Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Toxoplasma gondii in lactating animals: potential risk to milk consuming population in Khyber Pakhtunkhwa

Toxoplasma gondii em animais lactantes: risco potencial para população consumidora de leite em Khyber Pakhtunkhwa

S. Khan[a], K. Rafiq[a], M. N. Khabir[a], M. B. Khan[a], S. N. Khan[b], A. Khattak[a] and S. Attaullah[c*]

[a]University of Peshawar, Department of Zoology, Peshawar, Khyber Pakhtunkhwa, Pakistan
[b]Kohat University of Science and Technology Kohat, Department of Zoology, Kohat, Khyber Pakhtunkhwa, Pakistan
[c]Islamia College Peshawar, Department of Zoology, Peshawar, Khyber Pakhtunkhwa, Pakistan

Abstract

Toxoplasma gondii is an intracellular zoonotic protozoan parasite usually infects human and animal worldwide. This study aimed to analyze the sero-prevalence of *T. gondii* in blood of lactating animals and human living in close proximity and also to detect *Toxoplasma* DNA in unpasteurized milk of the studied animals. A total of 233 blood and milk samples were collected from lactating animals, and 735 blood samples were taken from humans in District Upper Dir, Khyber Pakhtunkhwa, Pakistan. The blood samples were analyzed through ELISA while the milk samples were analyzed by PCR for the presence of *T. gondii* DNA. A standard questionnaire was introduced to collect the data from the participants. In animals, the reported sero-prevalence was 32.18% for IgM, 17.16% for IgG, and 6.4% for both IgM and IgG. The reported positivity for *T. gondii* DNA in milk was 14.44%, 34.8%, 20%, and 26% in sheep, goats, cows, and buffaloes, respectively. In the human blood samples, 9.8% were found positive for IgM and 11.2% for IgG while none of the samples was found positive for both IgM and IgG. Overall sero-prevalence reported in females was significantly higher than the male ($p<0.05$) poor hygiene condition ($p < 0.0001$) were the significant risk factors associated with *T. gondii* infections in animals. In conclusion, *T. gondii* infection is prevalent in lactating animals and humans using their raw milk in the study area. It is suggested that raw milk should be considered as a vehicle for the transmission of *T. gondii* to humans. Proper pasteurization of milk is very useful in limiting the transmission of infection. Awareness and control programs should be implemented to prevent the infection.

Keywords *Toxoplasma gondii***,** sheep, goat, cow, buffalo, milk, ELISA, PCR.

Resumo

Toxoplasma gondii é um protozoário parasita intracelular zoonótico que geralmente infecta humanos e animais em todo o mundo. Este estudo teve como objetivo analisar a soroprevalência de *T. gondii* no sangue de animais lactantes e humanos que vivem em proximidade, além de detectar o DNA de Toxoplasma no leite não pasteurizado dos indivíduos estudados. Um total de 233 amostras de sangue e leite foram coletadas de animais lactantes e 735 amostras de sangue foram coletadas de humanos no Distrito Upper Dir Khyber Pakhtunkhwa, no Paquistão. As amostras de sangue foram analisadas pelo método ELISA enquanto as amostras de leite foram analisadas por PCR para a presença de DNA de *T. gondii*. Um questionário padrão foi introduzido para coletar os dados dos participantes. Em animais, a soroprevalência relatada foi de 32,18% para IgM, 17,16% para IgG e 6,4% para IgM e IgG. A positividade relatada para DNA de *T. gondii* encontrada no leite foi de 14,44%, 34,8%, 20% e 26% em ovelhas, cabras, vacas e búfalas, respectivamente. Nas amostras de sangue humano, 9,8% foram consideradas positivas para IgM e 11,2% para IgG, enquanto nenhuma das amostras foi considerada positiva para IgM e IgG. A soroprevalência geral relatada em mulheres foi significativamente maior do que em homens ($p < 0,05$). Neste estudo, contato com gatos ($p < 0,0001$), hábitos alimentares ($p < 0,0001$), fonte de água para beber ($p < 0,0001$) e más condições de higiene ($p < 0,0001$) foram os fatores de risco significativos associados a infecções por *T. gondii* em animais. Em conclusão, a infecção por *T. gondii* é prevalecente em animais lactantes e humanos que utilizam leite cru, isto é, não-pasteurizado, na área de estudo. Sugere-se que o leite não-pasteurizado seja considerado um veículo de transmissão do *T. gondii* para humanos. A pasteurização adequada do leite é muito útil para limitar a transmissão de infecções. Programas de conscientização e controle devem ser implementados para prevenir a infecção.

Palavras-chave: *Toxoplasma gondii*, ovelha, cabra, vaca, búfala, leite, ELISA, PCR.

*e-mail: sobia@icp.edu.pk
Received: 29 August, 2022 – Accepted: 15 January 2023

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular cyst forming apicomplexan parasite, infecting all warm-blooded animals including humans, with a cosmopolitan distribution (Rouatbi et al., 2019; Sharif et al., 2009) and is a causative agent of the geographically omnipresent infection known as toxoplasmosis (Rahimi et al., 2015; Sugden et al., 2016). The lactating animals are the significant hosts of *T. gondii* and thus considered most important transmission route of *T. gondii* infection. Usually, *T. gondii* infection is passed to grazing animals when coming in contact with the soil contaminated with *T. gondii* oocysts. Then these infected animals transmit tachyzoites through unpasteurized milk to their offspring as well as to human beings (Khamsian et al., 2021; Camossi et al., 2011; Sharif et al., 2009).

The disease is noticeable in both medical as well as veterinary field, as it result a significantly serious drain on productivity and the economy by damaging animal husbandry due to stillbirths and neonatal mortality in animals and stress on the health systems (Rahimi et al., 2015, Sharif et al., 2009). Many epidemics responsible for high mortality and morbidity and were reported in livestock sector of Pakistan (Ahmed et al., 2016). *Toxoplasma* infection in dairy animals may illustrate a real risk for transmission of parasite to human (Van den Brom et al., 2020; Omonijo et al., 2022), causes different complications such as Toxoplasmic encephalitis, blindness, stillbirth, abortion, fetal abnormalities, or even prenatal death in congenital cases, pneumonia, and sometimes heart infection (Chan and Smith, 2018). Globally prevalence of *T. gondii* is variable, different countries ranging from zero to hundred percent prevalence rates depending upon their civilizations, societies, inhabitant's life styles, animal's age and climate conditions (Lashari et al., 2018; Thai et al., 2019). The broad dissemination of this parasite may be attributed to various factors such as its co-evolution with multiple hosts (Mukhopadhyay et al., 2020; Alzaheb, 2018; Ding et al., 2017; Sanchez and Besteiro, 2021), multiple transmission routes (Foroutan et al., 2018), presence of diverse *T. gondii* genotypes (Shapiro et al., 2019), long viability in the moderate environment (Galvan-Ramirez et al., 2012), longer infectious time for about 18 months (Furtado et al., 2011) and resistant to freezing, drying and even disinfection (Rahimi et al., 2015). Milk is a highly consumable complete source of food as it contains all required nutrients in good proportion. Peoples need hygienic quality of milk for daily use and they fulfill their need from domestic and farm animals. The milk of these animals represents a source of infection for humans, therefore required to monitor prevalence of infection, aimed to reduce infections in them as much as possible. Better information regarding the environmental contamination of *T. gondii* is critical and vital to holistic determinations of potential health risks (Aguirre et al., 2019) and highlights the areas for further investigation so to implement effective measures to prevent *T. gondii* infections (Stelzer et al., 2019). Due to the economic importance of toxoplasmosis, this study aimed to analyze the sero-prevalence of *T. gondii* in blood of lactating animals and human living in close proximity and also to detect *Toxoplasma* DNA in unpasteurized milk of the studied animals in District Upper Dir, Pakistan.

2. Materials and Methods

2.1. Description of the study area

The study was conducted in Upper Dir District of Khyber Pakhtunkhwa, Pakistan, lies at latitude of 35° 9'55.89" N and 72° 2'48.54" E longitude having total areas of the district is 3,699 km^2 (Figure 1). People of the area mostly depend on domestic animals for the purpose of milk and different types of dairy products such as butter, cheese, yoghurt, and meat. These are the main sources of income for the people of the region. Basic educational and health facilities have also been provided by the Government to the people of the area.

2.2. Ethical considerations

The Advance Study Research Board (ASRB), University of Peshawar Khyber Pakhtunkhwa, Pakistan has approved the research Proposal. Special permission was obtained from the owner of the animals during sampling. Informed consent was obtained from all participants of the study.

2.3. Data collection

Data related risk factors of *toxoxoplasma* in animals (feeding habits, type of food, source of drinking water, hygienic conditions and age) and humans (age, contact with cats and gender) were collected through a predesigned questionnaire.

2.4. Collection of samples

2.4.1. Collection of blood and milk samples from animal

Blood and milk samples were collected from 233 lactating animals including sheep (n=76), goats (n=69), cows (n=50) and buffaloes (n=38)], from five different tehsils (Proper Dir, Wari, Barawal, Shringal and Kalkot) in district Upper Dir Khyber Pakhtunkhswa Pakistan. Blood samples (5mL) were collected from the jugular vein of lactating animals. Milk samples (5mL) were taken manually after disinfection of the teats with 70% ethanol. All the blood and milk samples were immediately transported to the

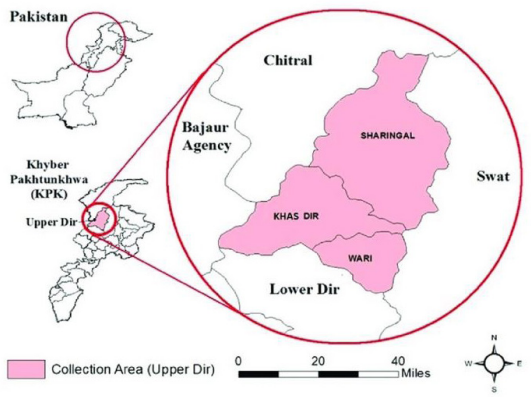

Figure 1. Map of Pakistan. The red area represents Upper Dir, Khyber Pakhtunkhwa (Ruhoollah et al., 2021).

Laboratory of Virology and Immunology, Department of Zoology, University of Peshawar. Serum was separated by centrifugation. All samples were stored at -20°C for serological and molecular assays.

2.4.2. Collection of blood samples from humans

A total of 735 blood samples (350 males and 385 females) were collected from the participants who live nearby the animals and consume their milk especially animal's owners and their family members.

All the blood samples were collected in 5 mL sterile test tubes and serum was separated and stored at -20°C for further serological examination.

2.5. Serological examination of T. gondii

The serum of human were analysed for *T. gondii* IgM antibodies and IgG antibodies using commercially available Toxo- IgM kit (BioCheck Inc. USA, Catalog Number: BC-1087) and Toxo-IgG kit (BioCheck, Inc. USA, Catalog Number BC-1085) according to the manufacturer's instructions. The microwell plate of ELISA was read by a Microplate reader with O.D at 450 nm (Microplate Reader, Model Dia 710 UK). The serum of animals were analysed by using Commercial ELISA Kits (ID Screen Toxoplasmosis Indirect®ID-VET Company, France) for the detection of Toxo-IgG and Toxo-IgM antibodies.

2.6. T. gondii DNA detection from milk

The extraction of DNA from milk was carried out by using a DNA isolation kit (Favorgen Tissue Genomic DNA Extraction Mini kit, USA). The extracted DNA was amplified by using primers of B1 gene for *T. gondii* following the protocol of Sadek et al. (2015). The reaction conditions consisted of one cycle of 95ºC for 5 minutes followed by 40 cycles of 94ºC for 30 seconds, 60ºC for 30 seconds, 72ºC for 1 minute, and final extension at 72ºC for 10 minutes, and then the amplified DNA were electrophoresed on 2% agarose gel. The amplified DNA 115 bp bands were visualized under UV- trans illuminator and compared with a 50 bp DNA ladder marker (thermoisher scientific, USA).

2.7. Data analysis

The data were analyzed by using SPSS version 20. Chi-square test (X^2) was used to assess the associations between *Toxoplasma* seropositive and the studied factors. A p-value less than 0.05 was considered statistically significant. The frequency of *Toxoplasma* DNA in milk samples was analyzed in four age groups of animals (≤5 years, 6-9 years, 10-12 years, and ≥13 years. Age-wise sero-prevalence of *T. gondii* was analyzed in the different age groups of humans (5-20 years, 21-35 years, 36-50 years, and ≥51 years).

3. Result

3.1. Toxoplasma in animals

The serological examination of the serum samples showed the presence of *T. gondii* antibodies (IgM and IgG) while PCR analysis showed the presence of *T. gondii* DNA in the unpasteurized milk samples of lactating animal. The overall prevalence for *T. gondii* IgM was 32.18% (n=75), IgG was 17.16% (n=40), while both IgG and IgM was 6.4% (n=15). Out of 76 sheep, IgM positivity was 38.16% (n=29), IgG was 21.05% (n=16), and both IgG and IgM was 6.5% (n=5). Of 69 goats, 34.78% (n=24) were positive for IgM, 21.74% (n=15) for IgG, and 8.6% (n=6) for both IgG and IgM. Of 50 cows , 24.4% (n=12), 10% (n=5), and 4% (n=2) were positive for IgM, IgG, and both IgG and IgM, respectively. In 38 buffaloes, 26.31% (n=10) were positive for IgM, 10.53% (n=4) for IgG and 26.0% (n=2) were reported positive for both IgG and IgM.

Through PCR, 23.60% (55/233) milk samples were found positive for *T.gondii* DNA. Of the positive samples, 14.47% (n=11) sheep, 34.80% (n=24) goats, 20% (n=10) cows and 26.31% (n=10) buffaloes. Interestingly, DNA was found positive in the milk samples of sheep (n=4), goats (n=14), cows (n=6) and buffaloes (n=3) whose blood were negative for IgM+IgG (p=0.477). Prevalence of *T. gondii* antibodies (IgM and IgG) and DNA in milk of lactating animals in Upper Dir district was shown in Table 1).

The distribution of positive antibodies in blood of animals and DNA of milk was showed in Table 2. The positive results of PCR (23.60%) were significantly higher than those of IgG (10.73%) but lower than IgM (25.75%). The proof of DNA in milk samples by PCR in both IgG positive animals and IgM positive animals were statistically significant (p=0.0002 and p=0.0092, respectively) in comparison to concurrent presence IgG and IgM. Goats were reported with high prevalence (34.8%) of *T. gondii* DNA in their milk as compared to other animals.

Table 1. Prevalence of *T. gondii* antibodies (IgM and IgG) and DNA in milk of lactating animals in Upper Dir district.

Animals (N)	IgM+ve	IgG+ve	IgG & IgM+ve	DNA +ve
	n (%)	n (%)	n (%)	n (%)
Sheep (n=76)	29 (38.16)	16 (21.05)	5 (6.5)	11 (14.47)
Goat (n=69)	24 (34.78)	15 (21.74)	6 (8.6)	24 (34.80)
Cow (n=50)	12 (24.0)	5 (10.0)	2 (4)	10 (20.00)
Buffalo (n=38)	10 (26.31)	4 (10.53)	2 (5.2)	10 (26.31)
Total (n=233)	75 (32.18)	40 (17.16)	15 (6.4)	55 (23.60)

+ve= positive samples.

Table 2. Comparative analysis of *T. gondii* antibodies and DNA in Upper Dir district.

Tests	Sheep	Goat	Cow	Buffalo	Total
IgG+IgM-DNA-	8	18	1	2	29
IgG+IgM+DNA-	5	-	-	-	5
IgG-IgM+DNA-	20	5	10	3	38
IgG+IgM-DNA+	3	4	2	-	9
IgG-IgM+DNA+	4	-	-	5	9
IgG+IgM+DNA+	-	6	2	2	10
IgG-IgM-DNA+	4	14	6	3	27
IgG-IgM-DNA-	32	22	29	23	106
Total	76	69	50	38	233

+ve = positive, -ve = negative.

Table 3. *T. gondii* DNA in lactating animals by PCR in Upper Dir district.

	Different Age groups of Lactating Animal								
Number	**≤5 years**		**6-9 years**		**10-12 years**		**≥13 years**		***p* value**
Lactating Animal	**N**	**+ve (%)**	**N**	**+ve (%)**	**N**	**+ve (%)**	**N**	**+ve (%)**	
Sheep	42	7 (16.66)	34	4 (11.76)		-		-	0.02
Goat	31	9 (29.03)	38	15 (39.47)		-		-	0.01
Cow	10	1 (10.0)	18	3 (16.66)	22	6 (27.27)		-	0.04
Buffalo	-	-	10	1 (10.0)	15	4 (26.66)	13	5 (38.66)	0.03
Total	83	17 (20)	100	23 (23)	37	10 (27)	13	5 (38)	

+ve = positive samples.

The frequency of Toxoplasma DNA in studied animals in age groups ≤5 years, 6-9 years, 10-12 years, and ≥13 years was 20.48%, 25.55%, 27.02%, and 38.49%, respectively. Statistically, T. gondii DNA positivity increased non-significantly (p=1.195) with age in all animals studied (Table 3).

Different risk factors of acquiring the parasite related to the IgM in positive and negative cases were also analyzed. It was found that contact with cats (p =0.9282), drinking water source (p=0.0955) and poor hygiene condition (p =0.39551) were not significant risk factor while feeding habits (p < 0.0001) of animals was associated with *T. gondii* infections (Table 4).

3.2. Toxoplasma in human

A total of 735 human blood samples (350 males and 385 females) were also analyzed for *T. gondii* infection. The overall seroprevalence of IgM and IgG were 9.8% (n=72) and 11.2% (n=82), respectively, while none was found positive for both IgM and IgG. In the male population, the prevalence of IgM was 8.5% (n=30/350) and IgG was 9.7% =(34/350), while in females, the seroprevalence of IgM was10.9% (n=42/385) and IgG was 12.4% (n=48/385). (Table 5).

The prevalence of *T. gondii* infection in the human population was given in Table 6. Results of this study indicated that toxoplasmosis infections significantly increase with age in both male (p=0.02) and female (p=0.01) populations.

4. Discussion

In the current study, the reported sero-prevalence of IgM was 32.18%, IgG was 17.16% and both IgM and IgG was 6.4% of the studied animals. The prevalence of *T. gondii* DNA in the milk of studied animals was 23.60%. The prevalence of *T. gondii* DNA in the milk samples varies in different age groups, ranging from 16.66% in animals aged ≤5 years, 11.76% in 6-9 years, and the prevalence increase with the age of the animals. The present study was consistent with a previous study from the United Kingdom that old age animals were less resistant to *T. gondii* infection than younger animals due to low immunity and high susceptibility to infection (Schares et al., 2017).

Several past studies reported low prevalence of *T. gondii* DNA in sheep milk samples than the present study; 3.4% prevalence rate reported from Italy (Fusco et al., 2007), 5% reported from Brazil (Camossi et al., 2011), 6.48% from Iran (Dehkordi et al., 2013), the difference between the present study and -mentioned studies might be due to variation in cat density, contaminated water and grasses with *T. gondii* oocysts, and geographic factors, etc. The current study supported result reported from Serbia that constant contact of sheep to the environment progressively increases the risk of *T. gondii* infection with the age of the sheep because the environment was contaminated with *T. gondii* oocysts

Table 4. Risk factors related prevalence for *T. gondii* in lactating animals in Upper Dir district.

Risk Factor	Risk Factors Nature	Total	Positive (IgM)	Percentage	*p* value
Presence of cat	Yes	170	55	32.35	0.9282
	No	63	20	31.74	
Feeding habit	Grazing	155	61	39.35	< 0.001
	Feeding at home	78	14	17.94	
Drinking water	Running	170	60	35.29	0.0955
	Stagnant	63	15	23.80	
Household hygienic conditions	Poor	185	62	33.51	0.39551
	Good	48	13	27.0	

(Stelzer et al., 2019). The sero-prevalence reported from Southern Punjab Pakistan in sheep was 19.88%, which was lower than the present study (Hanif and Tasawar, 2016). Likewise, sero-prevalence reported in sheep was 36% from Mohmand agency, Pakistan (Thai et al., 2019), and 31.45% from Ethiopia (Gebremedhin and Gizaw, 2014). The molecular prevalence of *T. gondii* DNA reported in sheep from Egypt was10.71% (Camossi et al., 2011), which was similar to current work.

In lactating goats in this study, the sero-prevalence rate of IgM was 34.78%, IgG was 21.74% and both IgM and IgG were 8.6% by ELISA. DNA of *T. gondii* detected in raw milk by PCR was 34.78%. The age of the lactating goats were evaluated for the relationship with *T. gondii* occurrence. Similar results were also reported from Ethiopia in old age caprine have high toxoplasmosis (Teshale et al., 2007), which justified the present study.

Data reported by several investigators in lactating goat milk from different countries of the world; such as from Egypt, the prevalence of *T. gondii* DNA through PCR was 22.73% (Camossi et al., 2011), and from El-Fayoum Governorate, Egypt was 25% (Ghoneim et al., 2009), from Brazil was 28.9% (Bisson et al., 2000), from Thailand was 27.9% (Jittapalapong et al., 2005), from Pakistan was 25.4% (Ramzan et al., 2009), and from Slovakia was 32.56% *T. gondii* DNA reported in goats milk (Spišák et al., 2010), which showed coherence with the present study.

Likewise, the prevalence of *T. gondii* DNA reported by several authors through PCR technique from different countries such as 6.05% in raw milk of goat from Brazil (Bezerra et al., 2015), and 13% of *T. gondii* DNA recorded in naturally infected lactating goats from Italy (Mancianti et al., 2013). Data reported from Northwest Tunisia that consuming raw goats' milk represents a real risk factor for human toxoplasmosis (Amairia et al., 2016). The reported prevalence of *T. gondii* DNA from Northwest of Iran was 9.44% in the raw milk of goats (Tavassoli et al., 2013). The sero-prevalence reported in goats from Multan Pakistan was 52% (Tasawar et al., 2011), while from Zimbabwe the prevalence rate reported was 67.9% (Hove et al., 2005) and from Egypt was 59.4% (Barakat et al., 2009). Detection of toxoplasmosis in different lactating species (goats, sheep, buffalos, cattle, and camels) was recently studied in Iran with different techniques and reported that the raw milk of the mentioned animal contains *T. gondii (*Dehkordi et al., 2013). Therefore, the unpasteurized milk of the said animals is a risk factor for *T. gondii* transmission to humans. In the current study, the molecular prevalence of *T. gondii* DNA was detected higher in goat milk as compared to other lactating animals included in the study population. A similar result was also reported by other authors that the milk of infected goat contains more *T. gondii* tachyzoites as compared to its meat (Spišák et al., 2010; Bezerra et al., 2015).

Table 5. Overall seroprevalence of *T.gondii* in human population in Upper Dir district.

Gender	IgM + n (%)	IgG+ n (%)
Male (n=350)	30 (8.5)	34 (9.7)
Female (n=385)	42 (10.9)	48 (12.4)
Overall (n=735)	72 (9.8)	82 (11.2)
p value	0.286	0.2363

+ve = positive samples.

The high prevalence of *T. gondii* in lactating goats in the present research may be due to different climatic factors such as high humidity, temperature change, the difference in agro-ecology, high density of dogs and cats.

In the current study, the examined sero-prevalence in lactating cows through ELISA; IgM was 24%, IgG was 10% and both IgM and IgG was 4%. DNA of *T. gondii* was detected in raw milk of cow by PCR with the prevalence of 20%. In the different age groups of cows showed that the prevalence of *T. gondii* DNA considerably increases with the age of the cow. The present research was coherent with the reported prevalence in cows from different countries such as 20% from Mohmand agency, Pakistan (Shah et al., 2013), 13.3% by ELISA from Sudan (Elfahal et al., 2013), 15.91% from Iran (Delgado et al., 2022), and all these support the present study. While the reported prevalence of *T. gondii* in cattle from Serbia the estimated prevalence of *T. gondii* was observed 76.3% in cattle (Klun et al., 2006), and from Brazil, 71% prevalence of *T. gondii* was reported in cattle (Santos et al., 2009) which was extremely higher than the present study.

Table 6. Age-wise sero-prevalence of *T. gondii* in human population in Upper Dir district.

Age	Age groups (years)								*p* value
	5-20		21-35		36-50		≥51		
Gender	IgM +/ Total (%)	IgG+/ Total	IgM+/ Total	IgG+/ Total	IgM+/ Total	IgG+/ Total	IgM+/ Total	IgG+/ Total	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
Male	5/80 (6.2)	6/80 (7.5)	7/85 (8.2)	8/85 (9.4)	9/95 (9.4)	10/95 (10.5)	9/90 (10)	10/90 (11.1)	0.02
Female	5/95 (5.2)	9/95 (9.4)	12/100 (12)	11/100 (11)	13/100 (13)	16/110 (14.5)	12/80 (15)	12/80 (15)	0.01

+ve = positive samples.

The sero-prevalence reported in lactating buffalo was 26.31% for IgM, 10.53% for IgG, and 6.4% for both IgG and IgM by ELISA. DNA of *T. gondii* detected by PCR was 26.0% in unpasteurized milk of buffalo. In the different age groups of buffalo, the prevalence of *T. gondii* DNA ranges such as, the positivity of 10% was reported in buffalo of age group II, 26.66% in age group III while 38.66% in age group IV. The frequency significantly increases with the age of the buffalo ($p<0.05$).

There are limited reports throughout the world on the sero-prevalence of *T. gondii* infection in buffaloes (Shah et al., 2013). Current study was supported by 27.2% sero-prevalence reported from Brazil (Oliveira et al., 2018), 7.8% reported from Trinidad (Persad et al., 2011), 8.8% from Iran (Anvari et al., 2018). Several risk factors including sex, diagnostic test, age, and climatic alterations probably take part in changing the prevalence in buffaloes throughout the globe (Ahmad and Qayyum, 2014). The high prevalence of *T. gondii* infection in the present study might be due to differences in agro-ecology, climate, cat and dog density in the study area, sample size, unhygienic condition, animal management, contaminated water with oocysts, transmission routes, age of the animals and type of serological tests applied, etc. Animals reared for dairy production and reproduction often lives much longer. Therefore, *T. gondii* infection was higher in the milk of adult cattle than in the young cattle mentioning the reason that animals with longer life expectancy have longer exposure to risk factors, thus more contaminated with *T. gondii* parasite (Abdallah et al., 2019; Stelzer et al., 2019), also supported current study. Moreover, in older age, animals are proven less resistant to pathogens due to low immunity and high susceptibility to infection than younger ones (Ahmad and Qayyum, 2014; Roberts et al., 2001; Ahmed et al., 2016).

The poor hygienic state may favor water and food to be contaminated with cat feces, hence it increased risk of *T. gondii* infection in the livestock. Because a small proportion of individuals acquired the infection, then pose a potential reservoir of infection. While the majority later infected by using contaminated food and drinking water. Thus good hygienic status and cleaning measures have a protective effect (Stelzer et al., 2019).

In this study, 738 humans tested for *T. gondii*, 9.80% and 11.80% showed seropositivity to IgM and IgG, respectively, corresponding to an overall prevalence rate of 20.95%. This showed that *T. gondii* infection is highly prevalent in the study area. This region is an agricultural region and the people kept domestic animals for economic purposes. Cat and other pet animals are also generally kept in-home or near home, unhygienic nutritional tradition and low education level contributes to infection. The prevalence rate of *T. gondii* infection throughout the globe, as well as within-country, is variable since its prevalence depends upon the environment (water quality, sanitation, coverage, etc.), climatic variations from one region to another, socioeconomic (hygiene, source of food, etc.), climatic factors (humidity, temperature), lifestyle of the inhabitants (dietary habits, method of cooking, hand washing, kind of food item cleaning, contact with cats and dogs or other domestic animals, contact with the soil, etc.) and difference in serological tests with variable specificity and sensitivity (Tenter et al., 2000; Robert-Gangneux and Dardé, 2012). However, a statistically significant correlation between positive serology for *T. gondii* in humans and ingestion of goat milk also had been reported in the literature (Sroka et al., 2017).

5. Conclusions

High prevalence of *T. gondii* in milk samples indicates a potential risk for the transmission of *T. gondii* to human through raw unpasteurized milk consumption. Therefore, awareness regarding the transmission and prevention of the disease is crucial. In general, integrated strategies and measures should be implemented to prevent and control *T. gondii* infection in dairy animals as well as in human in the study area.

Acknowledgements

We would like to thank all the participants and owners of animals for their cooperation.

References

ABDALLAH, M.C., KAMEL, M., KARIMA, B., SAMIR, A., DJAMEL, K., RACHID, K. and KHATIMA, A.O., 2019. Cross-sectional survey on Toxoplasma gondii infection in cattle, sheep, and goats in Algeria: seroprevalence and risk factors. *Veterinary Sciences*, vol. 6, no. 3, pp. 63. http://dx.doi.org/10.3390/vetsci6030063. PMid:31295942.

AGUIRRE, A.A., LONGCORE, T., BARBIERI, M., DABRITZ, H., HILL, D., KLEIN, P.N., LEPCZYK, C., LILLY, E.L., MCLEOD, R., MILCARSKY, J., MURPHY, C.E., SU, C., VANWORMER, E., YOLKEN, R. and

SIZEMORE, G.C., 2019. The one health approach to toxoplasmosis: epidemiology, control, and prevention strategies. *EcoHealth*, vol. 16, no. 2, pp. 378-390. http://dx.doi.org/10.1007/s10393-019-01405-7. PMid:30945159.

AHMAD, N. and QAYYUM, M., 2014. Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan. *Journal of Infection in Developing Countries*, vol. 8, no. 8, pp. 1022-1028. http://dx.doi.org/10.3855/jidc.4405. PMid:25116669.

AHMED, H., MALIK, A., ARSHAD, M., MUSTAFA, I., KHAN, M.R., AFZAL, M.S., ALI, S., MOBEEN, M. and SIMSEK, S., 2016. Seroprevalence and spatial distribution of toxoplasmosis in sheep and goats in North-Eastern Region of Pakistan. *Korean Journal of Parasitology*, vol. 54, no. 4, pp. 439-446. http://dx.doi.org/10.3347/kjp.2016.54.4.439. PMid:27658595.

ALZAHEB, R.A., 2018. Seroprevalence of Toxoplasma gondii and its associated risk factors among women of reproductive age in Saudi Arabia: a systematic review and meta-analysis. *International Journal of Women's Health*, vol. 10, pp. 537-544. http://dx.doi.org/10.2147/IJWH.S173640. PMid:30288126.

AMAIRIA, S., ROUATBI, M., RJEIBI, M.R., NOUASRI, H., SASSI, L., MHADHBI, M. and GHARBI, M., 2016. Molecular prevalence of Toxoplasma gondii DNA in goats' milk and seroprevalence in Northwest Tunisia. *Veterinary Medicine and Science*, vol. 2, no. 3, pp. 154-160. http://dx.doi.org/10.1002/vms3.29. PMid:29067190.

ANVARI, D., SAADATI, D., NABAVI, R. and ALIPOUR ESKANDANI, M., 2018. Epidemiology and molecular prevalence of toxoplasma gondii in cattle slaughtered in Zahedan and Zabol Districts, South East of Iran. *Iranian Journal of Parasitology*, vol. 13, no. 1, pp. 114-119. PMid:29963093.

BARAKAT, A.M.A., ELAZIZ, M.A. and FADALY, H.A., 2009. Comparative diagnosis of toxoplasmosis in Egyptian small ruminants by indirect hemagglutination assay and Elisa. *Global Veterinaria*, vol. 3, no. 1, pp. 9-14.

BEZERRA, M.J.G., KIM, P.C.P., MORAES, É.P., SÁ, S.G., ALBUQUERQUE, P.P.F., SILVA, J.G., ALVES, B.H.L.S. and MOTA, R.A., 2015. Detection of T oxoplasma gondii in the milk of naturally infected goats in the N ortheast of B razil. *Transboundary and Emerging Diseases*, vol. 62, no. 4, pp. 421-424. http://dx.doi.org/10.1111/tbed.12160. PMid:24034351.

BISSON, A., MALEY, S., RUBAIRE-AKIIKI, C.M. and WASTLING, J.M., 2000. The seroprevalence of antibodies to Toxoplasma gondii in domestic goats in Uganda. *Acta Tropica*, vol. 76, no. 1, pp. 33-38. http://dx.doi.org/10.1016/S0001-706X(00)00086-3. PMid:10913763.

CAMOSSI, L.G., GRECA-JÚNIOR, H., CORRÊA, A.P.F.L., RICHINI-PEREIRA, V.B., SILVA, R.C., DA SILVA, A.V. and LANGONI, H., 2011. Detection of Toxoplasma gondii DNA in the milk of naturally infected ewes. *Veterinary Parasitology*, vol. 177, no. 3-4, pp. 256-261. http://dx.doi.org/10.1016/j.vetpar.2010.12.007. PMid:21216534.

CHAN, M.Y. and SMITH, M.A., 2018. Infections in pregnancy. In C.A. MCQUEEN, ed. *Comprehensive toxicology*. USA: Elsevier, pp. 232-249. http://dx.doi.org/10.1016/B978-0-12-801238-3.64293-9.

DEHKORDI, F.S., BORUJENI, M.R., RAHIMI, E. and ABDIZADEH, R., 2013. Detection of Toxoplasma gondii in raw caprine, ovine, buffalo, bovine, and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran. *Foodborne Pathogens and Disease*, vol. 10, no. 2, pp. 120-125. http://dx.doi.org/10.1089/fpd.2012.1311. PMid:23441913.

DELGADO, I.L.S., ZÚQUETE, S., SANTOS, D., BASTO, A.P., LEITÃO, A. and NOLASCO, S., 2022. The Apicomplexan parasite *Toxoplasma gondii. Encyclopedia*, vol. 2, no. 1, pp. 189-211. http://dx.doi.org/10.3390/encyclopedia2010012.

DING, H., GAO, Y.M., DENG, Y., LAMBERTON, P.H. and LU, D.B., 2017. A systematic review and meta-analysis of the seroprevalence of Toxoplasma gondii in cats in mainland China. *Parasites & Vectors*, vol. 10, no. 1, pp. 27. http://dx.doi.org/10.1186/s13071-017-1970-6. PMid:28086987.

ELFAHAL, A.M., ELHASSAN, A.M., HUSSIEN, M.O., ENAN, K.A., MUSA, A.B. and EL HUSSEIN, A.M., 2013. Seroprevalence of Toxoplasma gondii in dairy cattle with reproductive problems in Sudan. *ISRN Veterinary Science*, vol. 2013, pp. 895165. http://dx.doi.org/10.1155/2013/895165. PMid:24171116.

FOROUTAN, M., DALVAND, S., DARYANI, A., AHMADPOUR, E., MAJIDIANI, H., KHADEMVATAN, S. and ABBASI, E., 2018. Rolling up the pieces of a puzzle: a systematic review and meta-analysis of the prevalence of toxoplasmosis in Iran. *Alexandria Journal of Medicine*, vol. 54, no. 3, pp. 189-196. http://dx.doi.org/10.1016/j.ajme.2017.06.003.

FURTADO, J.M., SMITH, J.R., BELFORT JUNIOR, R., GATTEY, D. and WINTHROP, K.L., 2011. Toxoplasmosis: a global threat. *Journal of Global Infectious Diseases*, vol. 3, no. 3, pp. 281-284. http://dx.doi.org/10.4103/0974-777X.83536. PMid:21887062.

FUSCO, G., RINALDI, L., GUARINO, A., PROROGA, Y.T., PESCE, A., GIUSEPPINA, M. and CRINGOLI, G., 2007. Toxoplasma gondii in sheep from the Campania region (Italy). *Veterinary Parasitology*, vol. 149, no. 3-4, pp. 271-274. http://dx.doi.org/10.1016/j.vetpar.2007.07.020. PMid:17764846.

GALVAN-RAMIREZ, M.L., TROYO, R., ROMAN, S., CALVILLO-SANCHEZ, C. and BERNAL-REDONDO, R., 2012. A systematic review and meta-analysis of Toxoplasma gondii infection among the Mexican population. *Parasites & Vectors*, vol. 5, pp. 271. http://dx.doi.org/10.1186/1756-3305-5-271. PMid:23181616.

GEBREMEDHIN, E.Z. and GIZAW, D., 2014. Seroprevalence of Toxoplasma gondii infection in sheep and goats in three districts of Southern Nations, Nationalities and Peoples' Region of Ethiopia. *World Applied Sciences Journal*, vol. 31, no. 11, pp. 1891-1896.

GHONEIM, N.S.S., HASSANAIN, N., ZEEDAN, G., SOLIMAN, Y. and ABDALHAMED, A., 2009. Detection of genomic Toxoplasma gondii DNA and anti-Toxoplasma antibodies in high risk women and contact animals. *Global Veterinaria*, vol. 3, no. 5, pp. 395-400.

HANIF, M. and TASAWAR, Z., 2016. Seroprevalence and risk factors associated with toxoplasmosis in sheep in Multan and Khanewal districts of Punjab (Pakistan). *Journal of Animal & Plant Sciences*, vol. 26, no. 6, pp. 1620-1627.

HOVE, T., LIND, P. and MUKARATIRWA, S., 2005. Seroprevalence of Toxoplasma gondii infection in goats and sheep in Zimbabwe. *The Onderstepoort Journal of Veterinary Research*, vol. 72, no. 4, pp. 267-272. http://dx.doi.org/10.4102/ojvr.v72i4.175. PMid:16562728.

JITTAPALAPONG, S., SANGVARANOND, A., PINYOPANUWAT, N., CHIMNOI, W., KHACHAERAM, W., KOIZUMI, S. and MARUYAMA, S., 2005. Seroprevalence of Toxoplasma gondii infection in domestic goats in Satun Province, Thailand. *Veterinary Parasitology*, vol. 127, no. 1, pp. 17-22. http://dx.doi.org/10.1016/j.vetpar.2004.08.019. PMid:15619370.

KHAMSIAN, E.M., HAJIMOHAMMADI, B., ESLAMI, G., FALLAHZADEH, M.H. and HOSSEINI, S.S., 2021. Toxoplasma gondii in milk of human and goat from the desert area in Central Iran. *Iranian Journal of Parasitology*, vol. 16, no. 4, pp. 601-609. http://dx.doi.org/10.18502/ijpa.v16i4.7873. PMid:35082889.

KLUN, I., DJURKOVIĆ-DJAKOVIĆ, O., KATIĆ-RADIVOJEVIĆ, S. and NIKOLIĆ, A., 2006. Cross-sectional survey on Toxoplasma gondii

infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. *Veterinary Parasitology*, vol. 135, no. 2, pp. 121-131. http://dx.doi.org/10.1016/j.vetpar.2005.08.010. PMid:16188388.

LASHARI, M.H., AFZAL, F., and FAROOQ, U., 2018. Hematological changes in free-range chicken (*Gallus domesticus*) naturally infected with *Toxoplasma gondii*. *Sarhad Journal of Agriculture*, vol. 34, no. 4, 973-978. http://dx.doi.org/10.17582/journal.sja/2018/34.4.973.978.

MANCIANTI, F., NARDONI, S., D'ASCENZI, C., PEDONESE, F., MUGNAINI, L., FRANCO, F. and PAPINI, R., 2013. Seroprevalence, detection of DNA in blood and milk, and genotyping of Toxoplasma gondii in a goat population in Italy. *BioMed Research International*, vol. 2013, pp. 905326. http://dx.doi.org/10.1155/2013/905326. PMid:24093106.

MUKHOPADHYAY, D., ARRANZ-SOLÍS, D. and SAEIJ, J.P., 2020. Influence of the host and parasite strain on the immune response during Toxoplasma infection. *Frontiers in Cellular and Infection Microbiology*, vol. 10, pp. 580425. http://dx.doi.org/10.3389/fcimb.2020.580425. PMid:33178630.

OLIVEIRA, J.P., CASSEB, A.R., RAMOS, A.S., ROLIM FILHO, S.T., NOGUEIRA, H.L., PINHO, R.O. and PEREIRA, W.L.A., 2018. Risk factors associated with the epidemiology of Toxoplasma gondii in cattle and buffaloes in the state of Pará, Brazil. *Semina: Ciências Agrárias*, vol. 39, no. 5, pp. 2029-2037. http://dx.doi.org/10.5433/1679-0359.2018v39n5p2029.

OMONIJO, A.O., KALINDA, C. and MUKARATIRWA, S., 2022. *Toxoplasma gondii* Infections in Animals and humans in southern africa: a systematic review and meta-analysis. *Pathogens (Basel, Switzerland)*, vol. 11, no. 2, pp. 183. http://dx.doi.org/10.3390/pathogens11020183. PMid:35215126.

PERSAD, A., CHARLES, R. and ADESIYUN, A.A., 2011. Frequency of toxoplasmosis in water buffalo (Bubalus bubalis) in Trinidad. *Veterinary Medicine International*, vol. 2011, pp. 705358. http://dx.doi.org/10.4061/2011/705358. PMid:22195295.

RAHIMI, M.T., DARYANI, A., SARVI, S., SHOKRI, A., AHMADPOUR, E., TESHNIZI, S.H., MIZANI, A. and SHARIF, M., 2015. Cats and Toxoplasma gondii: a systematic review and meta-analysis in Iran. *The Onderstepoort Journal of Veterinary Research*, vol. 82, no. 1, pp. e1-e10. http://dx.doi.org/10.4102/ojvr.v82i1.823. PMid:26017063.

RAMZAN, M., AKHTAR, M., MUHAMMAD, F., HUSSAIN, I., HISZCZYNSKA-SAWICKA, E., HAQ, A.U., MAHMOOD, M.S. and HAFEEZ, M.A., 2009. Seroprevalence of Toxoplasma gondii in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Tropical Animal Health and Production*, vol. 41, no. 7, pp. 1225-1229. http://dx.doi.org/10.1007/s11250-009-9304-0. PMid:19225903.

ROBERT-GANGNEUX, F. and DARDÉ, M.L., 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*, vol. 25, no. 2, pp. 264-296. http://dx.doi.org/10.1128/CMR.05013-11. PMid:22491772.

ROBERTS, C.W., WALKER, W., and ALEXANDER, J., 2001. Sex-associated hormones and immunity to protozoan parasites. *Clinical Microbiology Reviews*, vol. 14, vol. 3, pp. 476-488. http://dx.doi.org/10.1128/CMR.14.3.476-488.2001.

ROUATBI, M., AMAIRIA, S., AMDOUNI, Y., BOUSSAADOUN, M.A., AYADI, O., AL-HOSARY, A.A.T., REKIK, M., BEN ABDALLAH, R., AOUN, K., DARGHOUTH, M.A., WIELAND, B. and GHARBI, M., 2019. Toxoplasma gondii infection and toxoplasmosis in North Africa: a review. *Parasite (Paris, France)*, vol. 26, pp. 6. http://dx.doi.org/10.1051/parasite/2019006. PMid:30767889.

RUHOOLLAH, KHAN, W., AL-JABR, O.A., KHAN, T., KHAN, A., EL-GHAREEB, W.R., AGUILAR-MARCELINO, L., HUSSEIN, E.O.S., ALHIMAIDI, A.R. and SWELUM, A.A., 2021. Prevalence of gastrointestinal parasite in small ruminants of District Dir Upper Khyber Pakhtunkhwa Province of Pakistan. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e248978. https://doi.org/10.1590/1519-6984.248978.

SADEK, O.A., ABDEL-HAMEED, Z.M. and KURAA, H.M., 2015. Molecular detection of Toxoplasma gondii DNA in Raw Goat and Sheep milk with discussion of its Public Health Importance in Assiut Governorate. *Assiut Veterinary Medical Journal*, vol. 61, no. 145, pp. 166-177. http://dx.doi.org/10.21608/avmj.2015.170200.

SANCHEZ, S.G. and BESTEIRO, S., 2021. The pathogenicity and virulence of Toxoplasma gondii. *Virulence*, vol. 12, no. 1, pp. 3095-3114. http://dx.doi.org/10.1080/21505594.2021.2012346. PMid:34895084.

SANTOS, T.R., COSTA, A.J., TONIOLLO, G.H., LUVIZOTTO, M.C.R., BENETTI, A.H., SANTOS, R.R., MATTA, D.H., LOPES, W.D.Z., OLIVEIRA, J.A. and OLIVEIRA, G.P., 2009. Prevalence of anti-Toxoplasma gondii antibodies in dairy cattle, dogs, and humans from the Jauru micro-region, Mato Grosso state, Brazil. *Veterinary Parasitology*, vol. 161, no. 3-4, pp. 324-326. http://dx.doi.org/10.1016/j.vetpar.2009.01.017. PMid:19232473.

SCHARES, G., BANGOURA, B., RANDAU, F., GOROLL, T., LUDEWIG, M., MAKSIMOV, P., MATZKEIT, B., SENS, M., BÄRWALD, A., CONRATHS, F.J., OPSTEEGH, M. and VAN DER GIESSEN, J., 2017. High seroprevalence of *Toxoplasma gondii* and probability of detecting tissue cysts in backyard laying hens compared with hens from large free-range farms. *International Journal for Parasitology*, vol. 47, no. 12, pp. 765-777. http://dx.doi.org/10.1016/j.ijpara.2017.07.003. PMid:28870755.

SHAH, M., ZAHID, M., STHANADAR, A.A., PIR, A., KAUSAR, A. and JAN, A.H., 2013. Seroprevalence of *Toxoplasmagondii* infection in domestic animals of Mohmand agency, Pakistan. *Journal of Coastal Life Medicine*, vol. 1, pp. 70-73.

SHAPIRO, K., BAHIA-OLIVEIRA, L., DIXON, B., DUMÈTRE, A., DE WIT, L.A., VANWORMER, E. and VILLENA, I., 2019. Environmental transmission of Toxoplasma gondii: oocysts in water, soil and food. *Food and Waterborne Parasitology*, vol. 15, pp. e00049. http://dx.doi.org/10.1016/j.fawpar.2019.e00049. PMid:32095620.

SHARIF, M., DARYANI, A., NASROLAHEI, M. and ZIAPOUR, S.P., 2009. Prevalence of *Toxoplasma gondii* antibodies in stray cats in Sari, northern Iran. *Tropical Animal Health and Production*, vol. 41, no. 2, pp. 183-187. http://dx.doi.org/10.1007/s11250-008-9173-y. PMid:18473184.

SPIŠÁK, F., TURČEKOVÁ, Ľ., REITEROVÁ, K., ŠPILOVSKÁ, S. and DUBINSKÝ, P., 2010. Prevalence estimation and genotypization of *T. gondii* in goats. *Biologia*, vol. 65, no. 4, pp. 670-674. http://dx.doi.org/10.2478/s11756-010-0070-2.

SROKA, J., KUSYK, P., BILSKA-ZAJAC, E., KARAMON, J., DUTKIEWICZ, J., WOJCIK-FATLA, A., ZAJAC, V., STOJECKI, K., RÓŻYCKI, M. and CENCEK, T., 2017. Seroprevalence of Toxoplasma gondii infection in goats from the south-west region of Poland and the detection of T. gondii DNA in goat milk. *Folia Parasitologica*, vol. 64, pp. 023. http://dx.doi.org/10.14411/fp.2017.023. PMid:28783032.

STELZER, S., BASSO, W., SILVÁN, J.B., ORTEGA-MORA, L.M., MAKSIMOV, P., GETHMANN, J., CONRATHS, F.J., and SCHARES, G., 2019. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: risk factors and economic impact. *Food and Waterborne Parasitology*, vol. 15, e00037. https://doi.org/10.1016/j.fawpar.2019.e00037.

SUGDEN, K., MOFFITT, T.E., PINTO, L., POULTON, R., WILLIAMS, B.S. and CASPI, A., 2016. Is Toxoplasma Gondii Infection Related to Brain and Behavior Impairments in Humans? Evidence from a PopulationRepresentative Birth Cohort. *PLoS One*, vol. 11, no. 2,

pp. e0148435. http://dx.doi.org/10.1371/journal.pone.0148435. PMid:26886853.

TASAWAR, Z., LASHARI, H.M., HANIF, M. and HAYAT, S.C., 2011. Seroprevalence of *Toxoplasma gondii*in Domestic Goats in Multan Punjab, Pakistan. *Pakistan Journal of Life and Social Sciences*, vol. 9, no. 1, pp. 24-27.

TAVASSOLI, M., ESMAEILNEJAD, B., MALEKIFARD, F., SOLEIMANZADEH, A. and DILMAGHANI, M., 2013. Detection of T. gondii DNA in sheep and goat milk in Northwest of Iran by PCR-RFLP. *Jundishapur Journal of Microbiology*, vol. 6, no. 10, pp. e8201. http://dx.doi.org/10.5812/jjm.8201.

TENTER, A.M., HECKEROTH, A.R. and WEISS, L.M., 2000. Toxoplasma gondii: from animals to humans. *International Journal for Parasitology*, vol. 30, no. 12-13, pp. 1217-1258. http://dx.doi.org/10.1016/S0020-7519(00)00124-7. PMid:11113252.

TESHALE, S., DUMETRE, A., DARDE, M.L., MERGA, B. and DORCHIES, P., 2007. Serological survey of caprine toxoplasmosis in Ethiopia: prevalence and risk factors. *Parasite (Paris, France)*, vol. 14, no. 2, pp. 155-159. PMid:17645188.

THÁI, T.L., JUN, H., PARK, S.H., LÊ, H.G., LEE, J., AHN, S.K., KANG, J.M., MYINT, M.K., LIN, K., SOHN, W.M., NAM, H.W., NA, B.K. and KIM, T.S.. 2019. Seroprevalence of *Toxoplasma gondii* among School Children in Pyin Oo Lwin and Naung Cho, Upper Myanmar. *Korean Journal of Parasitology*, vol. 57, no. 3, pp. 303-308. http://dx.doi.org/10.3347/kjp.2019.57.3.303. PMid:31284355.

VAN DEN BROM, R., DE JONG, A., VAN ENGELEN, E., HEUVELINK, A. and VELLEMA, P., 2020. Zoonotic risks of pathogens from sheep and their milk borne transmission. *Small Ruminant Research*, vol. 189, pp. 106123. http://dx.doi.org/10.1016/j.smallrumres.2020.106123. PMid:32427176.