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TOXOPLASMOSIS IN FOUR CAPRINE BREEDS: A FUTURE RISK OF ZOONOSIS

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ABSTRACT

The present study was carried out to evaluate the seroprevalence of anti-*Toxoplasma* antibodies in four caprines breeds that are commonly reared in Cholistan desert, Rajan Pur and Rahim Yar Khan during the period between April 2011 and March 2012. The objective of the present study was to ascertain the infection rates of toxoplasmosis in different caprine breeds so that the disease resistant goat breeds could be recommended for rearing in the study area. Blood samples were collected from 865 goats reared in 21 flocks as well as from some domestically reared goats. The samples were examined through Latex Agglutination Test to assess *T. gondii* infection. The overall seroprevalence of *T. gondii* in caprines was 29.13%. Out of total four breeds of goats the highest infection rates 33.33% were found in Beetal followed in sequence by 30.98% in Nachi, 29.06% in Teddy and the lowest infection rates were found 21.02% in Dera Din Panah (DDP) breed. Non significant (P-Value= 0.5231) differences were observed in the overall high rates of toxoplasmosis in caprines. The uniform values of *Toxoplasma* infection in all the four caprine breeds might be associated with the reason that goats are allowed to breed naturally in the mixed flocks.

Key words: Toxoplasmosis, Latex agglutination test, goats.

INTRODUCTION

Domestic goats (*Capra hircus*) play an important role in the food chain and by and large a source of revenue of the countryside population (Lebbie, 2004) all over the world. The goats are vulnerable to a range of threats posed by infectious diseases caused by various parasites (Siddiki *et al*., 2010). Additionally, the parasitic infections lead to the decline in productivity due to early death in the embryonic life, mummification, abortion or stillbirths (Edwards and Dubey, 2013) thus causing the economic losses. These infections have become a serious threat to livestock (Lashari and Tasawar, 2011). One of these infectious diseases is toxoplasmosis caused by the parasite *Toxoplasma gondii* (abbreviated as *T. gondii*). It is widespread in its distribution and can be considered as one of the most successful protozoan parasites (Doskaya *et al.,* 2006) that can cause severe infections in all the mammalian species, including caprines (Dubey, 2009) as well as dogs the world over (Jadoon *et al.,* 2009). This disease is deleterious in terms of both economy of a country and health of its people (Kijlstra and Jongert, 2009). Approximately thirty three percent of animals and the human population of the world has been estimated to be infected with *T. gondii* at an average (Sensini, 2006) while according to Cook *et al.* (2000), the infection of toxoplasmosis prevails from 30 to 63% in the humans. This infectious disease has been established as a potential economic threat for goat farming business, particularly due to abortion all over the world (Innes *et al.,* 2009) and grave food hazard for human (Kijlstra and Jongert, 2009). Some authors are of the view that it the ignored disease of poverty (Hotez, 2008) or, to be concise, toxoplasmosis can be dubbed as the disease of "poor people" particularly those living in the underdeveloped countries such as Pakistan.

T. gondii is found in three morphologically different strains which are named as tachyzoite, bradyzoite, and sporozoite. The tachyzoites are active, proliferative forms found to exist in groups biologically called the clones), the bradyzoites exist as tissue cysts, and the sporozoites are found in oocysts in the environment either soil, water or air (Dubey, 1993). As *T. gondii* is a heteroxenous parasite, it requires more than one host to complete the life cycle (Fig. 1). The sexual or asexual phase of its life cycle is completed in cats, the definitive or primary hosts, both domestic and wild or any other member of the felidae family (Boothroyd, 2009). The asexual phase of life cycle of *T. gondii* is not dependent upon the sexual cycle (Su *et al.,* 2003). After the completion of sexual or asexual life cycle, felids shed the oocysts which harbor the sporozoites (Petersen and Schmidt, 2003). On ingestion of food contaminated with oocysts, the sporozoites present in the oocysts get entry into the gastrointestinal tract of secondary host (Fig. 1) that may be any kind of warm blooded animal including cattle, buffaloes, sheep, goats, mice, humans, and birds in which the asexual period of the life cycle is completed (Boothroyd, 2009).

The occurrence of toxoplasmosis varies with the differences in climatic conditions (Dubey *et al.,* 2004). For example, toxoplasmosis is more frequent, chiefly in warm and moist climates (Dubey *et al*., 2004) because the oocysts of *T. gondii* exhibit higher endurance and survival in the areas having warm and moist climate conditions in contrast to those which possess cold and dry

ones (Van der Puije *et al.*, 2000). Hypothesizing the climate conducive for proliferation of *T. gondii* infection, we chose the current study area that experiences a longer summer starting from mid of March and ending in the mid of October with the temperature rising up to 51°C leaving notable effects of stress on the flora and fauna of the region (Ali *et al.*, 2009). On the other hand, the average rainfall in the study area is less than 10 inches and the only source of drinking water is the meager rainfall water accumulated in natural depressions locally known as "Dhands" and/or according to Ali *et al.* (2009) some man-made ponds, named as "Tobas" in which water never lasts for the long period of time either due to seepage or evaporative loss (Ali *et al.*, 2009). Therefore, the animals inhabiting this area are left with meager vegetation to be used as food.

Owing to these reasons, the animals remain under the constant stress of harsh climatic conditions that perhaps facilitates the better survival of the parasite. In present survey the caprine breeds, namely Beetal, Teddy, DDP and Nachi were selected for assessment of toxoplasmosis because of being phylogenetically close relatives of one another, as has been revealed by their mtDNA investigation (Sultana *et al.*, 2003). The study was also aimed to establish the range of toxoplasmosis rates in different caprine breeds whereby the infection resistant breeds could be suggested for rearing in the study area so that the human health could be ensured and the parasite may not prove as the future health hazard.

MATERIALS AND METHODS

Study Area and Sampling Localities Distribution: The animals used for sampling were distributed in the following quadrants of study area:

a) The Cholistan Region Localities: In this region the sampling was carried out from Lesser and the Greater Cholistan sand dunes spanned between the border of India in the east and agricultural areas of Rahim Yar Khan in the west; b) Agricultural Region Localities: In Agricultural Region of District Rahim Yar Khan, the sampling was performed from caprines reared between the boundary of Rajan Pur district and the Lesser Cholistan desert in the east; and c) Reverine Region Localities: In this region the sampling localities were distributed between Agricultural region of Rahim Yar Khan and the eastern bank of river Indus.

Sample Size: A total of 865 blood samples were collected from goats (Table 1).

Samples Collection: In this study, the random sampling technique (Thrusfield, 2005) was performed during the collection of blood samples from following four caprine breeds that are commonly populated in the herds or reared domestically:

i. Beetal (also called Aseel)
ii. Dera Din Panah (abbreviated as DDP)
iii. Nachi (named so on the basis of its dancing gait)
iv. Teddy (a nickname given due to its dwarf size)

Exclusion Criteria: Following caprines fell in the ambit of exclusion criteria in the current study: a) Jamnapari and Juttal caprine breeds that were found in very small numbers. b) The goats that were suspected to suffer from some other disease were also excluded from sampling.

Sera Preparation: The blood samples (3 to 5 ml) collected from the jugular vein of each goat in vacuum tubes without the addition of anticoagulants were allowed for about one hour to coagulate and subsequently centrifuged at 3000 RPM for 10-15 minute for separation of serum from blood corpuscles. Sera were decanted in properly labelled, hygienic serum cups and stored at -20°C for analysis. These samples were ready for the detection of specific immunoglobulin G (IgG) through serological assay.

Serological Assay: The serological analyses of anti-*Toxoplasma* antibodies in sera were performed by using commercially available kits, "Toxoplasmosis Latex" manufactured by "ANTEC DIAGNOSTIC PRODUCTS-UK for 50 or 100 tests.

Seropositivity Reaction: When the drop of LAT reagent is added and mixed serum with antibodies anti-*Toxoplasma*, an antigen-antibody reaction occurs that can be expressed as under:

LAT + Serum (antibodies) = Agglutinate

Reagent and Controls: The commercial kit, "Toxoplasmosis Latex" for 50 or 100 tests that are available contains the following contents:

a. Latex reagent (the suspension of polystyrene particles sensitized/coated with *Toxoplasma* antigens in buffer containing bovine serum albumin < 0.1 % Na-azide.

b. Positive control (the positive control shows agglutination when added to the serum).

c. Negative control (the negative control does not show agglutination when added to the serum)

Serological Assay: During the serological assay, the steps followed were as under:

Both, the reagents and serum were brought at the room temperature prior to use. Sera were diluted 1:16 in 0.9% NaCl solutions (0.1 ml of serum + 1.5 ml of 0.9% saline). One drop (50ul) of diluted serum was placed onto the black area of the slide. The latex reagent was mixed well and one drop was added to each serum drop. Both drops were mixed with the aid of a stirrer and the slide was tilted. The presence or absence of agglutination was observed within the period no longer than three minutes.

The positive sera indicated the milky Latex agglutinates, while in negative sera no agglutination was noted.

Statistical Analysis: The statistical analysis of results were carried out by using Chi-square test for qualitative variables such as infection rates in sex and breed of goats via Pearson's test through SSPS version 20.

Results Interpretations: The results were interpreted as follows: a) the positive 1:16 sera indicated the milky Latex agglutinates and b) the negative sera showed no agglutination.

RESULTS AND DISCUSSION

Seroprevalence of toxoplasmosis via Latex Agglutination Test and other techniques has previously been reported in various animal species by many authors such as (Jittapalapong *et al.,* 2007) in caprines, (Chaudhary *et al.,* 2006) in buffaloes and humans, confirmed by PCR, (Jadoon *et al.,* 2009) in dogs, (Yang *et al.,* 2013) and (Lashari and Tasawar, 2010) in sheep and (Zewdu *et al.,* 2013) in caprines. In the present findings, the overall infection rates of toxoplasmosis 29.13% (252/865) as shown in Table 1 were in agreement with 27.9% observed by Jittapalapong *et al.* (2007) in Thai goats and 30.7% reported by Tzanidakis *et al.* (2012) in caprines farmed in Greece. The findings showed the prevalence values higher than 9.0% reported by Xu *et al.* (2014) in China, but lower than 42% recorded by Chikweto *et al.* (2011) in goats sampled from various Caribbean Islands. These disparities in the *T. godii* seroprevalence rates might be associated with the differences in study methods adapted by different authors (Yu *et al.,* 2007) and ecological factors prevailing in various study areas (Dubey *et al.,* 2004).

Among different breeds, the highest infection rates 33.33% (95/285) were found in Beetal followed in order by 30.98% (66/213) in Nachi, 29.06% (50/213) in Teddy and the lowest infection rates 21.02% (41/195) were found in Dera Din Panah abbreviated as DDP (Table 1). The current results revealed the non significant (P-Value= 0.5231) (Table 1) dissimilarities in seroprevalence of *T. gondii* between all the four caprine breeds in disagreement with (Van der Puije *et al.,* 2000). The non significant difference in infection rates of anti-*Toxoplasma* antibodies in different caprine breeds can be attributed to the mixed farming which is one of the potential determinants of uniform proliferation of *T. gondii* infection (Ali *et al.,* 2009).

In Beetal breed, the toxoplasmosis infection rates were found 38.35% (28/73) higher in bucks than 31.60% (67/212) in nannies (Table 2). There was no significant (P-Value= 0.7736; OR= 1.3466; CL= 0.7749, 2.3400) difference in the *T. gondii* prevalence rates in male and female caprines of Beetal breed (Table 2) consistent with findings of Xu *et al.* (2014) reporting non significant values of infections in females (9.58%) and males (8.48%). As Beetal bucks are commercially reared as favorable meat animals in almost all the parts of Pakistan and due to their handsome morphological features such as being Roman nosed, long eared and comparatively long tailed breed of caprines, these animals are the most purchased at the event of Eidul Azha festival (the annually celebrated religious rite of Muslims when animals are slaughtered for Divine will) and for general food consumption. The higher infection in the Beetal breed of caprines implies the health hazards for human through zoonotic transmission of parasite (Dubey *et al.,* 2005). The popularity of Beetal goat breed is also evident from the fact that the Agricultural region of current study area harbors the largest population of this breed of caprines in herds as well as in the mixed farms of live stock comprising cattle, buffaloes, sheep and goats. The uniformly higher infection rates in male and female Beetal breeds warrant the possibility of genital transmission of toxoplasmosis (Lopes *et al.,* 2013) in study area in general and particularly in the agri-based localities of Rahim Yar Khan region.

The overall infection rates in the Teddy caprine breed were found 29.06% (Table 1) in close agreement with toxoplasmosis rates in caprines 27.9% reported by Jittapalapong *et al.* (2007) in Thailand while the infection rates were higher than 19.7% reported by Zewdu *et al.* (2013) in caprines populated in Ethiopia. The Teddy bucks showed the overall 30.50% (18 /59) incidence rates of toxoplasmosis higher than female Teddy goats showing 28.31% (32/1133) positivity for *T. gondii* infection (Table 2) consistent with (Van der Puije *et al.,* 2000; Ramzan *et al.,* 2009). Nevertheless, the results revealed non-significant differences (P-Value = 0.9930; Odds Ratio= 1.1112; CL= 0.5580, 2.2128) between the infection rates prevailing in male and female Teddy caprines (Table 2). The higher values of toxoplasmosis established the poor management techniques (Zewdu *et al.,* 2013) adapted by Teddy goat breeders. This is pertinent to mention that present study was a de novo investigation to ascertain *Toxoplasma* infection at breeds' level in Teddy caprines populated in drought facing Cholistan desert and floods inflicted Rajan Pur region. The uniformity in incidence of toxoplasmosis in the bucks and does can also be explained through the evidence that the Teddy males are usually not made wether (sterile) unlike the males of other caprine breeds that are castrated in the early age to increase the body size. Such practice, it is suspected, provides the free hand to highly aphrodisiac Teddy bucks to have the chance of settling their fellow females Teddies leading to the genital transmission of toxoplasmosis (Salant *et al.,* 2013). The Teddy females being highly prolific might be responsible for the vertical transmission of *Toxoplasma* infection (Habibi *et al.,* 2012) transplacentally during the gestation and also to the

human after their freshening though milk. Apart from this, the Teddy goat breed was found reared in the mixed caprine herds suggesting the possible transmission of *T. gondii* from the other breeds of goats in the horizontal fashion (Lim *et al.,* 2013).

The overall seroprevalence rates recorded in DDP breed, so far not reported by any author, were 21.02% (Table 1). The gender wise statistical analysis showed that the infection rates in DDP were 24.13% and 20.48% in DDP breed male and females correspondingly (Table 2). The study results revealed non-significant (P-Value= 0.9777; OR= 1.2352; CL= 0.4878, 3.1279) (Table 2) seroprevalence differences in DDP bucks and nannies in agreement with (Ntafis *et al.,* 2007) who have also reported the non significant differences in the rates of *Toxoplasma* infections in females and male goats. The findings of this study disagreed with (Van der Puije *et al.,* 2000) in Ghana establishing significantly higher infections of toxoplasmosis in female caprines as compared with male animals, probably, due to the disparity in management strategies used for goat flocks in the respective areas of both studies (Zewdu *et al.,* 2013). The equal rates of infection speak of the possible natural inbreeding resulting in sexual transmission (Gilbert *et al.,* 2003) of toxoplasmosis in the DDP caprines due to less care for this less costly breed. The seroprevalence of *T. gondii* infection were less than the mean values, about 30% reported in the world over (Sensini, 2006) and the infection rates found in the overall caprine population sampled in the current investigations. An important justification of the lower rates of toxoplasmosis in this breed was the remoteness of its habitat in the Lesser Cholistan and the Greater Cholistan sand dunes localities with lesser access of cats than other regions of present study area (Hove *et al.,* 2005). The evenness in the seroprevalence of *T. gondii* infection in DDP caprines can be the source of the vertical (Lopes *et al.,* 2013) as well as horizontal transmission of disease (Asgari *et al.,* 2011) and impending zoonosis as well.

Out of total 213 Nachi breed of caprines sampled, 66 (30.98%) were found seropositive for infection of anti-*Toxoplasma* antibodies (Table 1). The seroprevalence rates in female 32.21% (48/149) and male animals 28.12% (18/ 64) were non-significant (P-Value= 0.9503; OR=0.8233; CL= 0.4325, 1.5673) (Table 2) in disagreement with (Van der Puije *et al.,* 2000) who have reported significantly higher values of toxoplasmosis infection in female goats as compared with males in Ghana. The varied infection rates might be the outcome of differential ecological factors prevailing in Ghana and the current study area (Dubey *et al*., 2004). The uniformly higher rates of toxoplasmosis infection can be attributed to the sexual transmission of infection within the animals (Lopes *et al.,* 2013), substandard management system (Zewdu *et al.,* 2013) and careless breeding of animals without screening of bucks and does before mating thus providing the possible chance of exchange of *T. gondii* tachyzoites' infestation during coitus. This suspicion about the sexual transmission is evident from the fact that the seminal samples taken from rams (Lopes *et al.,* 2013) and canines (Arantes *et al*., 2009) have been proven to be positive for *Toxoplasma* strains.

The uniform values of toxoplasmosis observed in all caprine breeds (Table 1) might be related with the reason that goats are allowed to breed naturally, except for the selection of morphologically sturdy bucks for the whole herd which is a sort of limited artificial selection, adapted by least number of farmers. Another aspect that justifies the uniformly higher values of *T. gondii* infection in caprines is the absolutely careless inter breeding between different breeds of goats, as was observed during the whole span of present investigation. Possibly, the interbreeding must have facilitated the parasite to make its way of dispersal in different breeds through genital passages (Habibi *et al.,* 2012). The higher infection rates bring to the hypothesis that zoonotic transmission (Dubey *et al.,* 2005) of *T. gondii* from caprines to human population might be taking place in nearby urban and remote areas of Pakistan where these animals are sold for human's consumption.

Table 1: Overall Prevalence of Toxoplasmosis in Four Caprine Breeds

Goat breeds	No. of goats examined	No. of goats infected	Prevalence (%)
Beetal	285	95	33.33
Teddy	172	50	29.06
DDP	195	41	21.02
Nachi	213	66	30.98
Total	865	252	29.13

Chi-Square = 5.122, DF = 3, P-Value = 0.163

Table 2. Relationship between Sex and Toxoplasmosis in Four Caprine Breeds

Breed	Gender	Total Tested	Total Positive	X2	P-Value	Odds Ratio	Confidence interval Lower limit	Confidence interval Upper limit
Beetal	Male	73	28	-	-	-	-	-
	Female	212	67	1.11418	0.77367	1.3466	0.774923	2.340015
Teddy	Male	59	18	-	-	-	-	-
	Female	113	32	0.09012	0.993	1.11128	0.558079	2.212846
DDP	Male	29	7	-	-	-	-	-
	Female	166	34	0.19873	0.97775	1.23524	0.487842	3.127964
Nachi	Male	64	18	-	-	-	-	-
	Female	149	48	0.35014	0.95032	0.82337	0.432544	1.567327
Total	Male	225	71	-	-	-	-	-
	Female	640	181	0.8645	0.9498	1.16914	0.841002	1.625348

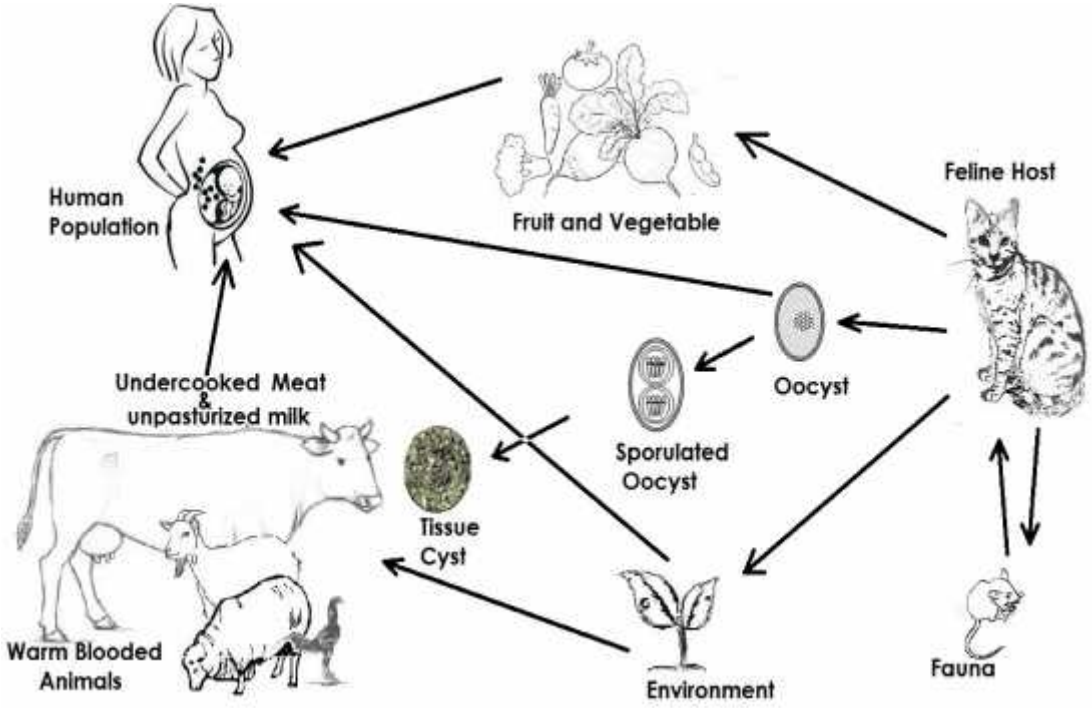

Fig. 1: Life cycle and mode of transmission of *Toxoplasma gondii*

Conclusion: The seroprevalence of toxoplasmosis in the caprine population of the study area recorded up to 29.13% demands the further study and application of control measures to prevent the proliferation of toxoplasmosis caused by parasite *T. gondii*. It was seriously noted that the basic health facilities and veterinarians in the study area were the insufficient. Thus the goats and other livestock animals are, by large, left at the mercy of parasitic infections. The gender wise uniform rates of infections in goats warrant the horizontal transmission of the disease in different caprine breeds because the parasitic infections have no boundaries.

Recommendations: The little work has been done in the study area to investigate *T. gondii* infection in the past, particularly in the livestock animal species. As *T. gondii* is transmitted to humans through zoonosis (Dubey *et al.*, 2005), it is warranted that the other species of meat producing animals must also be screened for occurrence of toxoplasmosis. Furthermore, mix farming of different caprine breeds must be discouraged. It is recommended

that the DDP breed of caprines showing less prevalence of toxoplasmosis is suggested for rearing in the study area.

Keeping in view, the high rates of toxoplasmosis in goats it is inferred that the *T. gondii* infection might be occurring in the human population of the study area. Therefore, it is also recommended that human population residing in Cholistan, Rajan Pur and Rahim Yar Khan Districts of Pakistan must also be screened for the *Toxoplasma* infection in the public interest.

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