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Seroprevalence of toxoplasmosis in slaughtered animals (Cow, Goats and Sheep) from butcher's shops in Mingora City Khyber-pakhtunkhwa Pakistan

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Abstract

This study was designed to evaluate the prevalence of *T. gondii* infection in abattoir animal of Mingora City from District Swat. Total 200 blood samples were collected 70 each from sheep and goat and 60 from cattle from August to December 2017. Samples were assessed through Latex agglutination test and Immunochromatographic test to establish the seroprevalence of toxoplasmosis in this region. The overall seroprevalence of Toxoplasmosis reported in this study area was 27.5% based on LAT. The prevalence of caattle was 18.3%, 27.14% in goats and 35.71% in sheep. Gender wise, seropositivity was higher in females in case of cattle and goats and males in case of sheep. Age-wise analysis revealed more prevalence of this infection adult species i.e. 3-5 years. Comparison of Latex agglutination test with Immuno chromatographic technique revealed that LAT is more effective in detecting the anti- *T. gondii* antibodies in animal sera. Majority of seropositive animals were identified in the acute stage of infection. We conclude that concludes that risk of toxoplasmosis is very high through contact with domestic animals used for human consumption in Mingora City. Frequent contact with these animals, especially sheep having highest seropositivity, will increase the risk of toxoplasmosis. Hence, measures should be taken to control the contact of these animals with other non-domestic wild animals.

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Introduction

Toxoplasma is a protozoonal parasite cause disease called toxoplasmosis.it decreseases the economical level by effecting population of veterinary , and human population . (hill *et al.*, 2005).it does not causes only abortion animals but it also has effect on public health by consuming contaminated and uncooked meat and milk that could help in dessimination of this parasites (bisson *et al.*, 2000). the mortility of neonatal and caprine abortion was increasing due to increasing the infection of toxoplasma gondii. as 50%. while in non clinical cses it causes low loss of neonatal. (radostits *et al.*, 1994).the use of goat and sheep increases day by day because of consideration good source of milk and meat by islamic people. (neto *et al.*, 2008). thetoxoplasma gondii insfection was considerably increases in islamic countries because of consuming unpasturazed milk in their traditional culture. (jittapalapong *et al.*, 2005). The farmer has big role in different countries including pakistan to increase the infection of toxplasmosis due to lack of knowledg about proper forming. Secondly slaughters also spreading this infection due to not taking care as they transport the meat from slaughter house so oocysyst in the sorrounding areas infect the meat by aerotr anmission open air mark*et al*so involve in dessimination of different infection like .t.gondii.because of mostly cats were identified there.thses foodstuff are the common source so spresad the infection. Different studies were reported about toxplasma gondii in different part of the world in animal (bisson *et al.*, 2000; ivana *et al.*, 2006; sharif*et al.*, 2006; Cattle have high natural resistance to this parasite. *T. gondii* causes subclinical infection in cattle (Dubey & Thulliez, 1994).

Cattle are considered a poor host for *T. gondii*, but serum antibodies to *T. gondii* have been found in cattle in many surveys worldwide, with prevalence ranging from 1 to 91.8% (Dubey, 2010). The overall seroprevalence of *T. gondii* in dairy cattle was 6.86% (Liu *et al.*, 2012) with a result that is slightly lower than what was reported in Anhui Province (11.50%), but higher than other regions reported in China (2.27-5.4%).

In Egypt, anti-*T. gondii* antibodies were detected in 10.8% of the cattle sera tested by enzyme-linked immunosorbent assay (ELISA)-based on shortened surface antigen 2 (TgSAG 2t) (Ibrahim *et al.,* 2009). When a modified agglutination test or ELISA was used, 43.7% sheep and 41.7% of goats were positive (Ghoneim *et al.,* 2010). A high seroprevalence of 65.6% was recorded in donkeys (El-Ghaysh, 1998) and 48.1% in horses (Ghazy *et al.,* 2007). Anti-*T. gondii* antibodies were detected in 17.4% of 166 camels (Hilali *et al.,* 1998). When poultry was tested, 47.2% of chickens and 50% of ducks were positive for anti-*T. gondii* antibodies (El-Massry *et al.,* 2000). The first study of toxoplasmosis in camels was done by El Din *et al.,* 1985 who reported an infection rate of 54% from slaughter-camel. Bornstein and Musa (1987) accounted 22.5%. Abbas *et al.* (1987) reported 12% via indirect haemagglutination test (IHA). Elamin *et al.* (1992) in Butana plains via LAT reported 67 %. Spisak *et al.*, 2010; berger-schoch *et al.*, 2011).

While in pakistan there are few reports available on sheep and goats in pakisatn like different parts punjab (ramzan *et al.*, 2009; lashari and tasawar, 2010) so this infection was questionable to date about in district swat kpk pakisatn. the current research study was conducted to fiind the most frequent source of t. gondii and its relation with age and sex to provide proper information about disease to the public and reduce the exposure.

1. To investigate the seroprevalence of *T. gondii* in animals from Butcher's shops of District Swat.
2. To compare the seroprevalence of *T. gondii* among different animal species.
3. To compare the efficiency of Latex agglutination test with Immuno chromatographic technique for detection of *T. gondii.*

Material and method

Study area

The current study was conducted in the Mingora City of District Swat at Parasitology Laboratory, Veterinary Research and Disease Investigation Center (VR & DIC) Balogram, Swat Khyber Pakhtunkhwa. Total 200 blood samples were collected: 70 each from

sheep and goat whilea 60 from cattle at Saidusharif slaughterhouse near Mingora City.

Collection of sera

5 to 10 ml blood was collected from every slaughtered animal in a syringe and kept overnight at 45°C. The serum was separated in 2ml Eppendorf tubes and brought to parasitology Laboratory Veterinary Research and Disease Investigation Center (VR & DIC) Balogram Swat for further processing. Samples were properly labeled with a specific number and background details were noted. Study duration was 4 months i.e. from August to November 2017.

Target animals

In this study goat, sheep and cattle were selected which were further divided into different age group, i.e. 0-2 young, 3-5 adult, ≥6 as per owner's information. The samples were further processed by LAT and ICT device.

Methodology

Latex Agglutination Test (LAT)

In this study, the latex agglutination test kit (SPINRER EACT, S. A. Ctra. Santa coloma, Spain) was used according to the manufacturer's guidelines. The kit comprised of toxo LAT reagent with positive and negative control. The toxo LAT reagent or buffer is a combination of polystyrene latex particles treated with soluble *T. gondii* antigen. This reagent permits naked observation of immunocomplex reaction. Pure agglutination was observed in the presence of *T. gondii* antibodies**.**

Principle and procedure

This kit works on an antigen-antibody reaction principle. Firstly, kit was checked whether it was working properly. One drop each of positive and negative control were added to the cord and 50µl toxo latex reagent was added to both of them. When kit worked properly, agglutination was formed in positive and vice versa. Serum sample was treated with 50 µl of toxoplasma reagent and mixed through stirrer on the surface of the cord circle and the cord was rotated for the examination of agglutination for five minutes. A naked agglutination was observed in case of toxoplasma antibodies presence while no agglutination was observed due to absence of Toxoplasma antibodies. The serum samples were diluted two-fold with normal saline i.e. 1:2. 1:4, 1:8, 1:6, 1:32, 1:64, 1:128. In majority of cases, positive reaction occurred at 1:16, which was taken as standard. Furthermore, all the samples were processed at this dilution by treating 50 µl of toxoplasma reagent (LAT Buffer) with 1:16 dilution of serum samples. Serum samples that displayed agglutination at 1:16 were taken positive.

Immunochromatographic test device

Immunochromatographic test device are commonly used for knowing the presence and absence of toxoplasma-specific IgM and IgG in serum sample.

Principle of ICT

The ICT device contains different elements, i.e. Sample pad, Capillary beds, Nitrocellulose membrane with burgundy colored conjugate (C CONTROL) and IgM and IgG band. These elements are involved to move the serum sample through sample pad to achieve IgM and IgG band. The primitive element is sample pad which contains microspores that are used to capture serum. The first time, when the serum sample are run on sample pad, the sample moves towards the next element (a burgundy colored conjugate pad). The conjugate PAD contains dried format of bioactive particles salt sugar matrix that are manufactured by manufacturer to speed up reaction between antigen and antibody. These salt and sugars are fixed on particle surface. When the sample fluid is run on the sample pad so serum samples absorbs the salt, sugar matrix and particles formed mixture (conjugate i.e. *T. gondii*) and this mixture is moved through porous membrane. When serum is passed on stripes, the strip area color is changed in the presence of specific antibody. There are three stripes which comprise of Control, IgM and IgG.

The control is indicator to ensure if the device is working functional. The second and third stripes are fixed with capture molecule like antibody and give reddish-purple color if the reaction is positive.

Procedure

l to 3 drops of sample were applied on sample pad of the strip. The serum sample was moved through capillary action. Formation of bands was seen in respective IgG and IgM columns. Formation of red-purple bands confirmed the presence of anti-*T. gondii* antibodies in the serum.

RESULTS

The current study was conducted in the Mingora City of District Swat to investigate seroprevalence of Toxoplasmosis in different slaughter animal species. In this study, total 200 blood samples were collected 70 each from sheep and goat while 60 from cattle at Saidu Sharifslaughterhouse near Mingora city. Samples were assessed by LAT and ICT. All the animals were further divided into different age group i.e. 0-2 young, 3-5 adult, older ≥6 on the basis of owner's information. The overall prevalence rate in different animals is given below in table 3.1.

Overall seroprevalence of Toxoplasmosis in different slaughter animal species

The overall seroprevalence of Toxoplasmosis reported in this study area was 27.5% based on LAT. The prevalence of cattle was 18.3%, 27.14% in goats and 35.71% in sheep. In this study, the highest percentage was reported in sheep which show that sheep may be most frequent reservoirs of toxoplasmosis reservoir in the area (Table 3.1).

Gender-wise seroprevalence of Toxoplasmosis based on LAT in different slaughter animal

Seropositivity of toxoplasmosis was also assessed gender-wise. In case of cattle, high seropositivity was seen in cows (22.5%) as compared to Oxen (10%). Similarly, female goats had high seropositivity (32.5%) as compared to bucks (20%). Conversely, seropositivity of sheep was higher (42.5%) as compared to Ewes (26.6%) (Table 3.2).

Distribution of serum and normal saline titer to toxoplasmosis on + ve reactions

20 serum samples were sampled from male cattle. In these 20 sera 2 (10%) were detected positive with concentration of 1 (0.5%) and 1(0.5%) by dilution of 1:2, and 1:4, as shown in table 3.2. In the 40 sera samples from female cattle, total 9 (22.5%) were positive with concentration or titer of 1(11.11%), 02 (22.22%), 0(0%), 4(44.44%), 02(22.22%) and 0(0%) by dilution of 1:2, 1:4 and 1:8,1:16,1:32,1:64 as given above in table 3.2. 30 male goat were studied in which 06(20%) were positive with concentration of, 03 (50%), 0(0%), and 02 (33.33%), 1(16.66%) by dilution of 1:2, 1:4, and 1:8, 1;16, 1:32 1:64,. 40 female goat were studied in which 13(32.5%) were positive with concentration of, 02 (15.38%), 0 (0%), and 5 (38.46%), 01(7.69%), 0(0%) , 05(38.46%) by dilution of 1:2, 1:4, and 1:8, 1;16, 1:32 1:64, as given above in table 3.2. 30, male sheep were studied in which 08(26.66%) were positive with concentration of 2 (25%), 0(0%), and 0 (0%), 04(50%), 0(0%), 2(25%) by dilution of 1:8, 1:16, and 1:32, as given above in table 3.2. 40 female sheep were studied in which 17 (42.5%) were positive with concentration of, 6 (33.33%), 0 (0%), and 0 (0%), 6(33.33%), 0(0%) 5 (27.77%) by dilution of 1:2, 1:4 and 1:8, 1:16, 1:32, 1:64 as given above in table 3.2

Age-wise seroprevalence of Toxoplasmosis based on LAT in different slaughter animal

Toxoplasmosis positive cases were mostly found in adults. Age from 3-5 is a grazing age of every animal and most of the animals face all situation of life like traveling, dirty places, openly grazing and come in contact with any other infected animal because of feces. The total positive male and female sample were distributed according to age distribution in which females were more than the males (Tables 4 to 9).

Comparison of Latex agglutination test with Immuno chromatographic technique for detection of T. gondii

In this study, ICT and LAT were used for the detection of *T. gondii*. ICT identified 41 out of 200 samples (20.5%), while LAT identified 55 out of 200 samples (27.5%). The LAT seemed more sensitive as compared to ICT that may be due to the fact that LAT can detect the smallest amount of antibodies to form agglutinates while ICT is specific to identify whether it is chronic or in acute stage but less sensitive. Although in the majority of cases, ICT showed similar results when compared to LAT, however, its

sensitivity was compromised in some cases in case of samples with low antibody titer (Table 5).

Detection of chronic and acute stage of toxoplasmosis by ICT

With the help of ICT, it is easily established if toxoplasmosis is chronic or acute by detecting the antibody isotype (IgM or IgG). Most animals detected as seropositive for toxoplasmosis had an acute stage of infection as shown in Table 3.11.

Out of 41 ICT positive samples, only 11 were identified during the chronic stage of infection (26.82%), whereas 30 out of 41 seropositive samples(73.71%) were at the chronic stage of infection (Table 3.11).

Table 1. Overall seroprevalence of Toxoplasmosis in different slaughter animal species based on LAT.

Species	No of sera tested	No of +ve reaction	No of -ve reaction	% Positivity
Cattle	60	11	49	18.3%
Goat	70	19	51	27.14%
Sheep	70	25	45	35.71%
Total	200	55	145	27.5%

Table 2. Gender –baseds eroprev alence of Toxoplas mosis based on LAT in different slaughter animal.

S.No	Animals	No. examined	seropositive	Seronegative	Percentage
1	Ox	20	02	18	10%
2	Cow	40	09	31	22.5%
3	Buck	30	06	24	20%
4	Goat	40	13	27	32.5%
5	Ewe	30	08	22	26.66%
6	Sheep	40	17	23	42.5%
Total		200	55	145	27.5%

Table 3. Distribution of serum and normal saline titer to toxoplasmosis on +ve reactions.

Species		No of sera tested	+ve reaction	Distribution of serum and normal saline titre to toxoplasmosis on +ve reactions						
				1:2	1:4	1:8	1:16	1:32	1:64	1:128
Cattle	Male	20	02	01	01	00	00	00	00	00
	Female	40	09	01	02	00	04	02	00	00
Goat	Male	30	06	03	00	02	01	00	00	00
	Female	40	13	02	00	05	01	00	05	00
Sheep	Male	30	08	02	00	00	04	00	02	00
	Female	40	17	06	00	00	06	00	05	00
Total		200	55	15	03	07	16	02	12	00

Table 4. Age group distribution of total positive samples in sheep.

Species Sheep	No of sera tested	+ve	-ve	Percentage
0-2	35	10	25	28.57%
3-5	27	12	15	44.44%
≥6 YEAR	08	03	05	37.03%
Total	70	25	45	35.71%

Table 4.1. Number of positive male and female samples distributed according to age groups in sheep.

Age distribution	Male	Female	Male +ve	Female +ve
0-2	15	20	03	07
3-5	10	17	04	08
≥6	05	3	01	2
TOTAL	30	40	8	17

Table 4.2. Age group distribution of total positive samples in Goats.

Species GOAT	No of sera tested	+ve	-ve	Percentage
0-2	35	08	27	22.85%
3-5	27	09	18	33.33%
≥6 YEAR	08	2	6	25%
Total	70	19	51	27.14%

Table 4.3. Number of positive male and female samples distributed according to age groups in goats.

Age distribution	Male	Female	Male +ve	Female +ve
0-2	15	20	03	05
3-5	10	17	02	07
≥6	05	03	01	1
TOTAL	30	40	6	13

Table 4.4. Age group distribution of total positive samples in Cattle.

Species Cattle	No of sera tested	+ve	-ve	Percentage
0-2	10	00	10	0%
3-5	30	8	22	26.66%
≥6 YEAR	20	03	17	15%
Total	60	11	49	18.33%

Table 4.5. Number of positive male and female samples distributed according to age groups in Cattle.

Age distribution	Total Males	Total Females	Male +ve	Female +ve
0-2	03	07	00	00
3-5	7	23	02	06
≥6	10	10	00	3
TOTAL	20	40	02	09

Table 5. Comparison ICT with LAT for detection of toxoplasmosis.

Animals	ICT +VE	LAT +ve
Ox	0	02
Cow	09	09
Buck	05	06
Goat	10	13
Ewe	05	08
Sheep	12	17
Total	41	55

Table 6. Samples with acute and chronic stages by ICT.

Animals type	Chronic	Acute
Cattle male	0	0
Cattle female	03	06
Goat male	0	05
Goat female	02	8
Sheep male	01	4
Sheep female	05	7
Total	11	30

Discussion

The *T. gondii* frequently exists in all parts of the world; however, there are very few reports available in Pakistan especially in Swat region. Different studies have been reported on this parasite in Pakistan has been reported in human subjects like women, young children; however, the studies on animals are neglected which are major reservoirs of this infection.Since no such studies have been reported previously especially in Mingora City, we designed this project to assess the current status of toxoplasmosis in this region in animals whose meat is utilized by the local population and estimate the risk of toxoplasmosis through transfer from these animals.

Overall, the seroprevalence of *T. gondii* in goat sheep and cattle was 27.5%, which was relatively higher as compared to the findings of Samad *et al.* (1993) who reported 16.1% of seroprevalence of this parasite in Bangladesh. The seroprevalence rate in Goat documented in our study was 27.5%. This is significantly lower than the findings of Jones *et al.* (2001) who reported 65.5% seroprevalence in North Central USA.

In sheep, 31.5% of seroprevalence rate was reported in this study. This was higher than the findings of Domy *et al.* (1992) who reported 22.3% seropositivity in sheep in Srilanka. These findings suggest that seropositivity of *T. gondii* shows huge variations in both developed and developing countries. It may be due to geographical conditions and different other factors likesample size, environmental factors, age, sex, breed and reliability of test performed. These factors may be the causes of differences in the prevalence of toxoplasmosis reported in different studies from different parts of the world.

Studies cited above showed variations in the seropositivity of toxoplasmosis in different regions of the world. On the other hand, there are several other studies which are in agreement with the findings of our results. Shah and Zeb *et al.* (2013) reported prevalence in male goats as 38.46% and female goat as 69.23%. Similarly, male sheep had seropositivity of 30.76% and female sheep were 41.66%.On the other hand, male Ox were 16.6 6% and female cows were 25%. Findings of our study are almost in line with the findings reported in their study. In addition, age-wise seropositivity of toxoplasmosis was slightly different to the findings of Shah and Zeb. According to our findings, more prevalence of this infection was reported in adult species i.e. 3-5 years, for example, sheep 44.44%, goat 33.33% and cattle 26.66%. According to their study, the ratein young goats was 20% and in older aged was 33.33%, while in young sheep it was 13.3% and older aged sheep had 36.36% seropositivity. Similarly, Elfahal *et al.* (2013) reported toxoplasmosis in younger age cows 36.4% and older cows 42.5%, which is also in agreement with the findings of our study.

In order to find the transmission of this zoonotic parasite, it is important to identify its life cycle and also know the frequent source of this parasite, both in wild and domestic animals (Lavikainen, 2010). This parasite causes infection in all warm-blooded hosts such as goat, sheep cow and human etc. A comparative study was conducted which showed that incidence of *Toxoplasma* is much higher in a warm climate, and at a lower altitude as compared to hilly regions. The outcome of the disease affects the immune status of the infected person (Gutierrez, Y. 1990). Therefore, we selected three animal groups that are used as food and are in close contact with the

human population. According to our findings, the major source of acquiring this parasite in our target area i.e, Mingora City is sheep. This may be due to the fact that sheep are mostly kept and moved in large herds so chances of transmission are very high among them. In addition, sheep in herds are in close contact with other wild animals like cats and dogs, major reservoirs of this parasite, which further increase the transmission and spread of toxoplasmosis. Hence, in order to control further spread, sheep-feline contact must be restricted.

Conclusion

This study concludes that risk of toxoplasmosis is very high through contact with domestic animals used for human consumption. Frequent contact with these animals will increase the risk of toxoplasmosis. Hence, measures should be taken to control the contact of these animals with other non-domestic wild animals, especially cats to control the spread in domestic animals which are directly in contact with the human population. Highly affected groups of animals were sheep. So measures should be taken to control the spread within sheep herds. Their contact with other wild reservoirs like cats and dogs must be controlled. Shepherds of these herds should be educated about how to control the spread of this disease. Further studies are required to define the effect of *T. gondii* disease on the health of human consumers through consumption of these animals.

References

Abbas B, Zubeir El, Yassin TM. 1987. Survey for certain zoonotic diseases in camels in Sudan. Revue d'élevage et de médecine vétérinaire des pays tropicaux **40(3),** 231-233.

Baerger-Schoch1 AE, Bernet D, Doherr MG, Gottstein B and Frey CF. 2011. *Toxoplasmagondii* in Switzerland: A serosurvey based on meat juice analysis of slaughtered pigs, wild boar, sheep and cattle. Zoonoses and Public Health.

Bisson A, Maley S, Rubaire-Akiiki CM, Watling JM. 2000. The seroprevalence of antibodies to *Toxoplasma gondii* in domestic goats in Uganda. Acta Tropica **76,** 33-38.

Bornstein S, Musa BE. 1987. Prevalence of antibodies to some viral pathogens, Brucella abortus and Toxoplasma gondii in serum from camels (Camelus dromedarius) in Sudan. Zoonoses and Public Health **34(1-10),** 364-370.

Domy P, Van-Aken D. 1992 Prevalence ofToxoplasma gondii antibodies in goat in Sri Lanka. Ann. Trop. Med. Parasitol **86,** 83-85

Dubey JP. 2010. Toxoplasmosis of Animals and Humans (CRC, Boca Raton, FL).Boca Raton, New York; Second pp. 1-313.

Elamin EA, Elias S, Daugschies A, Rommel M. 1992. Prevalence of Toxoplasma gondii antibodies in pastoral camels (Camelus dromedarius) in the Butana plains, mid-Eastern Sudan. Veterinary Parasitology **43(3-4),** 171-175.

El-Ghaysh A. 1998. Seroprevalence of Toxoplasma gondii in Egyptian donkeys using ELISA. Veterinary parasitology **80(1),** 71-73.

El-Massry A, Mahd, OA, El-Ghaysh A, Dubey JP. 2000. Prevalence of Toxoplasma gondii antibodies in sera of turkeys, chickens, and ducks from Egypt. Journal of Parasitology **86(3),** 627-628.

Ghazy AA, Shaapan RM, Abdel-Rahman EH. 2007. Comparative serological diagnosis of toxoplasmosis in horses using locally isolated Toxoplasma gondii. Veterinary parasitology **145(1-2),** 31-36.

Ghoneim NH, Shalaby SI, Hassanain NA, Zeedan GS, Soliman YA, Gracia-reguet N, Lebrun M, Fourmaux MN, Mercereau-puijalon o, Mann T, Beckers JM., Samin B, Van Beeuman J, Bout D & Dubremetz JF. 2010. The micronemeproteine MIC3 ofT.gondii is a secretory adhesionthat binds to both surface of host cells and the surface of parasites. Cell microbial **2,** 353-64

Gutierrez Y. 1990. Diagnostic pathology of infection with clinical correlation. Philadelphia PA.; Lea and Febiger PP.197-201.

Hilali M, Romand S, Thulliez P, Kwok OCH, Dubey JP. 1998. Prevalence of Neospora caninum and Toxoplasma gondii antibodies in sera from camels from Egypt. Veterinary parasitology 75**(2-3),** 269-271.

Hill DE, Chirukandoth S, Dubey JP. 2005. Biology and epidemiology of *Toxoplasmagondii* in man and animals. Animal Health Research Reviews **6,** 41-61.

Ibrahim HM, Huang P, Salem TA, Talaat RM, Nasr MI, Xuan X, & Nishikawa Y. 2009. Prevalence of Neospora caninum and Toxoplasma gondii antibodies in northern Egypt. The American journal of tropical medicine and hygiene **80(2),** 263-267.

Ivana L, Olgica DD , Sofija KK, Aleksandra N. 2006. Cross sectional survey of *Toxoplasma*m *gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. Veterinary Parasitology **135,** 121-131.

Jittapalapong S, Sangvaranond N, Pinyopanuwat W, Chimnoi W, Khachaeram S, Koizumi and Sharif M, Gholami Sh, Ziaei H, Daryani A, Laktarashi B, Ziapour SP, Rafiei A, Vahedi M. 2006. Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats slaughtered for food in Manzandaran Province, Iran. Journal of Animal and Veterinary Advances **5,** 188-190.

Jones JL, Kruszon-Moran D, Wilson M, McQuillan G, Navin T and McAuley JB. 2001. *Toxoplasma gondii*infection in the United States: Seroprevalence and risk factor. Am. J. Epidemiol **154(4),** 357-365.

Lashari MH and Tasawar Z. 2010. Seroprevalence of toxoplasmosis in sheep in Southern Punjab, and genotypization of *Toxoplasma gondii* in goats. Biologica **65,** 670-674.

Lavikainen A. 2010. Human medical view on zoonotic parasites. Acta Veterinaria Scandinavica **52(1),** S4.

Liu X, Liu C, Liu Y, Jin H, Zhao Y, Chen J, Liu Q. 2012. Seroprevalence of Toxoplasma gondii infection in slaughtered pigs and cattle in Liaoning Province, northeastern China. Journal of Parasitology **98(2),** 440-441.

Mahmood A, Hafeez MA. 2009. Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. Tropical Animal Healthand Production **41,** 1225-1229.

Maruyama S. 2005. Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Satun Province, Thailand. Veterinary Parasitology **1(27),** 17-22.

Neto JOA, Azevedo SS, Gennari SM, Funada MR, Pena HFJ, Araujo CSA, Batista ARCP, Silva MLCR, Gomes AAB, Piatti RM, Alves CJ. 2008. Prevalence and risk factor for anti-*Toxoplasma gondii* antibodies in goats of the Serido Oriental micro region, Rio Grande do Norte state, Northeast region of Brazil. Veterinary Parasitology **156,** 329- 332.

Ramzan MM, Akhtar F, MUhammad I, Hussain E, Hiszczyn*s*ka-Sawicka AU, Mahmood MS, Hafeez MA. 2009. Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. Tropical Animal Health and Production **41,** 1225-1229.

Samad MA, Rahman KB, Halder AK. 1993 Seroprevalence of Toxoplasma gondii in domestic ruminants in Bangladesh. Vet. Parasitol **47,** 157.

Spisak FL, Turcekova K, Reiterova S, Spilovska, Dubinsky P. 2010. Prevalence estimation Pakistan. Pakistan Veterinary Journal **30,** 91-94.