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Seroprevalence of caprine toxoplasmosis and associated risk factors in District Dir Upper, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Toxoplasmosis is a zoonotic disease caused by obligate, intracellular protozoan parasites, Toxoplasma gondii Nicole and Manceaux (Apicomplexa: Coccidia: Eucoccidiorida: Sarcocystidae) mainly causes abortion and still birth in humans and animals. The current research was conducted to determine the seroprevalence of toxoplasmosis and associated risk factors in goats, Capra hircus L in District Dir Upper (DU), Khyber Pakhtunkhwa (KP), Pakistan. Latex Agglutination Test (LAT) kit® was employed for this purpose. Agglutination at 1:16 dilution was considered positive. Antibodies were detected in 40 (n^+) serum samples of the total 212 (n_{total}) examined C. hircus showing an overall prevalence of 19%. The antibody titers to T. gondii positive sera were 7%, 5%, 5% and 2% by dilution of 1:16, 1:32, 1:64 and 1:128, respectively. Prevalence in males was 21% and in females 18%. There was no significant difference between toxoplasmosis and sex of C. hircus. Infection rate in young was 10% compared with 22% in adults. Difference between infection and age was statistically significant. Infection was higher in pregnant C. hircus than non-pregnant. There was statistically significant association between toxoplasmosis and history of abortion. Presence of cats, Felis catus L in vicinity of C. hircus and source of drinking water were found significant risk factors for toxoplasmosis. In conclusion, toxoplasmosis is common in C. hircus in DU and may be a risk for both animals and humans in this area. Therefore, appropriate measures should be taken to lower the risk of infection.

Key words: *Capra hircus*, seroprevalence, *Toxoplasma gondii*, toxoplasmosis, zoonotic disease.

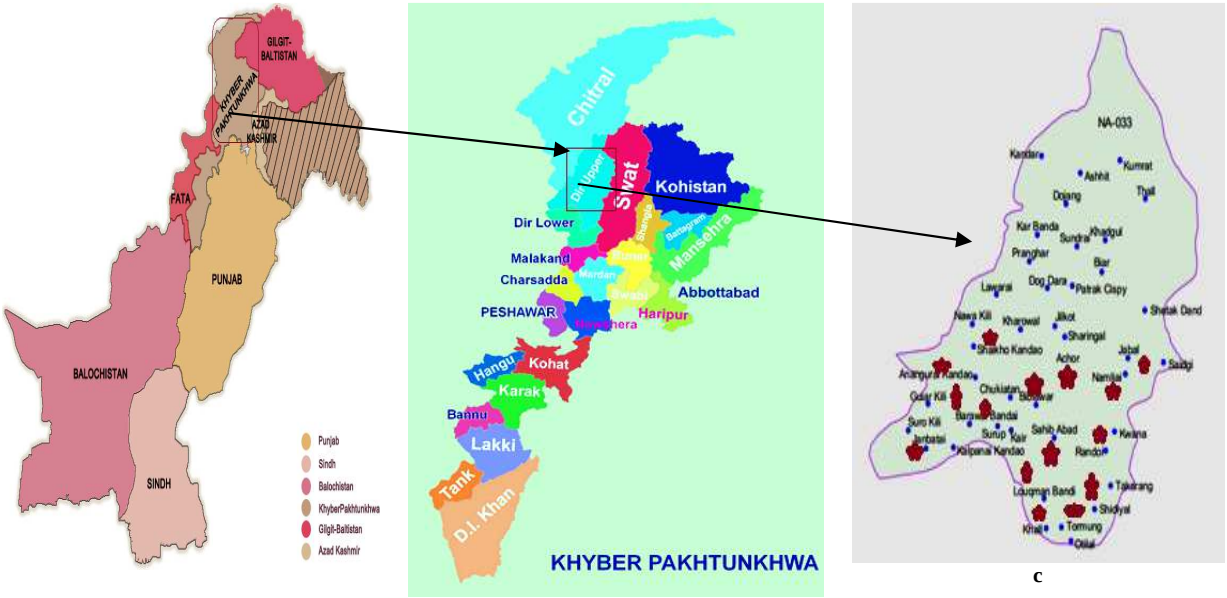
INTRODUCTION

The livestock especially goats, *Capra hircus* L, 1758 and sheep, *Ovis aries* L, 1758 are a main source of income for small farmers and poor, landless people in rural areas of Pakistan [1-2]. However, *C. hircus* are raised for milk, meat and leather production throughout the world [3]. Moreover, about 30-35 million rural population of Pakistan is engaged in livestock farming [4]. Further, Pakistan harbors about 53.8 million *C. hircus*. Furthermore, small ruminants are adversely affected by parasitic diseases [5-6]. Additionally, coccidian and helminthes are common parasites of *C. hircus* and *O. aries*. Likely, in small ruminants, toxoplasmosis is an important cause of reproductive loss [7]. Although, toxoplasmosis is the zoonotic disease caused by a coccidian protozoan, *Toxoplasma gondii* [8].

The systematic clinical signs of toxoplasmosis in *C. hircus* are abortion, still birth, death of neonates and fetuses depending upon the stage of pregnancy [9-10]. However, infection in the first half of pregnancy results in more serious consequences than in the second half. Moreover, tachyzoites have been isolated from the vaginal mucosa, saliva, nasal secretions and urine of *C. hircus* experimentally infected with parasites. Further studies have shown that tachyzoites can also be excreted in the milk of naturally infected *C. hircus* [9]. Furthermore, investigation of *T. gondii* infection is critical, as infection in these animals is a potential source of human toxoplasmosis [8]. In addition, prevalence of toxoplasmosis varies from 4-77% worldwide [11]. Although, different authors have investigated the prevalence of toxoplasmosis in domestic animals in Pakistan [12-17]. Published data about caprine

toxoplasmosis in DU, however, it is questionable to date. The present study was, therefore, planned to find out the seroprevalence of toxoplasmosis in *C. hircus* and associated risk factors in DU, KP, Pakistan. It is the first report from the study area and it will provide baseline information about the prevention and control of this zoonotic infection.

The Dir Upper (DU) is a mountainous region situated in the northern part of Khyber Pakhtunkhwa (KP), Pakistan. However, before merging into Pakistan, the whole Dir was a princely state ruled by Nawab Shah Jehan Khan. Moreover, in 1970, Dir was declared as a separate district of Pakistan and in 1996, the whole Dir was divided into 2 districts, i.e., DU and Dir Lower (DL). Further, DU comprises of 6 Tehsils and 28 Union Councils (UC). Furthermore, climate of the district is humid subtropical. Additionally, summer is moderate and warm. Although, winter is very cold and severe. While, rainfall is received throughout the year. According to livestock census (2006), there were 4069 *C. hircus* in DU (Figure 1) [18].



a b c

Figure 1 Map of the study area from where the blood samples were collected for the current research during January-May 2015; a: map of Pakistan [19]; b: map of Khyber Pakhtunkhwa [20]; c: map of Dir Upper (DU) [21] with the red stars show areas of DU from where the blood samples were collected for the present research

MATERIALS AND METHODS

The current research was carried out in Dir Upper (DU), Khyber Pakhtunkhwa, Pakistan from January-May 2015. It is situated at the 35.21° N and 71.88° E in Hindukush Mountains along the Afghanistan border between Chitral, Bajaur Agency (BA) and Dir Lower (DL). It lies at elevation of 1399 m from sea level with a total area 3699 km^2 (Online, 2015). According to population census (1998), its total population is 575858 (Figure 1) [18].

A questionnaire [22] was prepared, which was included all information about sex-age of goats, *Capra hircus* L area of sample collection, number of pregnancies and history of abortion etc have been collected information taken from *C. hircus* owners.

In order to estimate the potential risk factors of toxoplasmosis, information such as: a) presence of cats, *Felis catus* L, 1758 in households; b) sources of drinking water, e.g., rivers, streams or tap water etc; c) stagnant water sources, e.g., ponds or dams etc; d) type of managements: i) extensive: daily grazing in favorable weather conditions and returning to home at night or daily grazing with returning to home only in bad weather; ii) intensive: animal housed day and night were also noted on questionnaire at the time of sample collection.

Method

About 5 ml of blood was obtained in collecting tubes using disposable syringes and centrifuged for separation of serum [23]. The serum samples were then immediately tested for antibodies against *T. gondii* by using commercially available Toxo-Latex Test Kit® (Toxocell Latex Slide Agglutination, 100 tests, manufactured by DiaTech Diagnostic Technologies, Salerno, Italy) [24-25]. The test was performed according to the protocol given on kit. Before performing the test, reagents were tested with positive and negative control provided with the test kit to

check for validity [2, 26]. Agglutination at 1:16 dilution was considered positive. In case of agglutination at 1:16 dilution, serial dilutions at 1:32, 1:64 and 1:128 were prepared and tested again. The data were analysed through Chi-square test with a confidence interval of 95% using Statistical Package for Social Sciences (SPSS) version 16 [27].

RESULTS

In the present research, toxoplasmosis in *C. hircus* of DU, KP, Pakistan was determined. Both male and female *C. hircus* (n_t=212) were tested through Latex Agglutination Test (LAT) for the presence for *T. gondii* antibodies.

Antibodies were detected in blood of *C. hircus* [(n^+=40); (n_{total}=212)] showing an overall 19% prevalence of toxoplasmosis. Titers of antibodies to *T. gondii* were recorded up to maximum dilution of 1:128. The highest % of infection was found at 1:16 dilution. The infection % (at different dilutions) in descending order were: 7% (1:16) > 5% (1:32) = 5% (1:64) > 2% (1:128) (Figure 2).

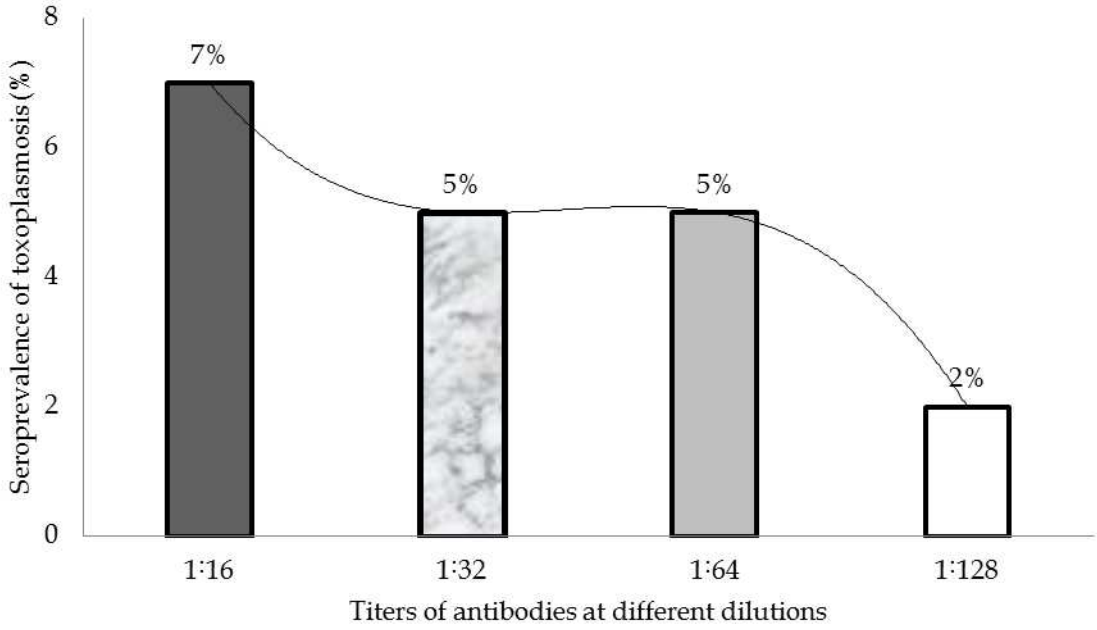


Figure 2 Seroprevalence of toxoplasmosis at different dilutions in sera of goats, *Capra hircus* L in Dir Upper, Khyber Pakhtunkhwa, Pakistan during January-May 2015; serum samples were tested by using commercially available Latex Agglutination Test (LAT) Kits®; agglutination at 1:16 dilution was considered positive; serial three-fold dilutions at 1:32, 1:64 and 1:128 were prepared in case of agglutination at 1:16 and tested again; n+=40: samples were positive; total samples were collected: n_{total}=212; %: data are shown in percentage; trend line: polynomial

In order to determine sex wise prevalence of toxoplasmosis in *C. hircus*, it showed that infection rate in males was 3% [(n_{total}=212); (n_{male}=56); (n_{female}=156)] higher than females. No significant association was found between toxoplasmosis and sex of *C. hircus* at *P*>0.05 (Table 1).

Table 1 Association of Toxoplasmosis with sex of goats, *Capra hircus* L in District Dir Upper, Khyber Pakhtunkhwa, Pakistan during January-May 2015[1]

SNo	Sex	n[2]	Positive (%)[3,4]	Negative (%)[3,4]
1	Males	56	21	79
2	Females	156	18	82
3	Total	212	19	81

[1]Serum samples were tested by using commercially available Latex Agglutination Test (LAT) Kits®; agglutination at 1:16 dilution was considered positive
[2]n: sample size (blood samples collected from number of C. hircus)
[3]Data were analyzed statistically by using Statistical Package for Social Sciences (SPSS) version 16 and were significant at P<0.05
[4]Pearson chi-square test was used to find association between toxoplasmosis and sex of C. hircus; %: data are shown in percentage; for the present data df: 1; χ^2: 0.33; P= 0.35

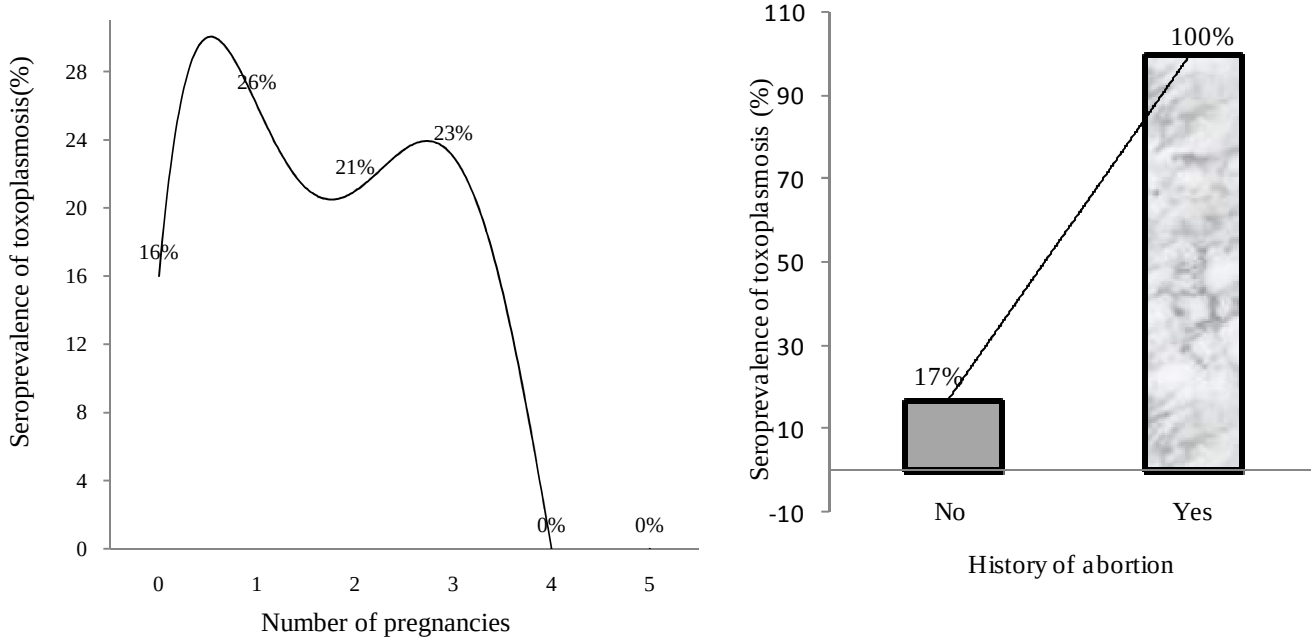
In order to determine age wise prevalence of toxoplasmosis, all *C. hircus* were divided into two groups, young: <1 year; adults: >1year. Seroprevalence of toxoplasmosis was higher in adults compared to young. Association of toxoplasmosis with age in descending order was: 22% (adults: >1year) >10% (young: <1 year). It was statistically significant at *P*<0.05 (Table 2).

Table 2 Association of toxoplasmosis with age of goats, *Capra hircus* L in District Dir Upper, Khyber Pakhtunkhwa, Pakistan during January-May 2015[1]

SNo	Age groups	n[4]	Positive (%)[6,7]	Negative (%)[6,7]
1	Young[2]	50	10	90
2	Adults[3]	162	22	78
3	T[5]	212	19	81

[1]Serum samples were tested by using commercially available Latex Agglutination Test (LAT) Kits®; agglutination at 1:16 dilution was considered positive
[2]Young'age: equal to or less than one year
[3]Adults'age: older than one year age
[4]n: sample size (blood samples collected from number of C. hircus in each age group)
[5]T: total sample size (blood samples collected from total number of C. hircus)
[6]Data were analyzed statistically by using Statistical Package for Social Sciences (SPSS) version 16 and were significant at P<0.05
Pearson chi-square test was used to find association between toxoplasmosis and age of C. hircus; %: data are shown in percentage; for the present data df: 1; χ^2: 3.36; P=0.04

Association of toxoplasmosis with number of pregnancies was also determined in *C. hircus*. Toxoplasmosis was higher in pregnant than non-pregnant. The highest % of infection was found in *C. hircus* with 1 pregnancy and the lowest with 4 and 5 pregnancies. The infection % with number of pregnancies in descending order was: 26% (1) > 23% (3) > 21% (2) >16% (0) > 0 (4) = 0 (5). However, there was no significant association between toxoplasmosis and number of pregnancy statistically at *P*>0.05 (Figure 3 a). Further, significant association was found between *Toxoplasma* seropositivity and history of abortion. All the *C. hircus* with history of abortion were found infected with *T. gondii* (Figure 3 b).



a **b**

Figure 3 Association of toxoplasmosis with number of pregnancies/history of abortion of goats, *Capra hircus* L in District Dir Upper, Khyber Pakhtunkhwa, Pakistan During January-May 2015; a: toxoplasmosis and number of pregnancies; b: toxoplasmosis and history of abortion; serum samples were tested by using commercially available Latex Agglutination Test (LAT) Kits®; agglutination at 1:16 dilution was considered positive; data were analyzed statistically by Statistical Package for Social Sciences (SPSS) version 16 and were significant at *P*<0.05; Pearson chi-square test was used to find association between toxoplasmosis and number of pregnancies/history of abortion of *C. hircus*; %: data are shown in percentage; trend line: polynomial; for number of pregnancies df: 6; χ^2: 8.32; *P* = 0.22; for history of abortion df: 2; χ^2: 9.28; *P* = 0.01; n=40; total samples were collected: n_{total}=212; %: data are shown in percentage; trend line: polynomial

In order to find out potential risk factors for toxoplasmosis in *C. hircus* from District DU, they are the following: a) presence of cats, *Felis catus* L in households; b) source of drinking water; c) type of management of *C. hircus* were analyzed statistically for acquiring infection with *T. gondii*. Among these, presence of *F. catus* in households of *C. hircus* and source of drinking water were found statistically significant *P*<0.01 (factors associated with acquisition of *T. gondii* infection). All the infected *C. hircus* had contacted with *F. catus*. Most of the *C. hircus* used running water for drinking, but infection rate was nearly 3 times higher in *C. hircus* that used stagnant water compared to running water. Additionally, the data revealed that the most of the *C. hircus* in DU were managed under extensive

system compared to intensive system, however, rate of infection was 9% higher in *C. hircus* managed under intensive system than extensive system (Table 3).

Table 3 The risk factors for toxoplasmosis were determined in goats, *Capra hircus* L from District Dir Upper, Khyber Pakhtunkhwa, Pakistan during January-May 2015[1]

SNo	Risk factors	Nature of risk factors	n[4]	Positive[5]	Prevalence (%)[6]
1	Cats, *Felis catus* in households	present	168	40	24
		absent	44	00	00
2	Source of drinking water	stagnant	50	18	36
		running	162	22	14
3	Type of management	extensive[2]	141	22	16
		intensive[3]	71	18	25

[1]Data were collected by filling printed questionnaire (n_Q=636) by the farmers
[2]Extensive: daily grazing in favorable weather conditions and returning to home at night or daily grazing with returning to home only in bad weather
[3]Intensive: Capra hircus housed day and night; chi-square test was used to find association between toxoplasmosis and risk factors
[4]n: sample size (blood samples collected from number of C. hircus)
[5]Data were analyzed statistically by using Statistical Package for Social Sciences (SPSS) version 16 and were significant at P<0.05
[6]Pearson chi-square test was used to find association between toxoplasmosis and risk factors; %: data are shown in percentage; for presence of Felis catus df: 1; χ^2: 12.91; P = 0.00; for source of water df: 1; χ^2: 12.55; P = 0.00; for type of management df: 1; χ^2: 2.93; P = 0.06

DISCUSSION

Domestic animals can act as source of toxoplasma infection to humans either through direct contact with them or through consuming their meat or products. Therefore, due to its public health importance, toxoplasmosis in goats, *Capra hircus* is studied extensively throughout the world [28]. Therefore, to keep in mind the same aim, the present study was conducted to provide awareness and education for safety of community of DU.

In the current study, overall prevalence of toxoplasmosis in *C. hircus* in Dir Upper found was 19%. However, different authors have reported different rate of toxoplasmosis from various regions of Pakistan, i.e., 53.84% from Mardan [14-15], 52% from Multan [29],14.32% from Pothwar, Punjab [30] and 6% from Lahore, Pakistan [17]. Moreover, outside Pakistan, 28.4% prevalence was reported from Iraq [31], 24% from Ethiopia [32], 21.1% from Brazil [33], 14.1% from China [34] and 13.4% from Palestine [35]. Further, several factors can affect the prevalence rate in different regions. Number of infected cats, *Felis catus* in the surrounding, susceptibility of different *C. hircus* breeds to infection, different husbandry practices and climatic conditions can be affected the prevalence of toxoplasmosis [36]. Prevalence rates were also varied among different countries due to difference in diagnostic techniques used and the number of samples tested [37]. The difference in seroprevalence of toxoplasmosis in *C. hircus* recorded in the present research when compared with the above-mentioned results can be attributed to differences in climatic conditions, husbandry practices, and serological techniques used in different regions.

At the present, males *C. hircus* showed higher prevalence of toxoplasmosis (21%) than females (18%). However, this was in contrast to studies from Punjab and Rahim Yar Khan, where higher infection rate was recorded in females than males [29]. Moreover, higher infection in female *C. hircus* than males was also reported from Ethiopia by Teshale et al. [38], while determining the prevalence and risk factors for toxoplasmosis in *C. hircus*. Generally, it is believed that female animals are more susceptible to infection with protozoan parasites compared to males [39]. Further, hormonal differences especially the sex hormones mainly affect the immunity of animals [40]. Furthermore, the male sex hormone, androgens suppresses both T-lymphocytes and B-lymphocytes thus render the male animals more prone to infections [41]. Another reason for lower infection in females than males that females *C. hircus* were usually reared inside homes for breeding and milk purposes in DU, therefore, they have less chances of contact with the oocysts of *T. gondii*.

Age of animal is an important variable and was discussed widely in literature [33]. During the present study, adult *C. hircus* with age greater than one year were found to be more infected (22%) than younger goats (10%). There was significant association between toxoplasmosis and age. Similar results were also reported by [29] from Punjab Pakistan and Ramzan et al. [12] from Rahim Yar Khan, Pakistan. Other authors had also reported higher infection of toxoplasmosis in older *C. hircus* [33, 38, 42-44]. Therefore, seroprevalence of toxoplasmosis was increased with increase in age of *C. hircus* [45] as exposure of animals to parasite in the environment increased with age [46-48]. Additionally, the immune system became weak in older age and the animal became more prone to infections [40]. Like these studies, at the present the higher rate of infection in adult *C. hircus* in DU found due to more chances of contact of these animals with outside contaminated environment compared to young ones, which usually stayed in homes in comparatively better hygienic conditions.

In the current research, pregnant *C. hircus* were found to be more infected than the non-pregnant ones. Immunity to infections usually becomes weak during pregnancy [48]. Therefore, during the present study, higher infection rate in pregnant than non-pregnant *C. hircus* recorded due to lower or gradually decreased in immunity during pregnancy.

In the present research, all *C. hircus* that had history of abortion were found infected with *T. gondii*. Previously, infection rate of 35.4% and 35.5% in *C. hircus* with history of abortion was reported from Egypt [12, 30]. It was also reported that toxoplasmosis can cause abortion in *C. hircus* at any time after about 9 days of infection [49]. However, rate of abortion in small ruminants due to toxoplasmosis was varied from 5-100% due to immune status of animal, exposure time and number of oocysts infecting the animal [50]. Moreover, the parasite suppressed the immune system in mother's placenta. Further, the developing fetus had insufficient immunity, therefore, the parasites continued to multiply in placenta and led to abortion [51]. Furthermore, abortion resulted when infection occurred in early gestation age [49]. Like the previous conducted studies, infection with *T. gondii* in 1st trimester of gestation may also be responsible for abortion in *C. hircus* in the current study.

During the present research, the presence of *F. catus* in vicinity of *C. hircus* was found a significant risk factor for acquisition of toxoplasmosis. This is in agreement to the results of Cavalcante et al. [33] who reported a higher correlation between presence of *F. catus* around the *C. hircus* herds and prevalence of toxoplasmosis in Brazil. Similar results were also observed by Neto et al. [52] from Poland and Ahmad et al. [30] from Pothwar region of Punjab, Pakistan. *Felis catus* acted as reservoir for *T. gondii* infection and were a main source of contamination of environment by releasing oocysts in faeces [53-55]. Similar to the above studies, the higher rate of infection was due to environmental contamination by infected *F. catus* faeces, which increased the chances of infection in *C. hircus*.

Drinking water can be regarded as an important source for transmission of toxoplasmosis [56]. However, significant association between toxoplasmosis and source of water used for drinking by *C. hircus* was studied during the current research. Drinking water was also reported a significant risk factor for toxoplasmosis in *C. hircus* by Ahmad et al. [30] from Pothwar region of Punjab, Pakistan. Contaminated water was acted as major source of infection in *C. hircus* than contaminated food [57]. Higher infection was recorded in *C. hircus* drinking stagnant water than those using running water in the present study. In Italy, Cenci-Goga et al. [58] reported significant higher rate of toxoplasmosis in sheep, *Ovis aries* L used stagnant water for drinking. The water contaminated with *T. gondii* can be then transmitted the infection to other animals by ingesting the infective oocysts [49]. Likewise, *F. catus* gained easier accessed to stagnant water compared to running water thus contaminated it with oocysts excreted in faeces. This contaminated water acted as the same as mentioned above in DU.

There was no significant association between toxoplasmosis in *C. hircus* and type of management. Similar results are also reported by Cenci-Goga et al. [58] in Italy and Fajardo et al. [59] in Brazil, who have investigated risk factors for toxoplasmosis in *O. aries*, *Bos indicus* L and other cattle, respectively. However, at the present, higher % of infection (25%) was found in *C. hircus* reared under intensive system compared to extensive system (16%). Most of the authors reported the opposite results in other animals [33, 43, 55, 60]. Prevalence of toxoplasmosis varied with differences in weather conditions, rearing practices, animal's age, life style, people's life style, differences in sensitivity and specificity of techniques used for diagnosis, felines number in the area and management type [90]. At the present, the differences in rate of toxoplasmosis recorded in *C. hircus* in DU from previous mentioned studies could be due to differences in weather conditions, age of animals and serological techniques used for diagnosis.

There were many limitations of the present research. Dir Upper is a mountainous region; therefore, access to all the remote areas of the district was not easy. Because, the area remained covered with snow and temperature was very low during the winter season. However, the most of people in DU involved with *C. hircus* farming were illiterate and did not know scientific ways of rearing [62]. Moreover, the most of *C. hircus* were kept in unhygienic conditions. Further, data for the present research was, therefore, collected from the lower parts of DU. Furthermore, the questionnaires were filled by asking the questions from owners orally. In future, studies using molecular methods such as Polymerase Chain Reaction (PCR) is recommended for determination of the seroprevalence of toxoplasmosis in animals, it will be great helped in confirming the seropositivity of *T. gondii* in livestock animals.

CONCLUSION

In *C. hircus* of DU, the toxoplasmosis was more prevalent in males, >1 year old, having 1 time pregnancy and history of abortion. Further, presence of *F. catus* in vicinity and source of drinking water were significant risk factors for toxoplasmosis.

Recommendations

In order to lower the prevalence and dissemination of toxoplasmosis in *C. hircus* in DU, suitable prophylactic measures should be undertaken. Further, rearing places of *C. hircus* and source of drinking water should be prevented from contamination by *F. catus* faeces. Moreover, the public especially the farmers should be educated about the spreading routes and acquisition of toxoplasmosis.

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REFERENCES

[1] J.A. Gadahi, M.J. Arshed, Q. Ali, S.B. Javaid and S.I. Shah, *Veterinary World* **2009**, 2, 2, 51-53
[2] F. Perveen, *Journal of Agricultural Science and Technology, USA* **2011**, A1, 73-80
[3] H.E. Hassan, K.M. Elamin, I.A. Yousif, A.M. Musa and M.A. Elkhairey, *Journal of Animal Science Advances* **2012**, 2, 4, 407-415
[4] K. Mehmood, M. Ijaz, M.S. Khan, M.A. Khan, A.J. Sabir and A. Rehman, *Proceedings of the first Asia Dairy Goat Conference* **2012**, 165-167
[5] G. Mulcahy, O. Sandra, D. Sheila, and J.P. Dalton, *Advanced Drug Delivery Reviews* **2012**, 56, 853-868
[6] F. Perveen, N. Ullah and I. Shabbir, *Proceedings of Parasitology* **2004**, 53, 25-36
[7] J. Kamani, A.U. Mani and G.O. Egwu, *Tropical Animal Health Production* **2010**, 42, 793-797
[8] Y. Hamzavi, A. Mostafaie and B. Nomanpour, *Iranian Journal of Parasitology* **2007**, 2, 1, 7-11
[9] M.A. Abu-Dalbou, M.M. Ababneh, N.D. Giadinis and S.Q. Lafi, *Iranian Journal of Veterinary Science and Technology* **2010**, 2, 2, 61-76.
[10] P. Xu, X. Li, L. Guo, B. Li, J. Wang, D. Yu, Q. Zhao and X. Liu, Parasites, **2014**, 21, 22-25.
[11] C.M. Hamilton, P.J. Kelly, P.M. Bartley, A. Burrells, A. Porco, D. Metzler, K. Crouch, J.K. Ketzis, E.A. Innes and F. Katze, *Parasites and Vectors* **2015**, 8, 166-171
[12] M. Ramzan, M. Akhtar, F. Muhammad, I. Hussain, E.H. Sawicka, A.U. Haq, M.S. Mahmood and M.A. Hafeez, *Tropical Animal Health Production* **2009**, 41, 1225-1229
[13] M. H. Lashari and Z. Tasawar, *Pakistan Veterinary Journal* **2010**, 30, 2, 91-94.
[14] M. Shah, M. Zahid, A. Alam, P. Asmat, A. Kausar and A. Jan, *Journal of Coastal Life Medicine* **2013 a**, 1, 1, 70-73
[15] M. Shah, M. Zahid, P. Asmat, A. Alam and A.A. Sthanadar, *International Journal of Biosciences* **2013 b**, 3, 7, 90-97
[16] Z. Tasawar, Z. Shafiq, M.H. Lashari and F. Aziz, Global Veterinaria **2013**, 11(5): 681-684.
[17] S.H. Khan, A. Maqbool, A. Anees, M.F. Qamar, K. Ashraf and N. Ahmad, *Pakistan Journal of Science* **2015**, 67, 1, 36-38
[18] Online, http//www.dirvalley.com **2015**; (Accessed: 14/4/ 2015)
[19] Online, http://nationalheritage.gov.pk/provinces.html **2015**; (Accessed: 27/7/2015)
[20] Online, http://swkpk.gov.pk/images/KP%20Map.jpg **2015**; (Accessed: 27/7/2015)
[21] Online, http://uelect.org.pk/constituency/33/NA-33+Upper+Dir **2015**; (Accessed: 27/7/2015)
[22] F. Perveen, Raqeebullah and M. Shahid, *International Journal of Medical Investigation* **2015**, 4, 2, 232-240
[23] Bakhtullah, F. Perveen, M. Shahid, A. Basit, M.A. Khan, S. Gul, I. Wazir, Raqeebullah, and K. Rahim, *Research Journal for Veterinary Practitioners* **2014**, 2 (4): 63-66.
[24] F. Perveen and M. Kashif, *Reseaarch Opinion in Animal and Veterinary Science* **2012** a, 2, 6, 412-417
[25] F. Perveen and M. Kashif, *Research Opinion in Animal and Veterinary Sciences* **2012** b, 2, 3, 145-147
[26] F. Perveen, M. Kashif and F.M. Bilqees, (2012) *Proceeding of Parasitology* **2012**, 54, 65-74
[27] F. Perveen, and Z. Hussain, *Basic Research Journal of Agricultural Science and Review* **2012**, 1, 1, 01-10
[28] J.F. Figueiredo, D.A.O. Silva, D.D. Cabral and J.R. Mineo, *Memorias do Instituto Oswaldo Cruz* **2001**, 96, 687-692
[29] Z. Tasawar, M.H. Lashari, M.H. Chaudhary and S. Hayat, *Pakistan Journal of life and Social Sciences* **2011**, 9, 1, 24-27

[30] N. Ahmad, Z. Iqbal, M. Mukhtar, M. Mushtaq, K.M. Khan and M. Qayyum, *Pakistan Journal of Zoology* **2015**, 47, 1, 161-167.
[31] J.M. Kader and Z.A.Y. Al-Khayat, *Iraqi Journal of Veterinary Sciences* **2013**, 27, 1, 21-23
[32] T. Negash, G. Tilahun, S. Patton, F. Prevot and P.H. Dorchies, *Veterinary Medicine Journal* **2004**, 155, 10, 486-487
[33] A.C.R. Cavalcante, M. Carneiro, A.M.G. Gouveia, R.R. Pinheiro and A.R.W. itor, *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **2008**, 60, 1, 36-41
[34] D. Zhou, F. Zhao, P. Lu, H. Xia, M. Xu, L. Yuan, C. Yan, S. Huang, S. Li and X. Zhu, *Parasites and Vectors* **2012**, 5, 48-52
[35] R.A. Othman and I.A. Zuheir, *Walailak Journal of Science and Technology* **2014**, 11, 1, 63-67
[36] A. Bisson, S. Maley, C.M.R. Akiiki and J.M. Watling, *Acta Tropica* **2000**, 76, 33-38
[37] A.A.H. Kadle, *Open Journal of Veterinary Medicine* **2014**, 4, 170-174
[38] S. Teshale, A. Dumetre, M.L. Darde, B**.** Merga and P. Dorchies, Parasites **2007**, 14, 155-159
[39] A. Alexander and W.H. Stinson, *Parasitology Today* **1988**, 4, 189-193
[40] C.W. Roberts, W. Walker and J. Alexander, *Clinical Microbiology Reviews* **2001**, 14, 476-488
[41] J.A.P. Da-Silva, *Annals of the New York Academy of Science* **1999**, 876, 102-118
[42] J.A. Mensah, K.M. Bosompem, E.A. Canacoo, J.M. Wastling and B.D. Akanmori, *Acta Tropica* **2000**, 76, 27-31
[43] W.N.A. Puije, K.M. Bosompem, E.A. Canacoo, J.M. Wastlaing and B.D. Akanmori, *Acta Tropica* **2000**, 76, 21-26
[44] M.M. Clementino, M.F. Souza and V.F.A. Neto, *Veterinary Parasitology* **2007**, 146, 199-203
[45] J.P. Dubey and D.S. Adams, *Journal of the American Veterinary Medical Association* **1990**, 196, 295-301
[46] S. Jittapalapong, A. Sangvaranond, N. Pinyopanuwat, W. Chimnoi, W. Khachaeram, S. Koizumi and S. Maruyama, *Veterinary Parasitology* **2005**, 127, 17-22
[47] L. Ivana, D.D. Olgica, K. K. Sofija and N. Aleksandra, *Veterinary Parasitology* **2006**, 135, 121-131
[48] M. Sharif, S. Gholami, H., Ziaei, A. Daryani, B. Laktarashi, S.P. Ziapour, A. Rafiei and M. Vahedi, *Journal of Animal and Veterinary Advances* **2006**, 5, 188-190
[49] J.P. Dubey and C.P. Beattie, (1988) *CRC Press Inc, Boca Raton, New York, USA* **1988**, 1, 220-225
[50] R.S. Younquist and W.R. Threlfall, *Saunders company, Philadelphia USA 2nd Edtn* **1997**, 1-1088
[51] T.G. Wegman and T.J. Gill, *Oxford university press, New York, USA* **1983**, 1-542
[52] J.O.A. Neto, S.S. Azevedo, S.M. Gennari, M.R. Funada, H.F.J. Pena, A.R.C.P. Araujo, C.S.A. Batista, M.L.C.R. Silva, A.A.B. Gomes, P.R.M. Iatti and C.J. Alves, *Veterinary Parasitology* **2008**, 156, 329-332
[53] J.P. Dubey and J.K. Frenkel, *The Journal of Protozoology* **1972**, 19, 155-177
[54] J. Dubey, *Journal of Parasitology* **2001**, 87, 215-219
[55] F.M. Lopes, D.D. Gonçalves, R.M. Bregano, R.L. Freire and I.T. Navarro, *The Brazilian Journal of Infectious Diseases* **2007**, 11, 5, 496-506
[56] M.M.C. Andrade, M. Carneiro, A.D. Medeiros, V.A. Neto and R.W.A. Vitor, *Parasites* **2003**, 20, 20-25
[57] G. Weilland and W. Dalchow, *Berliner und Münchener Tierärztliche Wochenschrift* **1970**, 83, 65-68
[58] B.T. Cenci-Goga, A. Ciampelli, P. Sechi, F. Veronesi, I. Moretta, V. Camniotti and P.N. Thompson, *Biomedical Central, Veterinary Research* **2013**, 9, 25-32
[59] H.V. Fajardo, S. D'ávila, R.R. Bastos, C.D. Cyrino, M. Detoni, J. Garcia, L.B. Neves, J.L. Nicolau and M.R. Amendoeira, *Parasites and Vectors* **2013**, 6, 1, 191-199
[60] L. Wang, H. Cheng, K. Huang, Y. Xu, Y. Li, J. Du, L. Yu, Q. Luo, W. Wei, L. Jiang and J. Shen, *Parasites and Vectors* **2013**, 6, 273-282
[61] J.P. Dubey, *Academic Press Elsevier Ltd. London, UK 1st Edtn* **2007**, 1-777
[62] F. Perveen and H. Shah. *International Journal of Zoological Investigations* 2015, 1(2), 118-127