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RESEARCH ARTICLE

Sero-epidemiology, Spatial Distribution and Phylogenetic Analysis of *Toxoplasma gondii* in Goats of Malakand Division of Pakistan

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ABSTRACT

The aim of the study was to use serological techniques to determine the seroprevalence and phylogenetic position of *Toxoplasma (T.) gondii* infecting goats of Malakand division of Pakistan. *Toxoplasma gondii* is an apicomplexan protozoan parasite infecting one-third of the population of the world, including humans, animals, birds, etc. Still, no information on *T. gondii* infection in goats regarding serological and the phylogenetic investigations are reported from the Malakand division. Randomly, 450 goat blood samples were collected from various districts. Risk factor information was recorded from the owners by questionnaire. ELISA test was used for the detection of *T. gondii* infection in goats, and positive samples were subjected to a PCR test using the ITS-1 gene. The overall seroprevalence of *T. gondii* infection in goats was 23.11% (104/450). The prevalence of parasite was higher in female goats (25.69%), non-aborted goats (25.85%), open grazing animals (25.38%), and the summer season months of June (42.55%), July (30.23%), and August (31.14%). Among different districts, the highest seroprevalence was found in district Lower Dir (38%). The BLAST analysis of partial sequences of PCR products of *T. gondii* based on ITS-1 showed 99% similarities with reported genotypes found in cattle and goats in Punjab and Brazil. It was concluded that a large number of goats may be positive for *T. gondii* in the study area. Risk factors like gender, age, area, etc. are strongly associated with the infection, which may play a key role in the spreading of the disease in animals and humans as well.

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INTRODUCTION

Toxoplasma (T.) gondii is an apicomplexan protozoan zoonotic parasite that causes t*oxoplasmosis* and infects about one-third of the world population (Taalay *et al.,* 2022). Cats are the definitive host of this parasite, and oocysts expelled by cats cause contamination of food and water and transmission of the disease (Aziz *et al.,* 2022). Under natural environmental conditions, the sporulation period of oocysts is one to five days. Ingestion of sporulated oocysts from contaminated soil, water, or plants is a common cause of infection in intermediate hosts (Ghaffari and Dalimi, 2019). Shortly after ingestion, tachyzoites occupy the central nervous system and muscles of the body, and finally, the tissue cysts are changed to bradyzoites (Dubey *et al.,* 2020). Animals, including humans, infected by *T. gondii* can cause severe diseases like cerebral calcification, seizer disorders, microcephaly, and chorioretinitis. It is also the major reason for pneumonitis, encephalitis, and myocarditis in immunologically weak people (Lepore, 2019). Compared to large ruminants like cattle and buffaloes, small ruminants, especially goats and sheep, are largely infested with *T. gondii* infection (Tasawar *et al.,* 2013). Infected animals and cattle usually do not show any clinical symptoms, but cysts developed in beef and meat play an important role in the transmission of *T. gondii* infection, as cyst development occurs in the muscular system of the body (Stelzer *et al.,* 2019). Improperly cooked cattle meat contains cysts increasing the chances of this parasite spreading from meat to humans (De-Berardinis *et al.,* 2017).

In the Pakistani human population, the infection rate of *T. gondii* has been reported to range from 12 to 28%

(Ali *et al.*, 2021). Livestock, especially small ruminants, are the main source of revenue for the poor farmers of Pakistan, as the trade of animals and animal products like milk, wool, and other products is a common source of income for farmers in rural areas (Ahmad and Qayyum, 2014). Serological detection by using the latex agglutination test for toxoplasmosis in cattle in Pakistan is reported to range from 19 to 52% (Khan *et al.*, 2017). Serological tests, especially ELISA, for the detection of *T. gondii* are generally considered the gold standards, which is rarely used for cattle (Taalay *et al.*, 2022). The seroprevalence of *toxoplasmosis* in Northern India, Bombay (India), Bangladesh, and Afghanistan was found in cattle at 19.3, 64.44, 12, and 15.74%, respectively (Akbar *et al.*, 2022). In Pakistan, the overall seroprevalence of toxoplasmosis is higher in humans 65% to 71% than rats 58.57%, goats 52%, dogs 28.43%, cats 26.43%, cattle 25% and sheep 24% (Akbar *et al.*, 2022).

In Khyber Pakhtunkhwa province, Malakand division is one of the economically important divisions of Pakistan, and goats are the chief source of revenue for the poor farmers (Khan *et al.*, 2021). The goats are a small farmer's choice due to their fast growth, best trading opportunities, and less hectic farming than other livestock (Irshad *et al.*, 2023). The demand for small ruminant meat is higher than that of cows and buffaloes, which is the main cause of forming in Pakistan generally and especially in the Malakand division (Khan *et al.*, 2021). Due to the large population of goats in plan and mountainous areas in this division, the epidemiology of blood parasites like theileriosis, anaplasmosis, babesiosis, etc. has been reported in many studies on cattle, goats, and sheep (Niaz *et al.*, 2021), but a lack of information regarding toxoplasmosis in the goat population in the Malakand division was a major factor in the decision to conduct this study. Therefore, the current investigation is the first documented report on the epidemiology and phylogenetic study of *T. gondii* infecting goats of the Malakand division of Pakistan.

MATERIALS AND METHODS

Study area: There are seven divisions and twenty-five districts in Khyber Pakhtunkhwa province of Pakistan, of which Malakand division is considered rich for livestock farming. This division covers a 952-square-yard area and lies approximately between 33° 59' 59.89" N attitude and 72° 56' 2.72" E longitude. This division shares borders with Peshawar in the east, Afghanistan in the west, Hazara division in the north and Gilgit Baltistan province in the south, and the total of nine districts as shown in (Fig.). Rainfall, temperature, and climatic conditions are diverse. The average rainfall was recorded at 8.4 inches. The hottest months are June and July, as well as the coldest months are January and February throughout the year, in which maximum temperatures recorded are up to 42ºC and -1ºC in the summer and winter months, respectively (Irshad *et al.*, 2023).

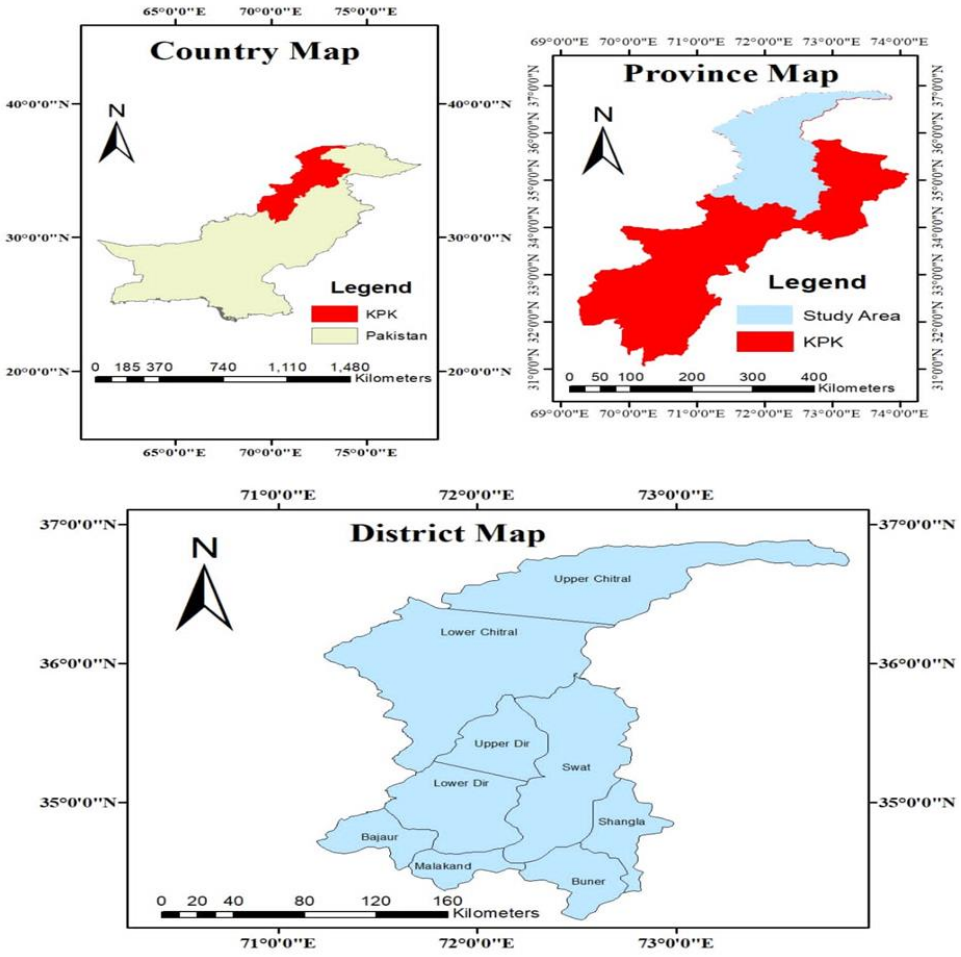

Fig. 1: Map of Malakand division, Khyber Pakhtunkhwa province of Pakistan.

Study design and sampling procedure: Randomly, a total of 450 blood samples of 5ml in a disposable syringe were collected from the jugular vein of hosuehold goats by visiting nine districts of the Malakand division of Pakistan during the study period (December 2020-November 2021) in order to investigate the epidemiology of *T. gondii* among goats in the study area. After collection, blood samples were stored in fresh EDTA tubes. From each district, 50 samples of goats were collected throughout the study period. During each visit, new goats were enrolled for sampling during the current study period. Epidemiological data were recorded in Excell sheet containing associated risk factors. The blood tubes were transported to the laboratory at 4ºC for further investigation.

Data collection: Epidemiological data were recorded in close-ended questionnaire containing associated risk factors (Thrusfield, 2018).

Serum isolation: Serums were isolated from a 3 ml blood sample by centrifugation at 3000 rpm for 10 minutes. The collected sera was stored at -20°C until further serological investigation by following the prescribed protocol (Ciuca *et al.,* 2020).

Serological investigation: Isolated serums were tested using a commercially available ELISA kit (IDvet Innovative Diagnostics, ID Screen®, France) for the detection of IgG antibodies against *T. gondii*, following the manufacturers' guiding principles available in the kit. Mean O.D was calculated for cut-off serum and an antibodies index was measured by following formula of ELISA kit (sample O.D/cut-off serum mean O.D.) × 10.

Parasite DNA isolation: DNA was isolated from a whole blood sample by following the prescribed protocol already followed by (Aziz *et al.,* 2022).

PCR amplification: ITS-1 gene primers F 5′ AGTTTAGGAAGCAATCTGAAAGCACATC 3 ′ R 5′ GATTTGCATTCAAGAAGCGTGATAGTAT-3′ were used for amplification of DNA through PCR for DNA-based identification and genetic diversity (Halová *et al.,* 2013). PCR was performed in a final volume of 25µl containing 13 mM Tris-HCl (pH 8.3), 65 mM of KCl, 25 mM of Mgcl2, 300µM of each dNTP, 1U of Taq DNA polymerase (Vivantas, USA), 0.5 µM of each primer and 5 µl (50-150ng) of template DNA. The PCR thermal profile contained an initial denaturation step of 94ºC for 3 minutes, followed by 30 cycles of denaturation at 94ºC for 30 seconds, primer annealing at 55ºC for 45 seconds, and extension at 72ºC for 30 seconds. A final extension at 72 ºC for 7 minutes was made according to (Halová *et al.,* 2013). The amplified PCR products of the ITS-1 gene with 267 base pairs were separated on 1.8% agarose gel through Electrophoresis and examined under a UV transilluminator.

Sequencing and phylogenetic analysis: Positive PCR products amplified from *T. gondii* DNA were sequenced. Randomly, four samples were sent for sequencing to Korea. Only two samples generated a proper sequence. The generated sequences of *T. gondii* were confirmed through NCBI-BLAST analysis. Further, the *T. gondii* ITS-1 gene homologous sequences were retrieved from the NCBI GenBank for phylogenetic study (Kumar *et al.,* 2018). The maximum Likelihood method and the Tamura 3-parameter model were used for inferring an evolutionary relationship by using MEGA-11 software. The two partial sequences of *T. gondii* isolates from goats based on the ITS-1 gene were submitted to GenBank, and accession numbers were obtained (PP082087 and PP082088).

Statistical analysis: Risk factor association and statistical analysis were carried out by SPSS.20 software using the Chi-square Fisher's exact test. A P-value <0.05 is considered statistically significant (Lashari *et al.,* 2020). For sequence alignment and phylogenetic tree construction, MEGA software version 11 was used.

RESULTS

Epidemiological findings: Error! Reference source not found. shows the associated risk factors. The overall prevalence of *T. gondii* infection in goats was found as 23.11% (104/450; CI: 1.729-1.808). According to gender, there was a significantly higher prevalence of *T. gondii* infection in female goats (25.69%; 83/323; CI: 0.102-0.111) as compared to those in male goats (16.53%; 21/127; CI: 0.893-0.900). Age-wise infection among goats was higher in group having age >5 years (26.85%; 29/108; CI: 0.895–0.910), and low prevalence was found in group having age less than one year (15.71%; 11/70; CI: 0.540–0.566). Area-wise, a high prevalence of *T. gondii* infection among goats in districts Lower Dir 38% (19/50; CI: 0.795–0.815) and Shangla 32% (16/50; CI: 0.783-0.804) was observed as shown in **Error! Reference source not found.** and Fig.). The high prevalence of *T. gondii* infection was found in non-aborted goats (25.85%; 91/352; CI: 0.601–0.626), and the lower was observed in aborted goats (13.26%; 13/98; CI: 0.092-0.107). A higher prevalence of *T. gondii* infection occurred in healthy goats (23.5%; 98/417; CI: 0.148-0.164) and an apparently weakend animals (18.18%; 6/33; CI: 0.202-0.226) The higher sero-prevalence of *T. gondii* infection was found in goats having no contact with cats (33.92%; 96/283; CI: 0.169–0.189) as compared to those having contact with cats (4.79%; 8/167; CI: 0.224–0.246). The infection rate of *T. gondii* in grazing goats was higher (25.38%; 83/327; CI: 0.191-0.212), and a lower prevalence was found in stall fed animals (17.07%; 21/123; CI: 0.478-0.504). There was no statistically significant difference in seroprevalence of *T. gondii* between the four sources of drinking water (P> 0.05).

Error! Reference source not found. is showing the prevalence of *T. gondii* infection among goats in the months of different seasons. In the summer season, a significantly higher prevalence of *T. gondii* occurred in the months of June (42.55%; 20/47; CI: 0.386–0.411), July (30.23%; 13/43; CI: 0.731–0.754), and August (31.14%; 19/61; CI: 0.793-0.813).

Molecular characterization and phylogenetic analysis: PCR results based on ITS-1 gene (267 bp) of Malakand

division of Pakistan isolates (PP082087 and PP082088) of *T. gondii* from goats confirmed the presence of the

Table 1: Epidemiological associated risk factors of *Toxoplasma gondii* infection among goats of Malakand Division of Pakistan.

Risk Factors	Variables	No. Infected	Sample Size	% Infected	Descriptive Statistics	
					95% CI	P-Value
Animal	Goat	104	450	23.11	1.729-1.808	0.004*
Gender	Male	21	127	16.53	0.893-0.900	0.014*
	Female	83	323	25.69	0.102-0.111	
Age	<1	11	70	15.71	0.540-0.566	0.185
	1-5	64	272	23.52	0.251-0.274	
	>5	29	108	26.85	0.895-0.910	
Area (Districts)	Buner	11	50	22	0.168-0.188	0.088
	Bajaur	12	50	24	0.565-0.591	
	Lower Chitral	5	50	10	0.756-0.778	
	Upper Chitral	6	50	12	0.918-0.932	
	Lower Dir	19	50	38	0.795-0.815	
	Upper Dir	8	50	16	0.409-0.434	
	Swat	13	50	26	0.888-0.903	
	Shangla	16	50	32	0.783-0.804	
	Malakand	14	50	28	0.387-0.412	
Abortion History	Aborted	13	98	13.26	0.092-0.107	0.044*
	Non-aborted	91	352	25.85	0.601-0.626	
Health Status	Healthy	98	417	23.5	0.148-0.164	0.003*
	Weak	6	33	18.18	0.202-0.226	
Condition of Household	Satisfied	42	163	25.76	0.528-0.553	0.087
	Unsatisfied	62	287	21.6	0.052-0.064	
Treatment	Treated	11	207	5.31	0.822-0.842	0.096
	Untreated	93	243	38.27	0.044-0.055	
Dogs with Animals	Yes	16	121	13.22	0.046-0.057	0.097
	No	88	329	26.74	0.263-0.286	
Cats with Animals	Yes	8	167	4.79	0.224-0.246	0.021*
	No	96	283	33.92	0.169-0.189	
Placement of Animals	Open	43	184	23.36	0.475-0.501	0.102
	Closed	61	266	22.93	0.047-0.058	
Sources of Drinking Water	Bore well	3	107	2.8	0.357-0.382	0.098
	Open Well	72	228	31.57	0.458-0.484	
	Tap	7	32	21.87	0.453-0.479	
	Drain	22	83	26.5	0.550-0.575	
Feeding System	Open Grazing	83	327	25.38	0.191-0.212	0.007*
	Stall Feeding	21	123	17.07	0.478-0.504	

* Indicates significant <0.05 risk factors

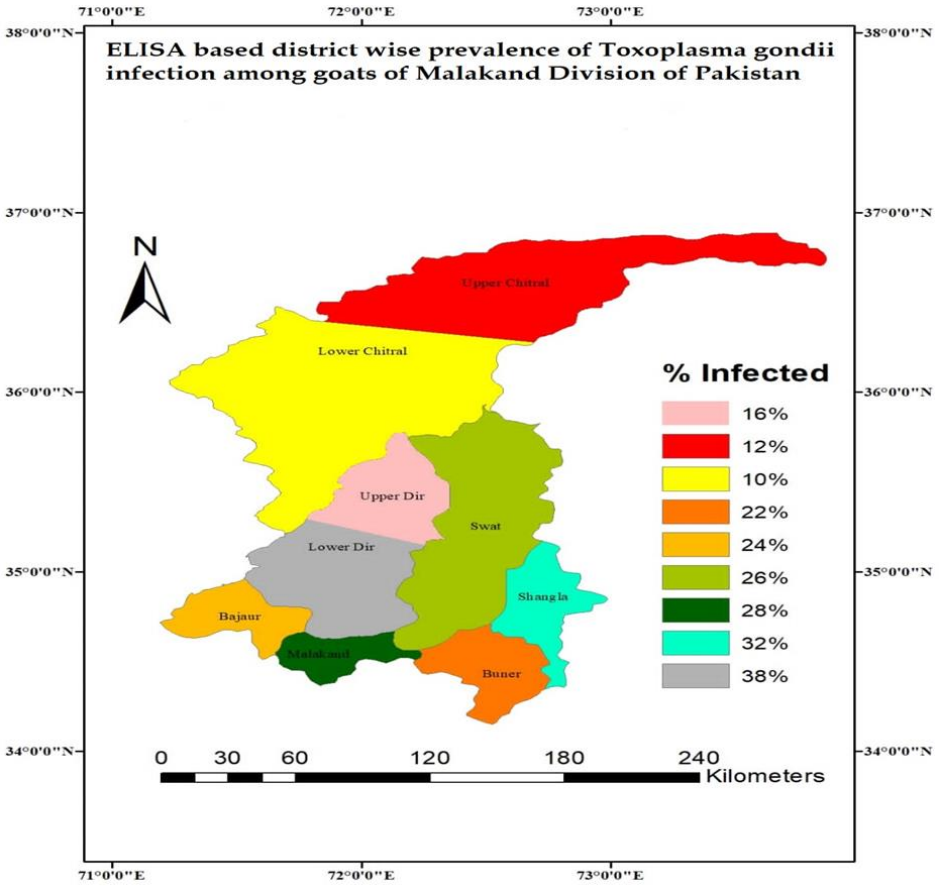

Fig. 2: ELISA based spatial distribution of *Toxoplasma gondii* infection among goats of Malakand Division of Pakistan.

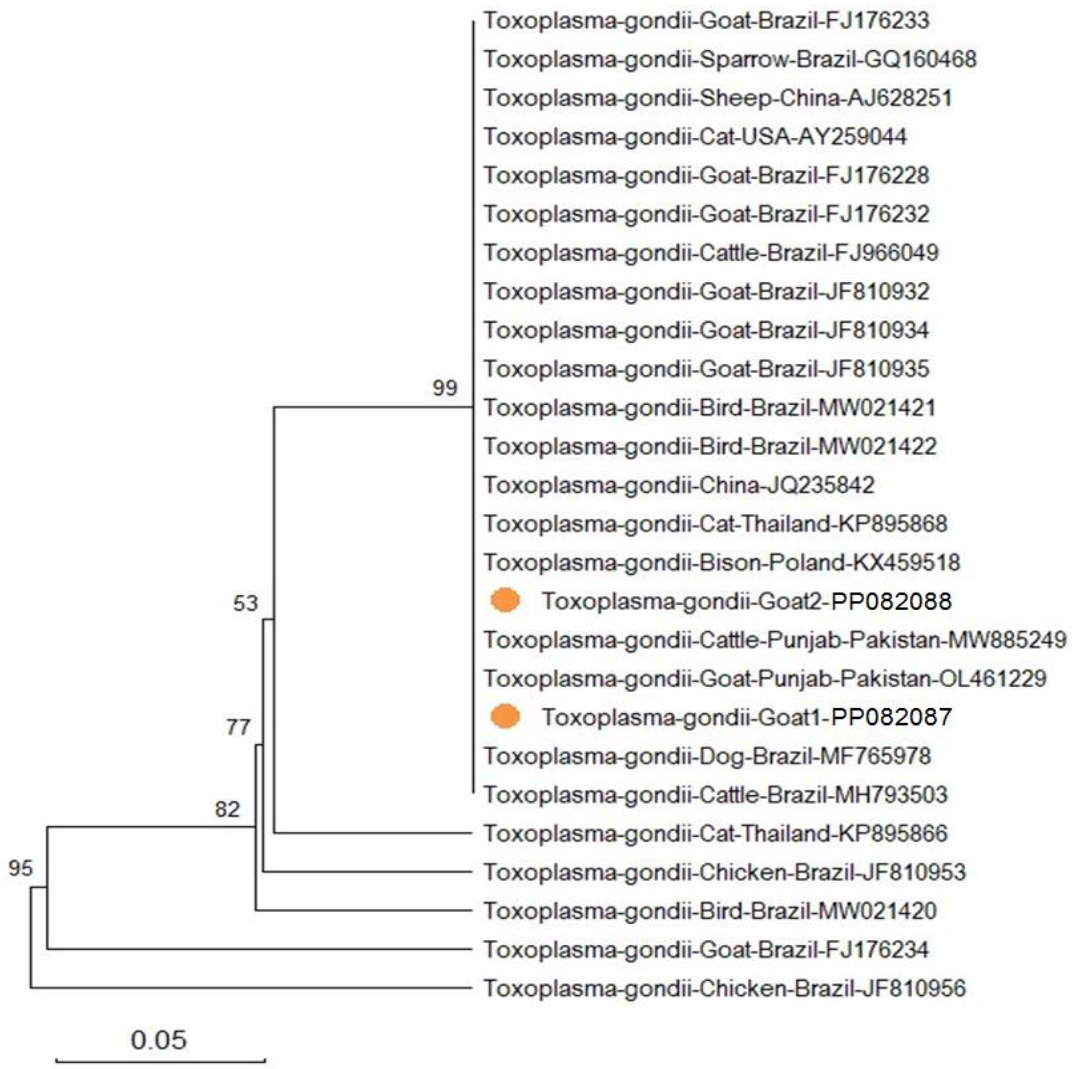

Fig. 3: Phylogenetic tree of two goat *Toxoplasma gondii* isolates (PP082087 and PP082088) based on the ITS-1 gene (267 bp) and related sequences obtained from GenBank at NCBI.

Table 2: Season and month wise prevalence of *Toxoplasma gondii* infection among goats of Malakand Division of Pakistan.

Season	Months	No. Infected	Sample Size	% Infected	Descriptive Statistics 95% CI	P-Value
Winter	Dec-2020	3	10	30	0.906-0.920	0.310
	Jan-2021	2	24	8.33	0.720-0.743	
	Feb-2021	1	20	5	0.784-0.805	
Spring	Mar-2021	4	23	17.39	0.355-0.380	0.009*
	Apr-2021	7	77	9.09	0.865-0.882	
	May-2021	18	63	28.57	0.149-0.168	
Summer	Jun-2021	20	47	42.55	0.386-0.411	0.017*
	Jul-2021	13	43	30.23	0.731-0.754	
	Aug-2021	19	61	31.14	0.793-0.813	
Autumn	Sep-2021	11	41	26.82	0.742-0.765	0.299
	Oct-2021	5	28	17.85	0.799-0.811	
	Nov-2021	1	13	7.69	0.527-0.540	

* Indicates significant <0.05 risk factors

parasite DNA by gel electrophoresis. BLAST analysis revealed 99% sequence homology of present amplified DNA sequences with the ITS-1 isolates of *T. gondii* recorded in GenBank (Fig. 3). These genotypes were identical to *T. gondii* isolates found in dogs and cattle from Brazil (MF765978 and MH793503, respectively) and to *T. gondii* isolates found in bison, goats and cattle from Poland and Punjab Pakistan (KX459518, OL461229 and MW885249, respectively) (Fig. 3). These sequences share their origin connection with those from neighbor regions and countries of the worlds as shown in phylogenetic in (Fig. 3).

DISCUSSION

Toxoplasma gondii is a widespread zoonotic disease that infects about one-third of the population throughout the world, including birds, cattle, and other hosts and cause still-birth, mental or physical retardation, abortion, central nervous damage, blindness, hydrocephalus and production losses in goats and even death in chronic cases (Majid *et al.,* 2021). In the present study, the overall prevalence of *T. gondii* infection among goats was found to be 23.11%. Seroprevalence has been reported ranging from 10 to 53% for small ruminants and between 5 and 55% for large ruminants in different districts and divisions of Pakistan (Ali *et al.,* 2021; Aziz *et al.,* 2022) while, throughout the worlds, the prevalence of *T. gondii* in cattle has been reported, e.g., 30% in Portugal (Almeida *et al.,* 2021), 8% in Brazil (Silva *et al.,* 2021), 13.5% in Egypt (Khattab *et al.,* 2022), 16–21.1% in Iran (Bahreh *et al.,* 2021), 19.3% in the north-west (Amdouni *et al.,* 2017), 10.2% in Poland (Sroka *et al.,* 2020), and 4.7% in Switzerland (Calero-Bernal *et al.,* 2023). Globally, the differences in the prevalence of *T. gondii* are mainly due to differences found in the management strategies of livestock, feeding patterns, geographical position, watering system, and grazing system (Almeida *et al.,* 2021). On the other hand, variation in climatic conditions may also play an important role in the occurrence of *T.*

gondii (Bahreh *et al.,* 2021). Additionally, it might be associated with varied approaches utilized in each investigation to find *T. gondii* (Aziz *et al.,* 2022).

In our study, the gender-wise prevalence of *T. gondii* infection revealed noteworthy distinctions. Female goats were found to be highly infected (25.69%) compared to male goats (16.53%). Our finding is in line with the reports of Abdallah *et al.* (2019) and Rafique *et al.* (2022), which represented a higher prevalence in female cattle (33%) than in male cattle (19.5%). This higher prevalence of this parasite in females may be due to their low immune system, espacially during pregnancy. Female animals are commonly retained for breeding and milk production purposes, while male animals are commonly sold to slaughter at less than 1.5 years of age, resulting in less exposure time to protozoan parasites than females (Rêgo *et al.,* 2016).

In this study, age-wise *T. gondii* infection rate among goats in age group <1 was 15.71%, 1-5 years was 23.52%, and in >5 years was found to be 26.85%. Our results are in line with the findings of Abdallah *et al.* (2019) who demonstrated a higher prevalence in the adult animals (52.43% cattle, 31.5% sheep, and 31.48% goats) than in the young animals (14.51% cattle, 20.66% sheep, and 9.12% goats). Our findings are similar to those of Jirapattharasate *et al.* (2021) and Tilahun *et al.* (2018) who reported a low prevalence in young animals, while higher prevalence was reported in adults. The seroprevalence of the *T. gondii* antibody has been found to increase with age, no matter the animal species. This could be due to the longer exposure of the adults to the *T. gondii* infection. Animals that have lived longer are more likely to be having *T. gondii* antobodies. Abortion history indicated that aborted goats were 13.26% infected, while, non-aborted goats were 25.85% infected. However, a lower prevalence of *T. gondii* was observed in non-aborted goats as compared to those of aborted gaots in India (Kalambhe *et al.,* 2017). The high prevalence rate in non-aborted goats may be due to asymptomatic infestation in animals, which cannot show physical symptoms, but serological evaluation may show the presence of infection. In the present study, most of the non-aborted goats were found serologically positive for the disease, which may be due to the asymptomatic infection, while ELISA-based detection showed a high prevalence in non-aborted goats.

The genetic diversity information regarding *T. gondii* in goats from the Malakand division of Pakistan has not been reported yet. Therefore, for the first time, we characterized PCR products of *T. gondii* isolates from goats by targeting the ITS-1 gene for phylogenetic study, as the ITS-1 gene is a sensitive molecular tool for *Toxoplasma* species (Taalay *et al.,* 2022). PCR-positive samples based on the ITS-1 gene (267 bp) confirmed the presence of *T. gondii* in targeted animals. BLAST analysis of the obtained sequences reveals the relationship with *T. gondii* isolated from Bison, Poland (Gozdzik *et al.,* 2016), goats and cattle of Punjab (Aziz *et al.,* 2022), and dogs and cattle from Brazil (Santos *et al.,* 2010). Nevertheless, the sequence analysis showed closed relationships with up to 99% sequence similarities with those isolates reported from neighboring countries.

Conclusions: From the current investigation, it was concluded that *T. gondii* is more prevalent among goats in the Malakand division of Pakistan. The associated risk factors like gender, abortion history, health status, keeping cats with livestock, feeding system and summer seasons were strongly associated with occurrence of this parasite in the study area. The findings of the present research indicate the need for comprehensive research to explore the genetic diversity of *T. gondii* in goats, sheep, cattle, buffalos, water sources, and other food sources in different parts of Pakistan to find transmission routes.

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Conflicts of Interest: The authors declare no conflict of interest.

Author Contributions: Conceptualization: AI, SN, WK, AR; Methodology: AI, SN, WK, AR; Validation, AI, SN; Analysis: AI, WK, AR; Research: AI, SN, WK, AR; Data Curation: AI, SN, WK, AR; Writing the Articale: AI, SN, WK, AR; Critical Review: AI, SN, AR; All authors have read and agreed to publish this version of the manuscript.

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REFERENCES

Abdallah MC, Kamel M, Karima, *et al.,* 2019. Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep, and goats in Algeria: seroprevalence and risk factors. Vet Sci 6:1-15.

Ahmad N and Qayyum M, 2014. Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan. J Infect Dev Ctries 8:1022-1028.

Akbar H, Shabbir MZ, Ullah U, *et al.,* 2022. Serological investigation of bovine toxoplasmosis using commercial and indigenous ELISA kits while validating cattle toxo IgG ELISA Kit. Animals 12:20671-13.

Ali A, Omer T, Ullah A, *et al.,* 2021. Epidemiological survey of *Toxoplasma gondii* and associated risk factors in ruminant species of the Khyber Pakhtunkhwa Province of Pakistan. J Parasitol Res 8:6653239.

Almeida D, Quirino J, Barradas PF, *et al.,* 2021. A 2-Year Longitudinal Seroepidemiological Evaluation of *Toxoplasma gondii* Antibodies in a Cohort of Autochthonous Sheep from Central Portugal. Pathogens 10:402-6.

Amdouni Y, Rjeibi MR, Rouatbi M, *et al.,* 2017. Molecular detection of *Toxoplasma gondii* infection in slaughtered ruminants (sheep, goats and cattle) in Northwest Tunisia. Meat Sci 133:180-184.

Aziz MN, Iqbal RK, Irfan M, *et al.,* 2022. First report on molecular epidemiology, seasonality and phylogeny of *Toxoplasma gondii* infecting goats from Khanewal district in Punjab, Pakistan. Acta Trop 228:106304.

Bahreh M, Hajimohammadi B and Eslami G, 2021. *Toxoplasma gondii* in sheep and goats from Central Iran. BMC Res Notes 14:461-5.

Calero-Bernal R, Gennari SM, Cano S, *et al.,* 2023. Anti-*Toxoplasma gondii* antibodies in european residents: A systematic review and meta-analysis of studies published between 2000 and 2020. Pathogens 12:1430-1438.

Ciuca L, Borriello G, Bosco A, *et al.,* 2020. Seroprevalence and clinical outcomes of Neospora caninum, *Toxoplasma gondii* and Besnoitia besnoiti infections in water buffaloes (Bubalus bubalis). Animals 10:5321-11.

De-Berardinis A, Paludi D, Pennisi L, *et al.,* 2017. *Toxoplasma gondii*, a foodborne pathogen in the swine production chain from a European perspective. Foodborne pathog Dis 14:637-648.

Dubey J, Murata F, Cerqueira-Cézar C, *et al.,* 2020. Public health significance of *Toxoplasma gondii* infections in cattle: 2009–2020. J Parasitol 106:772-788.

Ghaffari AD and Dalimi A, 2019. Molecular identification of *Toxoplasma gondii* in the native slaughtered cattle of Tehran province. J Food Qua Haz Cont 6: 153-161.

Gozdzik K, Cabaj W, Bien J, *et al.,* 2016. In vitro isolation and molecular identification of the first *Toxoplasma gondii* isolate from new born European bison (Bison banasus banasus L.). Polish Aca Sci Poland 2:8-18

Halová D, Mulcahy G, Rafter P, *et al.,* 2013. *Toxoplasma gondii* in Ireland: seroprevalence and novel molecular detection method in sheep, pigs, deer and chickens. Zoonoses Public Health 60:1-13.

Irshad A, Ullah A, Khan MF, *et al.,* 2023. Identification and prevalence of gastrointestinal parasites in goats and sheep of District Lower Dir, Pakistan. J Xi'an Shiyou Uni, Nat Sci Ed 11:251-256.

Jirapattharasate C, Udonsom R, Prachasuphap A, *et al.,* 2021. Development and evaluation of recombinant GRA8 protein for the serodiagnosis of *Toxoplasma gondii* infection in goats. BMC Vet Res 17:271-9.

Kalambhe D, Gill J and Singh BB, 2017. Molecular detection of *Toxoplasma gondii* in the slaughter sheep and goats from North India. Vet Parasitol 241:35-38.

Khan M, Rashid I, Akbar H, *et al.,* 2017. Seroprevalence of *Toxoplasma gondii* in South Asian countries. Rev Sci Tech Off Int Epiz 36:3981-996.

Khan T, Nasreen N, Shater AF, *et al.,* 2021. Risk factor analysis for the prevalence of gastrointestinal parasites found in large ruminants in Lower Dir Khyber Pakhtunkhwa Pakistan. Saudi J Bio Sci 28:7022-7026.

Khan W, Al-Jabr O, Khan T, *et al.,* 2021. Prevalence of gastrointestinal parasite in small ruminants of District Dir Upper Khyber Pakhtunkhwa Province of Pakistan. Brazilian J Bio 83:e248978.

Khattab RAH, Barghash SM, Mostafa OMS, *et al.,* 2022. Seroprevalence and molecular characterization of *Toxoplasma gondii* infecting ruminants in the North-West of Egypt. Acta Trop 225:106139.

Kumar S, Stecher G, Li M, *et al.,* 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Bio Evo 35:1547-1556.

Lashari M, Farooq U, Mubeen S, *et al.,* 2020. Seroprevalence of *Toxoplasma gondii* and associated hematological alterations in small ruminants of DG Khan district of Southern Punjab, Pakistan. Arq Bras Med Vet Zootec 72:1698-1704.

Lepore T, 2019. Specific diagnostic tools for protozoan infection of ruminants. Royal (Dick) Sch Vet Stu 1842:36047-256.

Majid A, Ahmad N, Haleem S, *et al.,* 2021. Detection of toxoplasmosis in pets and stray cats through molecular and serological techniques in Khyber Pakhtunkhwa, Pakistan. BMC Vet Res 17:1-7.

Niaz S, Rahman ZU, Ali I, *et al.,* 2021. Molecular prevalence, characterization and associated risk factors of Anaplasma spp. and Theileria spp. in small ruminants in Northern Pakistan. Parasite 28:1-13.

Rafique A, Nasir S, Ashraf A, *et al.,* 2022. Sero-surveillance and risk factors analysis of caprine toxoplasmosis in Faisalabad Punjab, Pakistan. Pak Vet J 42:102-06.

Rêgo W, Paul N, Vitor R, *et al.,* 2016. Risk factors for *Toxoplasma gondii* infection in goats and sheep raised in the State of Piauí in northeast Brazil. Small Ruminant Res 141:17-23.

Santos SL, de-Souza CK, Gondim LQ, *et al.,* 2010. Investigation of *Neospora caninum, Hammondia* sp., and *Toxoplasma gondii* in tissues from slaughtered beef cattle in Bahia Brazil. Parasitol Res 106:457–461.

Silva BM, Queiroz WCC, Maia MO, *et al.,* 2021. Seroprevalence and risk factors of *Toxoplasma gondii* in cattle from Unai, Minas Gerais state, Brazil. Vet Parasitol: Reg Stu and Reports 25:100610.

Sroka J, Karamon J, Wójcik-Fatla A, *et al.,* 2020. *Toxoplasma gondii* infection in slaughtered pigs and cattle in Poland: seroprevalence, molecular detection and characterization of parasites in meat. Parasit Vectors 13:1-11.

Stelzer S, Basso W, Silván JB, *et al.,* 2019. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food Waterborne Parasitol 15:e00037.

Taalay I, Iqbal RK, Asif M, *et al.,* 2022. Molecular survey of *Toxoplasma gondii* in cattle and buffaloes and phylogenetic position of Pakistani isolates based on ITS-1 gene. Comp Immunol Microbiol Infect Dis 84:101782.

Tasawar Z, Shafiq Z, Lashari M, *et al.,* 2013. Seroprevalence of *Toxoplasma gondii* in cattle, Punjab, Pakistan. Global Vet 11:681-684.

Thrusfield M, 2018. Veterinary epidemiology. John Wiley and Sons. 2:1-59.

Tilahun B, Tolossa YH, Tilahun G, *et al.,* 2018. Seroprevalence and risk factors of *Toxoplasma gondii* infection among domestic ruminants in East Hararghe zone of Oromia Region, Ethiopia. Vet Med Int 10:1-8.