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 felid 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...
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. gondii 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; 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 (Ntasis *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 interbreeding 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**

<table>
<thead>
<tr>
<th>Goat breeds</th>
<th>No. of goats examined</th>
<th>No. of goats infected</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beetal</td>
<td>285</td>
<td>95</td>
<td>33.33</td>
</tr>
<tr>
<td>Teddy</td>
<td>172</td>
<td>50</td>
<td>29.06</td>
</tr>
<tr>
<td>DDP</td>
<td>195</td>
<td>41</td>
<td>21.02</td>
</tr>
<tr>
<td>Nachi</td>
<td>213</td>
<td>66</td>
<td>30.98</td>
</tr>
<tr>
<td>Total</td>
<td>865</td>
<td>252</td>
<td>29.13</td>
</tr>
</tbody>
</table>

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