EXPLORING THE PRESENCE AND GENETIC MAKEUP OF *TOXOPLASMA GONDII* IN HOUSEHOLD CATS: A FASCINATING INVESTIGATION

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Abstract. Toxoplasmosis, caused by the intracellular parasite *Toxoplasma gondii*, poses a significant zoonotic risk. With its adaptability to diverse environments and a wide range of warm-blooded hosts, *T. gondii* remains a persistent concern for public health worldwide. In this study, we investigated 250 fecal samples from cats across various pet clinics in Hyderabad. Microscopic examination revealed *T. gondii* infection in 38 out of 250 samples, indicating a 15.2% prevalence rate among cats. We delved deeper to identify factors contributing to disease development and found intriguing patterns. Breed-wise analysis showed higher infection rates in Persian cats (19.79%), followed by Bengal (16.67%) and crossbreeds (9.09%). Notably, younger cats exhibited significantly higher infection rates (39.06%) compared to adults (6.99%) (P = 0.0001). Surprisingly, male cats showed no infections, while female cats harbored a 26.03% infection rate. Further analysis revealed a correlation between infection and symptoms, with dry hair-coated cats showing a 31.91% infection rate compared to those with healthy coats (5.13%). Additionally, fecal consistency appeared to influence infection rates, with semi-solid samples exhibiting a prevalence of 100%. Morphological characterization of positive samples revealed bi-layered oocyst walls with oval and spherical shapes, while molecular analysis via PCR confirmed the presence of a 469 bp fragment of the B1 gene of *T. gondii*. Sequencing confirmed a 99-100% similarity with *T. gondii* from various hosts and locations. Phylogenetic analysis indicated close relatedness to isolates from rats and sheep in Nigeria and Mexico, respectively. These findings underscore the common prevalence of *T. gondii* among pet cats, posing a significant risk to pet owners. The alarming baseline data generated in this study calls for strategic management of toxoplasmosis in public health initiatives.
Keywords: *Toxoplasma gondii, household cats, prevalence, molecular characterization, zoonotic disease, risk factors*

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Introduction

Toxoplasmosis, caused by the parasitic organism *Toxoplasma gondii*, poses a significant global health concern as a zoonotic disease affecting various host species, including humans (Njuguna et al., 2017). Despite its widespread impact, toxoplasmosis often remains overlooked due to its chronic nature (Smith et al., 2021). Domestic cats, along with other members of the Felidae family, serve as the definitive hosts of *T. gondii*, serving as primary sources of infection for humans and other susceptible hosts (Kvac et al., 2017; Li et al., 2019). Interaction with these definitive hosts or reservoirs, as well as other companion animals, poses a considerable risk of infection. Although toxoplasmosis typically manifests as a chronic condition, it can lead to severe consequences, including abortion, pneumonia, encephalitis, and muscular disorders (Smith et al., 2021). Additionally, economic losses due to abortion, meat condemnation, and birth defects in livestock highlight its significance in both public health and agricultural sectors (Stelzer et al., 2019). The prevalence of *T. gondii* varies among populations due to differing management conditions, with domesticated animals, especially pets, representing potential sources of infection for humans (Dubey et al., 2020). *T. gondii* reproduces through both sexual and asexual phases, with cats serving as definitive hosts for sexual reproduction and various warm-blooded animals, including humans and birds, as intermediate hosts for asexual reproduction (Dubey, 2010; Lappin et al., 2010). The parasite exists in three infectious stages–oocysts, tachyzoites, and bradyzoites–which can be transmitted through various routes, including ingestion of contaminated food or water, congenital transmission, or contact with contaminated soil (Dubey, 2006; Freppel et al., 2019). While many infections remain asymptomatic or result in mild illness, immunocompromised individuals and vulnerable populations, such as the very young or elderly, may develop severe symptoms (Dubey, 2006; Cuomo et al., 2013; Egorov et al., 2018). Cats infected with *T. gondii* are usually diagnosed through fecal examination through microscopy, while humans are diagnosed with serological test or by tissue biopsy followed by microscopy. In general, human case are preferably diagnosed by serological examination due to their less-invasive nature and higher accuracy (Jones et al., 2001; Montoya, 2002). Given the zoonotic potential of *T. gondii*, understanding its prevalence in pet animals, such as household cats, is crucial for assessing the risk of human infection (Balder et al., 2015; Smith et al., 2021). This study aims to investigate the prevalence of *T. gondii* in household cats in Hyderabad, along with morphological and molecular characterization of the parasite. These findings will provide valuable insights into the epidemiology of *T. gondii* in pet animals and inform strategies for its management and control. Prevalence of Toxoplasmosis based on the morphological characteristics has also been reported from different countries (Blader et al., 2015). However, accurate identification of *T. gondii* on the basis of morphology is unreliable due to high similarity between oocysts of Toxoplasma, Hammondia and Neospora species and is difficult to differentiate during mixed infection (Monteiro et al., 2008). Therefore, an alternative strategy is required to control the Toxoplasmosis in cats on the basis of confirmatory diagnosis as these can serve as a main source of infection to the humans. Therefore, the present study aims to investigate the prevalence of *T. gondii* infecting household cats with their morphological and molecular characterization of the different genotypes involved in the infection. These baseline data will further help to understand epidemiology of *T. gondii* in pet animals for planning better treatment and control strategies. The key objective of research was to assess the prevalence of *T. gondii* infection in household cats and evaluate its impact on feline health, morphologically characterize *T. gondii* isolates

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obtained from household cats and molecularly characterize isolated *T. gondii* strains to understand genotype diversity and evolutionary relationships.

Materials and methods

Study area and sample collection

Fecal samples were directly collected from the rectum of 250 cats presented at various pet clinics in Hyderabad. Samples were handled using disposable gloves, placed in ice containers, and transported to the laboratory at the Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. Sample data, including animal condition, fecal consistency, age, sex, breed, and management system, were recorded during collection.

Fecal examination

Each fecal sample (3 g) was mixed with 50 ml of saturated sugar solution (SG = 1.27) for parasitological investigation. After sieving and standing for 20 min, the mixture was observed under a microscope at 10× and 40× magnification for the presence of *T. gondii*. Positive samples were preserved at -20°C for further analysis.

Sporulation of T. gondii

Positive fecal samples were processed to isolate parasites, which were washed in tap water and then placed in 2.5% potassium dichromate solution for sporulation over 7 days. Sporulated oocysts were observed under a microscope for morphological characterization.

Determination of infection severity

All fecal samples were analyzed using the McMaster technique to determine infection severity. Fecal solutions were prepared and counted to calculate oocyst per gram of feces.

Purification of oocysts

For molecular characterization, oocysts were isolated from positive fecal samples using centrifugation and washing steps to obtain a purified sediment.

Genomic DNA extraction

Genomic DNA was extracted from oocysts using the phenol-chloroform method. The extracted DNA was stored at -20°C until further use.

Primers

Universal primers targeting 18S ribosomal RNA gene regions and *T. gondii*-specific primers targeting the B1 gene region were used for PCR amplification.

PCR amplification

PCR reactions were performed using extracted DNA and specific primers. Amplification conditions included denaturation, annealing, and extension cycles.

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Agarose gel electrophoresis

PCR products were separated on agarose gels and visualized using UV transillumination.

PCR product purification

PCR products from positive samples were purified using a gel extraction kit and sent for sequencing.

Sequence and phylogenetic analysis

Sequenced DNA was compared with sequences in the NCBI database, and alignments were performed using Clustal W. Phylogenetic trees were constructed using Neighbor-joining (NJ) analyses with bootstrap analyses to assess tree topology reliability.

Statistical analysis

To assess prevalence rates and differences among groups, the data underwent rigorous statistical analysis using SPSS (Version 20.0). The Chi-square test was employed for categorical variables, while the Student's t-test was utilized for continuous variables. This comprehensive approach ensured robust evaluation and comparison of the data across various parameters.

Results

The objective of this study was to assess the prevalence of toxoplasmosis among cats presented for treatment at various clinics in District Hyderabad. Over a five-month period, from August 2022 to December 2022, two hundred and fifty (250) fecal samples were collected from cats at pet clinics. Samples were obtained without discrimination between diseased and healthy animals. Data on various factors potentially contributing to disease development, including animal condition, fecal consistency, age, sex, breed, and management system, were recorded. Fecal samples were analyzed at the Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. Initial diagnosis relied on microscopic examination to identify Toxoplasma infection based on morphological characteristics, followed by PCR amplification for precise species identification. Results indicated that out of 250 samples, 38 cats were infected, yielding an overall prevalence rate of 15.2% for toxoplasmosis in cats in District Hyderabad (refer to *Table 1*).

Table 1. *Prevalence of toxoplasmosis among cats in District Hyderabad during the study period*

Total no. of samples	Positive samples	Negative samples	Prevalence
250	38	212	15.2%

Breed-wise prevalence of toxoplasmosis in cats

During our study, we investigated whether different cat breeds exhibited variations in the prevalence of toxoplasmosis. Cats were divided into three categories based on their breed: Persian, Bengal, and crossbreeds. Results showed that Persian cats had the highest

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infection rate (19.79%), followed by Bengal cats (16.67%), and crossbreeds (9.09%) (*Table 2; Fig. 1*). Most of the cats examined were household pets, kept in clean environments in close proximity to humans. These findings suggest that cats of various breeds are equally susceptible to toxoplasmosis, potentially serving as a source of infection for humans due to the parasite's zoonotic nature. However, statistical analysis revealed no significant difference in infection rates among the different cat breeds (P = 0.1661).

Table 2. *The breed-wise prevalence of toxoplasmosis in cats at district Hyderabad during the present study*

Breed type	No. of observed cats	No. of infected cats	Prevalence %	Frequency %
Cross breed	88	8	9.09	21.05
Persian	96	19	19.79	50.00
Bengal	66	11	16.67	28.95
Total	250	38	15.2	100.00

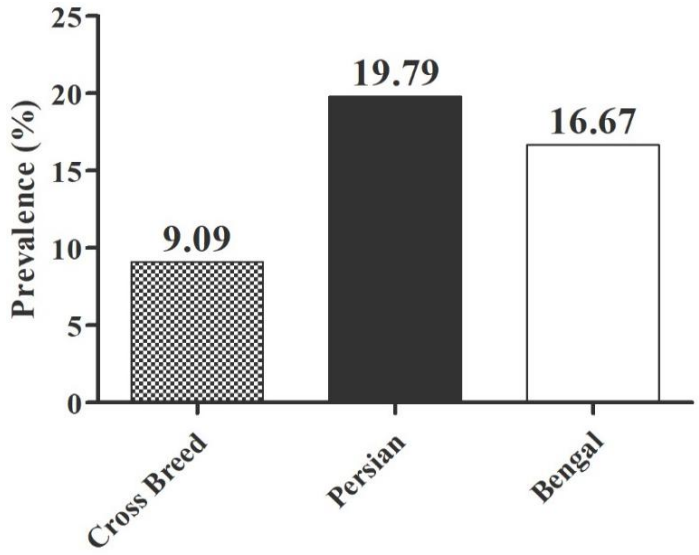

Figure 1. *The breed-wise prevalence of toxoplasmosis in cats at district Hyderabad during the present study. df = 2; P value: 0.1661 (non-significant > P = 0.05)*

Animal age-wise prevalence of toxoplasmosis

During the sampling process, cats were divided into different age groups: young cats (<1 year old) and adult cats (>1 year old), in order to assess the impact of age on the prevalence of *T. gondii* infection. The findings revealed a significant difference (P = 0.0001) in infection rates between young and adult cats. Among the sampled young cats, 25 out of 64 tested positive for *T. gondii*, resulting in a prevalence rate of 39.06%. In contrast, only 13 out of 186 adult cats tested positive, with a prevalence rate of 6.99%. The data indicates a higher susceptibility to *T. gondii* infection among young cats compared to adults, with infection rates of 65.79% and 34.21%, respectively (*Table 3; Fig. 2*).

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Table 3. *Age-wise prevalence of toxoplasmosis in cats in district Hyderabad*

Age group	No. of observed cats	No. of infected cats	Prevalence %	Frequency %
Young (<1 Y)	64	25	39.06	65.79
Adult (>1 Y)	186	13	6.99	34.21
Total	250	38	15.20	100.00

df = 1; P value: 0.0001 (significant < P = 0.05)

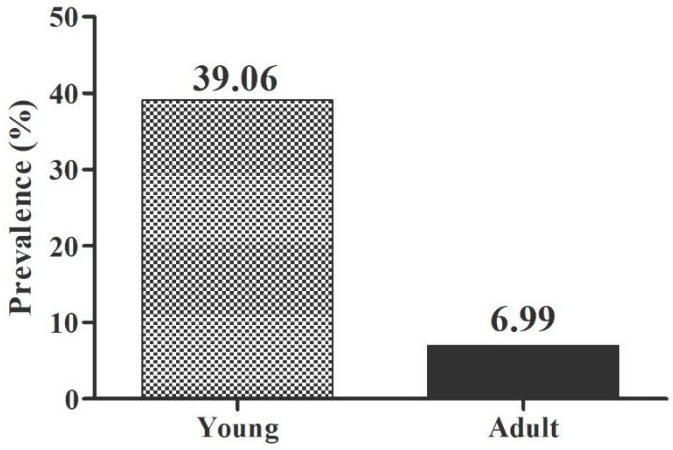

Figure 2. *Age-wise prevalence of toxoplasmosis in cats in district Hyderabad*

Sex-wise prevalence of toxoplasmosis

During the study, it was noted that a majority of pet owners prefer female cats over males. A total of 104 fecal samples were collected from male cats, while 146 samples were obtained from female cats. Despite the smaller sample size for males, none tested positive for toxoplasmosis. In contrast, out of the 146 samples from female cats, 38 were positive for the parasite. Furthermore, a notable difference in infection rates was observed between young and adult female cats, with a higher infection rate of 58.14% in young females compared to 12.62% in adults. Statistical analysis revealed significant differences in infection rates between male and female cats, as well as between young and adult female cats (P = 0.0001) (*Table 4; Fig. 3*).

Table 4. *Sex-wise prevalence of toxoplasmosis in cats from district Hyderabad*

Gender	Age group	No. of observed cats	No. of infected cats	Prevalence %	Frequency %
Male	Young	21	0	0	0
	Adult	83	0	0	0
	Total	104	0	0	0
Female	Young	43	25	58.14	65.79
	Adult	103	13	12.62	34.21
	Total	146	38	26.03	100.00
Total		250	38	15.2	

df = 1; P value: 0.0001 (significant < P = 0.05)

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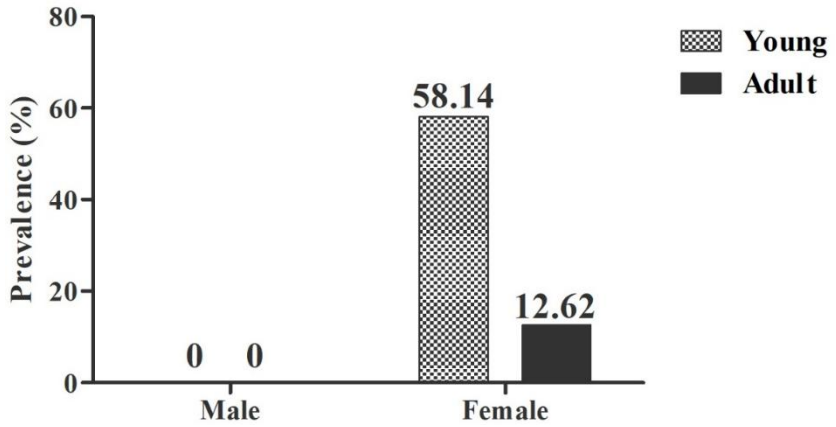

Figure 3. *Sex-wise prevalence of toxoplasmosis in cats from district Hyderabad*

Animal condition-wise prevalence of toxoplasmosis

During the study, various factors contributing to disease development or resulting as consequences of the disease were considered to determine the risk factors associated with its occurrence. One such factor observed was the body condition of the animals. Cats were categorized based on their coat conditions, distinguishing between those with normal, healthy coats and those with dry and rough coats. It was found that only 5.13% of cats with a healthy coat tested positive for toxoplasmosis, possibly indicating subclinical infection. In contrast, cats with dry and rough coats showed a significantly higher infection rate of 31.91% (*Table 5*; *Fig. 4*). This suggests a potential association between coat condition and susceptibility to *T. gondii* infection.

Table 5. *The overall prevalence of toxoplasmosis based on animal condition of cats in district Hyderabad*

Group	No of observed cats	No of infected cats	Prevalence %	Frequency%
Healthy coat	156	8	5.13	21.05
Dry coat	94	30	31.91	78.95
Total	250	38	15.20	100.00

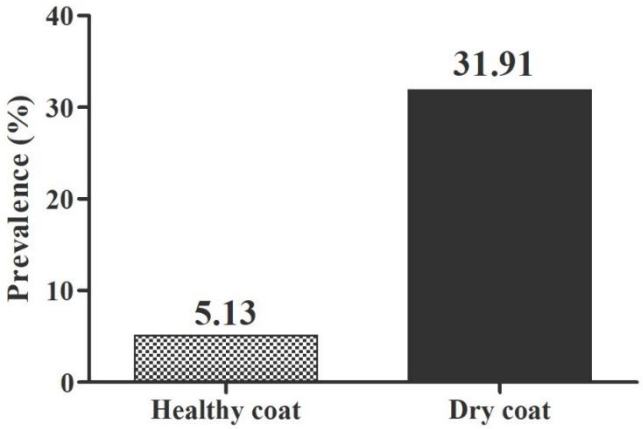

Figure 4. *The overall prevalence of toxoplasmosis based on animal condition of cats in district Hyderabad. df = 1; P value: 0.0001 (significant < P = 0.05)*

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Stool consistency-based prevalence of toxoplasmosis

The study involved observing cats for infections in various types of stools to determine if toxoplasmosis is causing clinical signs in the animals. Samples were categorized as normal, diarrheic, or semi-solid. Out of 122 observed samples with diarrhea, 30 were found infected, representing a prevalence rate of 24.59% in cats. In contrast, only 6 out of 126 samples with normal consistency were infected, indicating a prevalence rate of 4.76%. Additionally, all 2 samples of semi-solid feces were infected with *T. gondii* (*Table 6; Fig. 5*). Overall, there was a significant difference in the prevalence rate among the groups with different fecal consistencies (P = 0.0001). These findings suggest that cats with diarrhea exhibit a higher prevalence rate compared to those with normal feces. This underscores the potential impact of *T. gondii* on the digestive system, as parasitic infections can disrupt digestive balance, leading to diarrhea initially and potentially resulting in weakness in later stages of the disease.

Table 6. *The overall prevalence of toxoplasmosis based on stool consistency of cats in district Hyderabad*

Stool group	No. of observed cats	No. of infected cats	Prevalence %	Frequency %
Diarrhea	122	30	24.59	78.95
Normal	126	6	4.76	15.79
Semi solid	2	2	100.00	5.26
Total	250	38	15.2	100

df = 2; P value: 0.0001 (significant < P = 0.05)

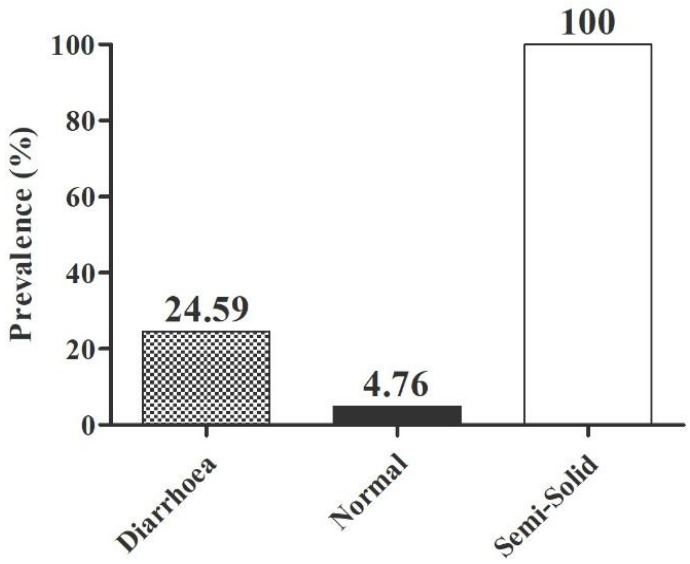

Figure 5. *Prevalence of toxoplasmosis based on stool consistency of cats in district Hyderabad*

Morphological characterizations of Toxoplasma gondii

The parasitic species initially collected underwent microscopic examination to identify the specific species. All specimens that tested positive were morphologically identified as *T. gondii*. This identification was based on the presence of a bi-layered oocyst wall and

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an oval or spherical shape with a diameter ranging from 10 to 12 µm (*Fig. 6*). To induce sporulation, the oocysts were subjected to potassium dichromate and maintained at room temperature (12-25°C). After 7 days, sporulation occurred, and the sporulated oocysts were examined for their shape, size, presence of sporocysts, and the bi-layered oocyst wall.

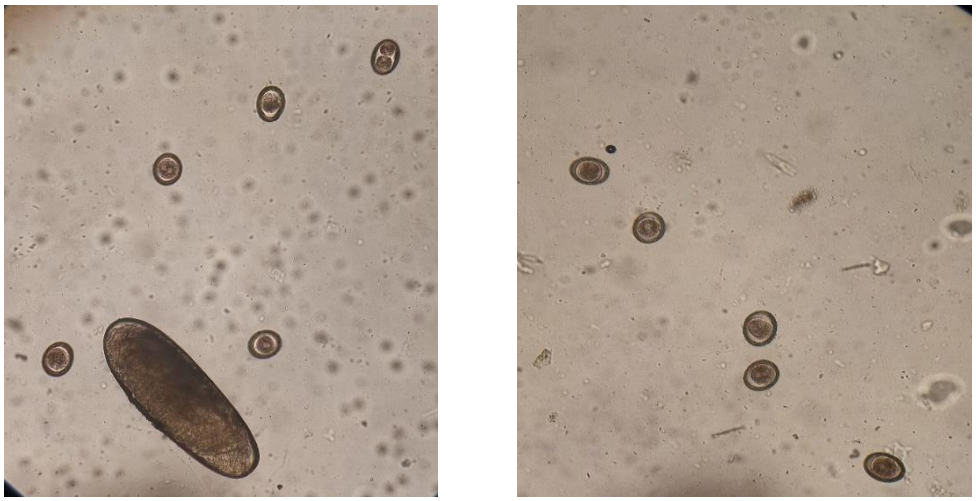

Figure 6. *Sporulated and un-sporulated T. gondii oocysts in fecal samples collected from cats in district Hyderabad. Oocysts were observed under 40× magnification*

Molecular identification of T. gondii

Additionally, the six microscopically positive samples underwent DNA extraction followed by PCR amplification to accurately identify the species and strain. The DNA extracted from these samples was amplified using primers targeting the B1 gene from the *T. gondii* genome to identify the organism responsible for the infection. PCR amplification of the selected samples resulted in a 469 bp fragment size band on agarose gel for all samples, indicating successful amplification through PCR and confirming the presence of *T. gondii* in the infected cats (*Fig. 7*).

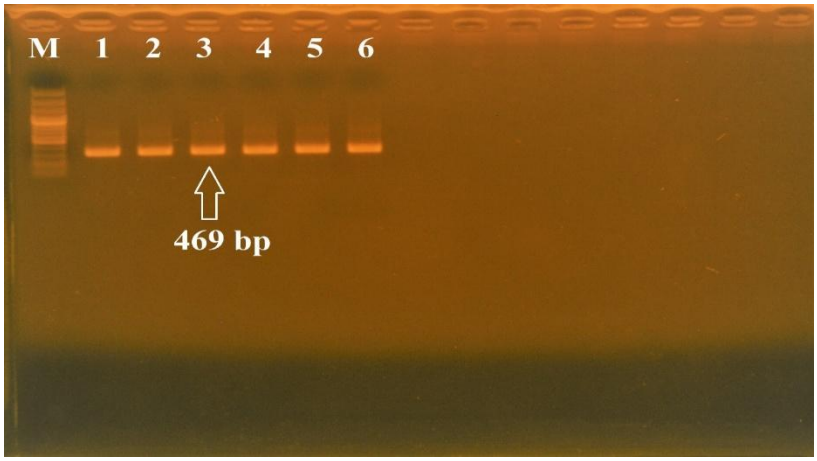

Figure 7. *PCR amplification of T. gondii samples using primer set targeting B1 gene. Figure shows the PCR amplification of samples from cats with sample no. 1, 2, 3, 4, 5, 6 indicates a 469 bp band on the agarose gel. Lane M: 100 bp DNA ladder molecular weight marker*

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Sequencing

The PCR-positive samples underwent further sequencing to ensure accurate identification at the species and strain level. The sequencing results revealed that the amplified fragments were 469 base pairs in length. Notably, only one identical sequence type was identified from both samples, and they all corresponded to *T. gondii*. These findings strongly suggest that the cats in the study area are indeed infected with *T. gondii*. Below is the raw sequencing results obtained including the raw sequencing data here.

Sample: Tg1

AAAAATGTGGGAATGAAAGAGACGCTAATGTGTTTGCATAGGTTGCAGT
CACTGACGAGCTCCCCTCTGCTGGCGAAAAGTGAAATTCATGAGTATCTGTG
CAACTTTGGTGTATTCGCAGATTGGTCGCCTGCAATCGATAGTTGACCACGA
ACGCTTTAAAGAACAGGAGAAGAAGATCGTGAAAGAATACGAGAAGAGGT
ACACAGAGATAGAAGTCGCTGCGGAGACAGCGAAGACTGCGGATGACTTC
ACTCCCGTCGCACCAGCAGCAGAGGAGTGCCGGGCAAGAAAATGAGATGC
CTAGAGGAGACACAGCGTGTTATGAACAAATCTATTGAGGTTTCGCGAAGA
GGAGGGAACATATTATATACAGAAGAAGAACAAGAGACGTGCCGCATGTC
GCTAAGCCATCGGAAGGGATGCTCAGAAAATGGCACAGTATCACATTACAG
TTCCGTTGATTCGT

Sample: Tg2

AAAAATGTGGGAATGAAAGAGACGCTAATGTGTTTGCATAGGTTGCAGT
CACTGACGAGCTCCCCTCTGCTGGCGAAAAGTGAAATTCATGAGTATCTGTG
CAACTTTGGTGTATTCGCAGATTGGTCGCCTGCAATCGATAGTTGACCACGA
ACGCTTTAAAGAACAGGAGAAGAAGATCGTGAAAGAATACGAGAAGAGGT
ACACAGAGATAGAAGTCGCTGCGGAGACAGCGAAGACTGCGGATGACTTC
ACTCCCGTCGCACCAGCAGCAGAGGAGTGCCGGGCAAGAAAATGAGATGC
CTAGAGGAGACACAGCGTGTTATGAACAAATCTATTGAGGTTTCGCGAAGA
GGAGGGAACATATTATATACAGAAGAAGAACAAGAGACGTGCCGCATGTC
GCTAAGCCATCGGAAGGGATGCTCAGAAAATGGCACAGTATCACATTACAG
TTCCGTTGATTCGT

Sequence comparison

The sequences obtained from the PCR amplification using the primer set targeting the B1 gene consistently produced a fragment size of 469 base pairs. Given the identical band size and morphology observed, one representative sequence from each sequence group was selected for sequencing and subsequently deposited in the GenBank of the National Center for Biotechnology Information (NCBI) with accession numbers. Subsequently, the sequences obtained from this study were aligned with each other for comparison (*Fig. 8*). The multiple alignment of sequences revealed a high degree of sequence similarity among all the sequences obtained and the species collected from the study area. Furthermore, comparing both types of sequences obtained demonstrated their identical nature, suggesting that the source of infection may be uniform, and the cats likely acquired the infection from the same geographical area (*Fig. 9*). Furthermore, it is essential to compare the obtained sequencing with sequences from various localities to

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gain a broader perspective. To achieve this, sequences from different geographical areas were retrieved from the NCBI database and aligned for comparison (*Fig. 9*).

Figure 8. *Multiple alignments of obtained sequences. The comparison of both obtained sequences shows a base pair match*

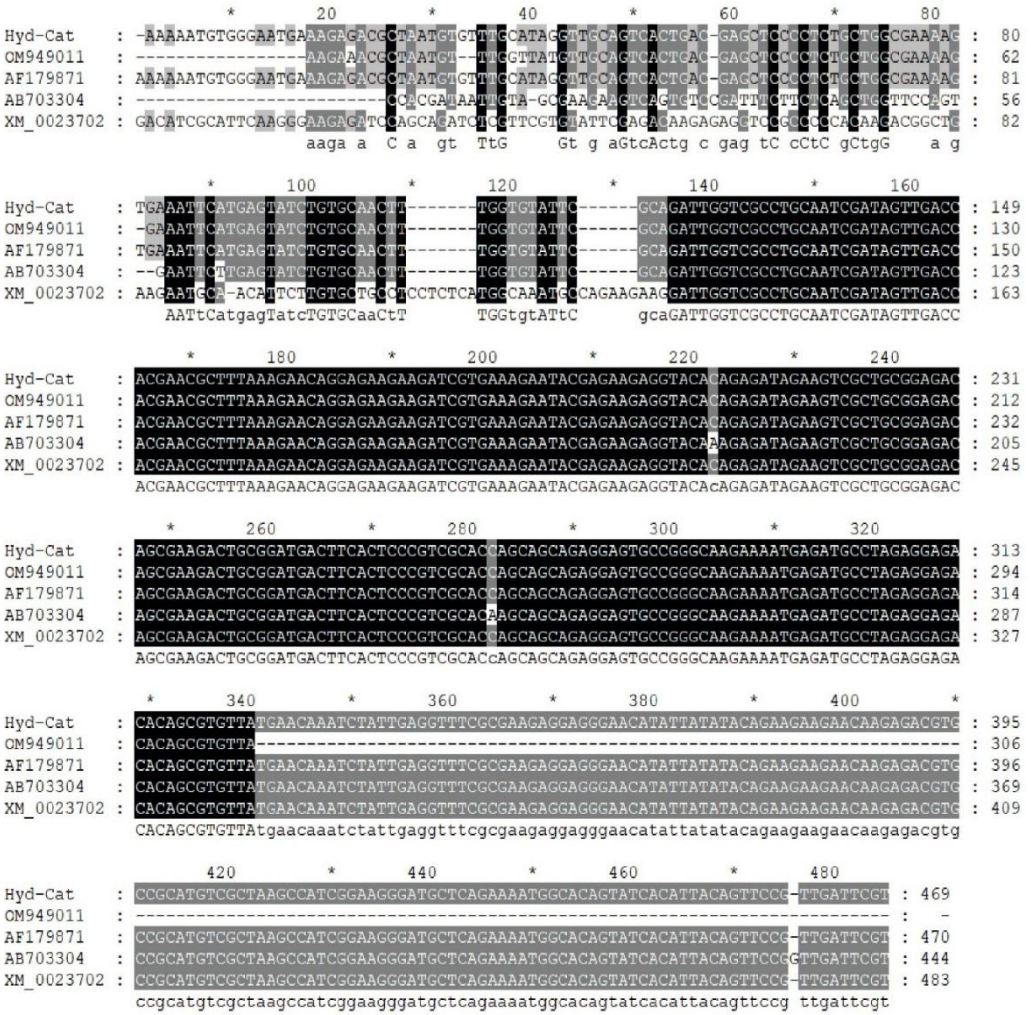

Figure 9. *Multiple alignment of the B1 gene of T. gondii amplified during the present study along with other related strains. The conserved residues are specified in black*

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Sequence similarity analysis

Additionally, the analysis of sequence similarity and identity reveals that the species amplified through targeting the B1 gene exhibit a high degree of sequence similarity, ranging from 99% to 100%. This indicates that they belong to the same species and strains (*Table 7*). Furthermore, a global sequence similarity and identity analysis demonstrates that these species are closely related to other isolates of *T. gondii*, with similarity indices ranging from 0.99 to 0.98. These findings further confirm the close relationship of the identified species and isolates with the group of *T. gondii* (*Table 8*).

Table 7. *Sequence similarity percentage of the B1 gene of the gDNA amplified from the different isolates of T. gondii*

SIMILARITY RESULTS						
***T. gondii* (Hyderabad-Cat)**	**1**	100				
***T. gondii* (XM_002370240)**	**2**	100	100			
***T. gondii* (AB703304)**	**3**	99.02	99.02	100		
***T. gondii* (OM949011)**	**4**	100	100	99.02	100	
***T. gondii* (AF179871)**	**5**	100	100	99.02	100	100
		1	**2**	**3**	**4**	**5**

Table 8. *Global sequence similarity and identity index of the B1 gene of the gDNA amplified from the T. gondii isolates as compared to the other related strains*

GLOBAL SIMILARITY RESULTS						
***T. gondii* (Hyderabad-Cat)**	**1**	1	1	0.99	1	1
***T. gondii* (XM_002370240)**	**2**	1	1	0.99	1	49
***T. gondii* (AB703304)**	**3**	0.98	0.98	1	0.98	0.98
***T. gondii* (OM949011)**	**4**	1	1	0.99	1	1
***T. gondii* (AF179871)**	**5**	1	1	0.99	1	1
		1	**2**	**3**	**4**	**5**

Polygenetic tress analysis

Furthermore, to identify the species and analyze their evolutionary relationships with other related species and strains, phylogenetic analysis was conducted using amplified sequences. The analysis of the B1 gene phylogeny reveals that the *T. gondii* isolates from our study were sub-clustered within the same clade as isolates obtained from rats in Nigeria (*Fig. 10*). Additionally, sequences from other species within the same clade were sub-clustered with isolates collected from various host animals. However, *T. gondii* strains formed a separate cluster due to their high sequence similarity, yet they remained within the same major clade. These findings suggest that based on the B1 gene, the specimens collected belong to *T. gondii*, as all strains were clustered alongside other related isolates in the major clade, distinct from isolates collected from different host species (*Fig. 10*). In summary, the sequence similarity and phylogenetic tree analysis strongly support the conclusion that the collected specimens belong to the species *T. gondii*. These *T. gondii* species infecting cats are closely related to other strains prevalent in different geographical areas.

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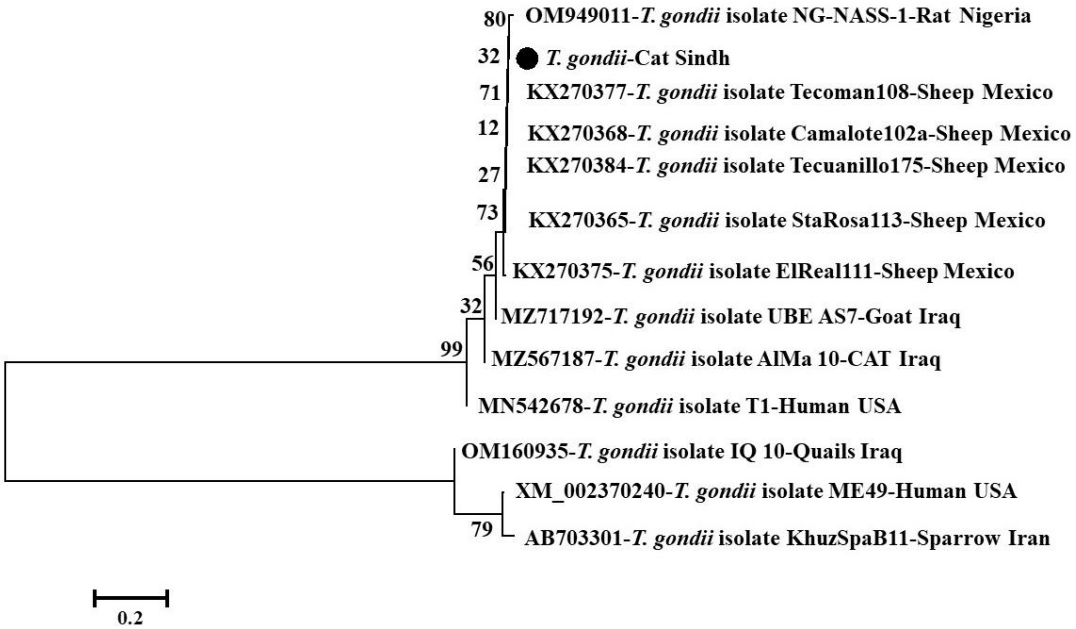

Figure 10. *Phylogenetic and molecular evolutionary analysis of the B1 gene of T. gondii (469 bp) was performed using Neighbor-joining (NJ) analyses with 1000 bootstraps employing MEGA 6 software. The marked sequence, along with related sequences and their accession numbers, are provided to infer an evolutionary relationship among different isolates*

Discussion

Toxoplasmosis is a major zoonotic disease affecting public health as well as livestock production (Kijlstra and Jongert, 2009; Hampton, 2015; Belluco et al., 2018). Poor hygienic conditions and increased urbanization, coupled with the trend of keeping pets as companion animals, contribute to its consistent prevalence (Kijlstra and Jongert, 2009). The disease, caused by the unicellular protozoa *T. gondii*, primarily spreads through contaminated food and water, often via infected cat feces (Kijlstra and Jongert, 2009). Human transmission can occur through direct contact with pets or consuming undercooked meat from infected animals, with severe consequences for immunocompromised individuals and pregnant women (Hampton, 2015; Belluco et al., 2018). The present study aimed to investigate the prevalence of toxoplasmosis in cats in the Hyderabad district, utilizing morphological and molecular techniques for species characterization. Findings revealed a worrisome 15.2% infection rate among cats presented at various clinics, highlighting the potential risk to pet owners (Karakavuk et al., 2021). Previous studies in Pakistan and other countries have reported varying prevalence rates, indicating persistent infection and the need for vigilance (Ahmad et al., 2014; Ahmad and Tasawar, 2015; Khan et al., 2018). Interestingly, higher infection rates were observed in younger cats, consistent with findings from other regions. However, some studies reported no significant difference in infection rates among different age groups, suggesting complex factors at play (Jung et al., 2015; Karimi et al., 2022; Khodaverdi and Razmi, 2019; Kwak and Seo, 2020; Rahman et al., 2014). Conversely, human populations showed an increased infection risk with age, likely due to increased pet exposure and weakened immune systems (Daryani et al., 2014; Wyman et al., 2017). Gender-wise analysis in this study showed only female cats were infected, which was unexpected and may be attributed to sample size limitations. Breed-wise analysis revealed

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varying infection rates, with Persian cats showing higher prevalence in some studies. Geographic and temporal variations could contribute to these differences (Miró et al., 2011; Must et al., 2017). Climatic factors play a significant role in disease transmission and persistence, with warmer, humid environments favoring parasite survival. However, there's variability in findings regarding the impact of age and region on infection persistence, highlighting the need for further research (Kantzoura et al., 2013; Rouatbi et al., 2016). Molecular analysis targeting the B1 gene provided insights into the genetic diversity and evolutionary relationships of *T. gondii* isolates. While B1 gene analysis revealed high sequence similarity among isolates, limitations in genetic polymorphism hindered complete genotype distinction. Phylogenetic analyses supported the close relationship between isolates from different hosts, suggesting potential transmission pathways (Ivović et al., 2012; Sroka et al., 2019). Limitations of this study include a small sample size and reliance on clinic-presented cats, which may not represent the entire population. Future research with larger sample sizes and broader sampling strategies is needed to validate these findings and assess infection risk factors among pet owners.

Conclusion and recommendations

The present study delves into the molecular diversity of *Toxoplasma gondii* infection in household cats, marking the first investigation of its kind in the Sindh province. Our research revealed that 15.2% of cats brought to various clinics in Hyderabad were infected with *T. gondii*. Notably, young and female cats exhibited a higher susceptibility to infection, while cat breeds did not significantly influence infection rates. Additionally, cats displaying symptoms such as diarrhea and rough dry coats were more likely to harbor the infection, indicating a correlation between clinical presentation and *T. gondii* infection. Our molecular characterization of *T. gondii* isolates unveiled a high degree of sequence similarity and evolutionary linkage with isolates from rats and sheep, shedding light on potential origins and transmission patterns. These findings contribute to a better understanding of the risk factors predisposing both humans and animals to *T. gondii* infections, enabling timely diagnosis and treatment of asymptomatic animals. To mitigate the spread of the parasite and safeguard human and animal health, we propose several measures. Firstly, household cats should receive appropriate treatment with antiparasitic drugs to eliminate asymptomatic infection or active shedding. Additionally, managing stray cat populations from a hygienic perspective is crucial to reduce the risk of disease transmission. Confining household cats can prevent hunting and potential infection from wildlife, while regular cleaning of litter boxes helps minimize environmental contamination. Furthermore, disposing of pet litter away from gardens or grazing areas can prevent further contamination. Future research should focus on assessing the viability of *T. gondii* in human populations and the environment to facilitate comprehensive risk assessment analyses. By implementing these recommendations, we can effectively mitigate the spread of *T. gondii* infections, ensuring the health and well-being of both humans and animals. Continued research in this area will deepen our understanding of *T. gondii* transmission dynamics and inform the development of more effective preventive strategies.

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