats are rarely fed cultivated ration. On special circumstances, these animals

were fed with concentrates (oil seed cakes mostly). In the morning, flocks were taken out for grazing on the roadsides and communal lands. In the afternoon, these goats were kept in their kraal of mud walls and thorny bushes to confine animals at night and to protect them from predators.

Collection and analysis of blood: Adopting all precautionary and safety measures for blood collection, approximately 3-5 ml of blood sample was drawn aseptically through jugular venepuncture of each animal and shifted to gel-clot activator containing vacutainer (Xinle, china). After collection, samples were subjected to centrifugation (4000 rpm for 7-10 minutes), serum was harvested in cryovials (1.5 ml: Imec, China) and preserved at -20°C until subjected to latex agglutination test for *T. gondii* (Antec Diagnostic Products @ UK/*T. gondii*) by following the manufactures instructions. A cut-off value (1:32) of agglutination reaction was considered for a positive test (Elaminet *et al.*, 2012).

Statistical analysis: The number of positive samples from different tehsils in relation to different factors like sex, age and lactation number of goat, grazing management, water supply, definitive host, housing management, neonatal death time and reproductive problems (abortion, still birth, repeat breeding and fetal mummification) were computed. Quantitative association of different determinants with reference to toxoplasmosis seroprevalence was determined through multi variant analysis and odd's ratio at $\pm 95\%$ confidence interval by using Minitab Ver. 18.

RESULTS

A total of 384 goats were screened in the current study. The overall prevalence of toxoplasmosis in goats of district Faisalabad was found to be 33.59%. Among five tehsils of Faisalabad District, maximum prevalence of *T. gondii* was observed in Tehsil Faisalabad (44.44%), followed by Jaranwala (38.04%), Jhumra (31.50%), Samundri (28.39%) and Tandlianwala (24.24%). Odd's ratio showed that chances of occurrence of *Toxoplasma* were 0.77, 0.57, 0.50 and 0.40 times less in animals from Jaranwala, Jhumra, Samundri and Tandlianwala, respectively as compared to Tehsil Faisalabad. However, Tehsil Faisalabad, Jhumra, and Jaranwala showed significantly higher ($p < 0.05$) prevalence than that of Tehsil Samundri and Tandlianwala. However, the difference among

Table 1. Sero-Prevalence of *Toxoplasma gondii* in Beetal goats belonging to different tehsils of district Faisalabad.

Tehsil	Total Samples	Positive Samples	Prevalence (%)	Odd's Ratio	Confidence Interval
Faisalabad	72	32	44.44 ^a	-	-
Jaranwala	92	35	38.04 ^a	0.77	0.41-1.44
Jhumra	73	23	31.50 ^a	0.57	0.29-1.13
Samundri	81	23	28.39 ^b	0.50	0.25-0.97
Tandlianwala	66	16	24.24 ^b	0.40	0.19-0.83
Total	384	129	33.59		

Values with different letters for each parameter differ significantly ($p \leq 0.05$).

former three tehsils was non-significant and the same was true for the latter two tehsils (Table 1).

Of different determinants age, water supply and presence of definitive host were found to be statistically associated ($\chi^2=6.212$, $P=0.045$; $\chi^2=10.723$, $P=0.001$; $\chi^2=9.737$, $P=0.002$ respectively) with toxoplasmosis in Beetal goats. Animals 2-4 years and >5 years in age showed significantly higher prevalence as compared to <2 years old animals. Moreover, goats supplied with canal water for drinking and those reared in the presence of definitive host (cat) showed significantly higher ($p<0.05$) prevalence of toxoplasmosis as compared to their counterparts. All other risk factors including body condition scoring, lactation number of goats, sex, grazing and housing management were not associated ($p>0.05$) with prevalence of toxoplasmosis in goats (Table 2).

The sero-prevalence of *T. gondii* was 41.55, 38.77, 35.00 and 32.35% in animals with reproductive problems i.e abortion, repeat breeding, fetal mummification and still birth,

respectively (Table 3). The difference in the prevalence of *T. gondii* among the animals having different reproductive problems was non-significant. The odd ratio revealed that the animals having history of repeat breeding, foetal mummification and still birth had 0.79, 0.76 and 0.67 times less chances of seropositivity for toxoplasmosis, respectively than the animals with history of abortion, though the difference was non-significant. When the neonatal death time was considered, maximum prevalence (66.66%) was observed at 36 hours, followed by 24 hours (39.39%), the minimum prevalence was 48 at hours (11.11%), the difference was non-significant (Table 3).

DISCUSSION

The study describes sero-epidemiological investigation of toxoplasmosis in goats of five tehsils of Faisalabad district. Several studies have documented prevalence of

Table2. Potential risk factors for seropositivity of *Toxoplasma gondii* in Beetal goats by multivariant analysis.

Risk factors	Categories	Total samples	Positive samples	Prevalence (%)	Odd ratio	Confidence interval	P Value
Age (Years)	< 2 Years	168	45	26.78 ^b	-	-	Chi=6.212
	2-4 Years	137	53	38.68 ^a	1.72	1.06-2.80	P=0.045
	>5 Years	79	31	39.24 ^a	1.77	1.00-3.11	
B.C.S	< 2.5	195	69	35.38 ^a	-	-	Chi=0.571
	2.5-3.5	132	42	31.81 ^a	1.17	0.73-1.88	P=0.752
	> 3.5	57	18	31.57 ^a	0.99	0.51-1.93	
Lactation no.	1-2	123	44	35.77 ^a	-	-	Chi=2.915
	3-4	132	41	31.06 ^a	0.59	0.32-1.08	P=0.233
	>4	67	29	43.28 ^a	0.73	0.40-1.34	
Sex	Female	322	114	35.40 ^a	-	-	Chi=2.929
	Male	62	15	24.19 ^a	0.58	0.31-1.09	P=0.087
Grazing management	Grazing	309	109	38.51 ^a	-	-	Chi=2.005
	Non grazing	75	20	13.33 ^a	0.67	0.38-1.17	P=0.157
Water supply	Canal water	285	109	38.24 ^a	-	-	Chi=10.723
	Tap water	99	20	20.20 ^b	0.41	0.24-0.71	P=0.001
Definitive host	Presence of cat	294	111	37.75 ^a	0.41	0.23-0.73	Chi=9.737
	Absence of cat	90	18	20.00 ^b	-	-	P=0.002
Housing management	Intensive	73	22	30.13 ^a	-	-	Chi=0.483
	Semi intensive	311	107	34.40 ^a	1.22	0.70-2.11	P=0.487

Values with different letters for each risk factor differ significantly ($p\leq0.05$)

Table 3. Prevalence of toxoplasmosis in animals with reproductive problems and neonatal death time.

Risk factors	Categories	Total Samples	Positive Samples	Prevalence percentage	Odd ratio	Confidence interval
Reproductive problems	Abortion	77	32	41.55 ^a	-	--
	Still birth	34	11	32.35 ^a	0.67	0.29-1.57
	Fetal mummification	40	14	35.00 ^a	0.76	0.34-1.67
	Repeat breeding	49	19	38.77 ^a	0.79	0.39-1.76
Neonatal death time (hours)	24	33	13	39.39 ^a	-	-
	36	15	10	66.66 ^a	3.08	0.85-11.1
	48	9	1	11.11 ^a	0.19	0.02-1.72

toxoplasmosis in goats ranging from 28.9% to 92.0% in Brazil and 42.0, 59.8, 80.61, 63.0, 27.9, 11.6 and 12.5% in Germany, Bulgaria, Turkey, Spain, Thailand, Ethiopia and Italy, respectively (Ramzan *et al.*, 2009). In the current study, overall prevalence of 33.59% was recorded in all five tehsils of District Faisalabad. Seroprevalence reported from different studies in Pakistan were 25.4% from Rahim Yar Khan (Ramzan *et al.*, 2009), 14.32% from Pothohar (Ahmad *et al.*, 2015), 42.8% from North-Eastern Pakistan regions (Ahmed *et al.*, 2016) and 42.28% from District Mardan (Shah *et al.*, 2013).

In female goats, the prevalence observed was numerically higher as compared to male goats but differences were statistically non-significant. Xu *et al.* (2014 and 2015) also reported non-significant difference in *T. gondii* infection between females and males (9.58 vs 8.48% and 18.3 vs 13.1%, respectively). However, few studies revealed a significantly higher incidence of *T. gondii* in females than males (Van der Puijeet *et al.*, 2014; Lahmar *et al.*, 2015; Ahmad *et al.*, 2016). Moreover, Tilahun *et al.* (2018) also observed a significantly higher sero-prevalence of *T. gondii* in female sheep, goats and cattle than males in east Hararghe zone of Oromia Region, Ethiopia. Females seem to be more prone to protozoan diseases as compared to males, most probably due to stressors (gestation and lactation). Males are usually sold just after one year of age and the exposure time to protozoa is less (Jittapalapong *et al.*, 2005). It is generally assumed that, hormones particularly sex specific hormones, directly influence the immune system of an individual (Alexander and Stimson, 1988; Roberts *et al.*, 2001).

In this study, animals of more than two to five years showed significantly higher prevalence of Toxoplasmosis. Spisak *et al.* (2010) and Tilahun *et al.* (2018) reported that age was directly related with prevalence of *T. gondii*, because in older animals there is regular exposure to risk factors for a longer period than the younger animals (O' Donoghue *et al.*, 1987; Al-mabruk *et al.*, 2013). Similar findings were reported in sheep and goats by other workers (Lashari and Tasawar, 2010; Boughattas *et al.*, 2015; Lahmar *et al.*, 2015; Xu *et al.*, 2015; Ahmed *et al.*, 2016).

The results of the present study are supported by those of the previous studies (Gazzonis *et al.*, 2015), in grazing system animals graze from dawn to dusk on pastures, fields after harvesting the crops and consume water from ponds to fulfil their nutritional requirement. The oocytes being shed by the cats contaminate the fields, pastures and water sources so, it acts as a potential source for the spreading of *T. gondii* infection among the goats (Neto *et al.*, 2008; Tzanidakis *et al.*, 2014; Tilahun *et al.*, 2018). Meanwhile, the animals raised on non-grazing and tap water supply showed lower incidence rate because of appropriate hygienic standards and less chances of oocyte contamination (Anderlini *et al.*, 2011; Ahmad and Tasawar, 2016).

Recent studies by Bawm *et al.* (2016), Zhang *et al.* (2016) and Tilahun *et al.* (2018) observed that the cat's contact was a potential risk factor for occurrence of toxoplasmosis among goats. Cats act as the definitive and final host of life cycle of *T. gondii* parasite. Cats excrete the oocytes of *T. gondii* in their faeces and contaminate the fodder and water source and goats raised semi-intensively had greater chance to get infection from contaminated fodder and water. This contaminated fodder and water are the risk factors for the animals and human being as well (Neto *et al.*, 2008; Dubey, 2009). On the other side, goats raised intensively (good management practices) and in the absence of cats had low chances to get infection because of good hygienic environment provided intensively rearing system and absence of free roaming cats mean no or less oocytes contamination (Lopes *et al.*, 2010; Wang *et al.*, 2011; Bawmet *et al.*, 2016). Dalbough *et al.*, (2012) reported that abortion was one of the important risk factors for transmission of *T. gondii* among the goats. In this study attempt was also made to see, if there is any correlation between prevalence of toxoplasmosis and various reproductive disorders such as abortion, repeat breeding, fetal mummification and still birth. However, no significant correlation between these variables could be seen. This could have been due to the affect that number of goats having history of still birth and fetal mummification were very small which might have affected the results.

Conclusion: In the current study significantly higher prevalence of *Toxoplasmosis* was observed in beetal goats with age more than five years. The animals reared on canal water showed higher prevalence rate than animals kept on tap water. Significantly high prevalence rate was observed in animals that were having cats (definitive host) in their vicinity. Moreover, no significant association between prevalence of *T. gondii* in beetal goats in district Faisalabad and various reproductive disorders such as abortion, fetal mummification, still birth and repeat breeding. Goats may be directly or indirectly involved in the transmission of toxoplasmosis to humans. Moreover, feral cats are also at risk of becoming probable reservoirs of infection because of consuming goat meat from slaughterhouses. In Pakistan, it is a common practice to keep different species in the same vicinity. It is, therefore, necessary to conduct sero-epidemiological investigations in all production animals including humans to detect probable infection reservoirs.

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