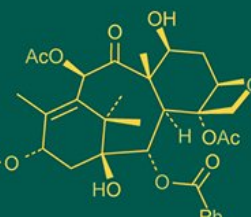
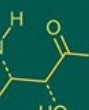
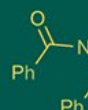


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Epidemiological investigations and molecular characterization of *Toxocara canis* from Jammu region

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Abstract

Toxocara canis is one of the most prevalent parasites that have been found in pregnant bitch and pups. Reduced body weight, unthriftiness, anaemia, loose faeces are the common implications of *Toxocara canis*. The current study was conducted on 305 dogs (≤ 3 months) to determine the prevalence, associated risk factors and molecular characterization of canine toxocarosis in Jammu region. Overall prevalence of GIT parasites were 52.13% highest in monsoon (69.35%) and lowest in summer (33.82%). Prevalence of *Toxocara canis* was highest in monsoon and lowest in winters with the risk of 4.74 and 2.66 times in monsoon as compared to winter and summer. Dog aged less than a month had highest prevalence and gender based prevalence showed non-significant ($p > 0.05$) difference. The prominent clinical signs viz, pasty faeces (47.76%), rough hair coat (77.61%) and pale mucous membrane (85.07%) were observed in affected dogs. The positive PCR samples for COI gene were sequenced commercially, and allocated with accession number as; *Toxocara canis* COI gene (ON911829). *Toxocara canis* COI gene (ON911829) as Jammu isolate showed identity with other isolates of *Toxocara* and other species: [98.25% with *Toxocara canis* of Russia (KX365898); 98.25% with *Toxocara canis* of Heilongjiang, China (KC998807). This study involved first ever molecular analysis of *T. canis* in dogs from Jammu region and adds new information through the use of COI gene

Keywords: COI gene, ITS-2, Larval migration, *Toxocara canis*

Introduction

Toxocara canis is one of the most prevalent and harmful parasite that infect dogs globally. (Chattha *et al.*, 2009; Traversa *et al.*, 2012) [6, 39]. The nematode is a member of the family Ascaridae, found in the small intestine of the dog (Glickman and Schantz, 1981; Yarsan *et al.*, 2003) [12, 42]. It causes a common helminthic disease in dogs that has been seen in few different parts of the nation, with varying range of clinical signs in infected animals. Foetal pups acquire *T. canis* infection *in utero* via trans-placental route when hypobiotic larvae from the somatic tissue stores are activated in the late period of gestation. The larva can also enter mammary glands and then spread via colostrum and milk in newborn pups (Overgaauw *et al.*, 1997) [23]. In pups (≤ 6 months of age), ingestion of infective eggs of *T. canis* most likely leads to hepato-tracheal migration of larva followed by a patent infection while the older dogs are less likely to get patent infection as they develop immunity against tracheal migration of larvae (Greve, 1971 and Dubey, 1978) [13, 9]. Somatic migration occurs in older dogs (Sprent, 1958) [34]. However, few older dogs ≥ 6 months do shed *T. canis* eggs, posing a risk of zoonosis (Sager *et al.*, 2006; Claerebout *et al.*, 2009; Barutzki and Schaper, 2011) [27, 7, 3]. Confirmatory diagnosis of the infection can be done by qualitatively detecting ascarid eggs in faeces using the floatation method and quantitatively estimating the eggs per gram using the Mc. Master method (Habluetzel *et al.*, 2003) [15]. However, precise identification of *Toxocara* species and their clear distinction from one another, as well as from other ascaridoid nematodes responsible for visceral larva migrans (VLM) and ocular larva migrans (OLM), is essential for investigations related to taxonomy, epidemiology, population genetics, diagnosis, and disease control. Molecular detection of *Toxocara* infection using ELISA and PCR techniques have also proven to be the diagnostic tools (Elsemore *et al.*, 2017; Oge *et al.*, 2019) [10, 22].

PCR technique has the ability to selectively amplify DNA from nematode eggs, larval or adult stages and therefore can be employed for rapid and specific diagnosis (Smith *et al.*, 2009; Gaser, 2013) [31]. Despite the veterinary and zoonotic importance of *T. canis* information on its prevalence, associated risk factors, clinical manifestation, and molecular characteristics in young dogs from Jammu region remains limited. Therefore, the present study was undertaken to determine the prevalence of *T. canis* infection and associated risk factors in dogs from the selected area of Jammu, to assess the major clinical manifestations associated with infection, and to characterize the parasite molecularly using ITS-2 and COI gene markers. The study also aimed to evaluate the phylogenetic relationship of the Jammu isolate with previously reported *T. canis* isolates from different geographical regions.

2. Materials and Methods

The research was approved by IAEC (Institutional Animal Ethics Committee) registered with CPCSEA (Committee for the purpose of control and supervision of experiments in animal) under no. 08/IAEC-20/2021.

2.1 Experimental design: The epidemiological investigations of *Toxocara canis* infection in canines was conducted on domesticated and stray pups from various rural and urban areas of Jammu district of Union Territory of Jammu & Kashmir. Sick and healthy pups presented at the small animal medicine OPD under division of teaching veterinary clinical complex (TVCC), SKUAST-J and private based veterinary clinics in Jammu region were included in this study. Prevalence of *Toxocara canis* infection was studied on the basis of qualitative and quantitative faecal examination. A total of 300 pups aged $\leq$ 3 months using simple random sampling method were considered. The calculation of sample size was done based on formula described by (Thrushfield, 2007) [36].

2.2 Evaluation of epidemiological data: The evaluation of epidemiological data viz; age, gender, faecal consistency, body weight, body coat and co-infection for analysis of association with *T. canis* infection was done. The research period was divided into four seasons, according to the Indian Meteorological Department, Pune: summer (March–June), monsoon (July–September), post monsoon (October–November), and winter (December–February). Along with that the consistency of the faecal sample was scored using the Waltham faeces scoring chart

(https://www.waltham.com/dyn/_assets/_pdfs/other-resources/waltham-scoring.pdf). And, body weight of pups was measured with digital weighing scale.

2.3 Faecal processing and examination: The examinations of faecal samples were done grossly for adult worm, microscopically by floatation method as per method described by (Soulsby, 1982) [33]. Quantitative faecal examination was done to obtain accurate information with regard to severity of an infection. Egg counting method was used to determine number of eggs per gram of faeces (EPG), for this purpose, Mc-Master technique was performed as per method described by (Soulsby, 1982) [33].

2.4: Molecular characterization of *Toxocara canis* of dogs in Jammu and subsequent phylogenetic analysis with other *Toxocara* species

The infected pups were given oral piperazine citrate @ 200mg/kg body weight (Actipar) and adult worms were collected from their faecal samples. The worms were thoroughly washed in PBS and fixed in 70% ethanol and later stored at -20°C until genomic DNA was extracted. Isolation of DNA was done from small portion of adult *T.canis* specimen using the Wizard® genomic DNA purification kit (Promega, USA, Cat. no. A1120) with few modifications. The ITS-2 (internal transcribed spacer) located within nuclear ribosomal gene was selected and analyzed (Table1) (Mahdy *et al.*, 2020) [20]. Additionally, Cytochrome C Oxidase subunit I (COI) primers were retrieved from the national centre for Biotechnology information (NCBI) database and utilized for the analysis (Table 1). The reaction mixture was prepared with PCR master mix (Thermo scientific, K0171) and supporting components required for the amplification of the desired DNA segments (Table 2). The DNA amplification was done with conventional PCR assay using T100™ thermal cycler (Bio-rad, USA, Serial no. 621BR41692) using specific cyclic conditions for each primer set (Table 3). The amplified PCR products were confirmed with gel electrophoresis using 1.5% of molecular grade agarose. The molecular weight were computed with 100-1500 bp DNA Ladder and visualized under UV exposure in the gel documentation system (E-BOX, Vilber). The positive PCR products were purified using SureExtract® PCR clean-up/ gel extraction kit (Genetix Biotech Asia Pvt Ltd., and subsequently sent to Biokart India Pvt. Ltd. for Sanger sequencing.

Table 1: Sequence of primer used for this study

Gene	Type	Sequence	Source
ITS-2	Forward	5'-CGGTGGATCACTCGGCTCGT-3'	Mahdy <i>et al.</i> , (2020) [20]
	Reverse	5'-CCTGGTTAGTTTCTTTTCCTCCGC-3'	
Cytochrome C oxidase subunit I (COI)	Forward	5'-TGGGTTGTGGTCTGGTATGG-3'	https://www.ncbi.nlm.nih.gov
	Reverse	5'-GAGCCCCCAACATCAAAGGT-3'	

Table 2: Formulation of Reaction mixture

S. No	Component	Volume
1.	DNA extract	3 μ l
2.	PCR master mix (Thermo scientific, K0171)	30 μ l
3.	Forward primer	2 μ l
4.	Reverse primer	2 μ l
5.	Nuclease-free water	23 μ l

Table 3: The following were the cycle conditions used to amplify *Toxocara* spp. Genes

Steps	Process	Temperature & Time
Step 1	Initial denaturation	94°C for 3 minutes
Step 2	Denaturation	94°C for 30 s
Step 3	Annealing	50 °C for ITS-2 for 30 s 58 °C for COI for 30 s
Step 4	Elongation	72°C for 1 minute
Repeat step 2-4 for 31 cycles		
Step 5	Final elongation	72°C for 5 minutes

3. Statistical analysis

The data obtained on the prevalence of *Eimeria* spp. infection in relation to age, season and sex of dogs were compared by Chi square test. The mean oocyst per gram of faeces was compared by ANOVA test using SPSS 16.0. Here, *p* value of <0.05 was considered significant.

Results and Discussion

Prevalence of *Toxocara canis* in dogs of Jammu region based on faecal floatation examination

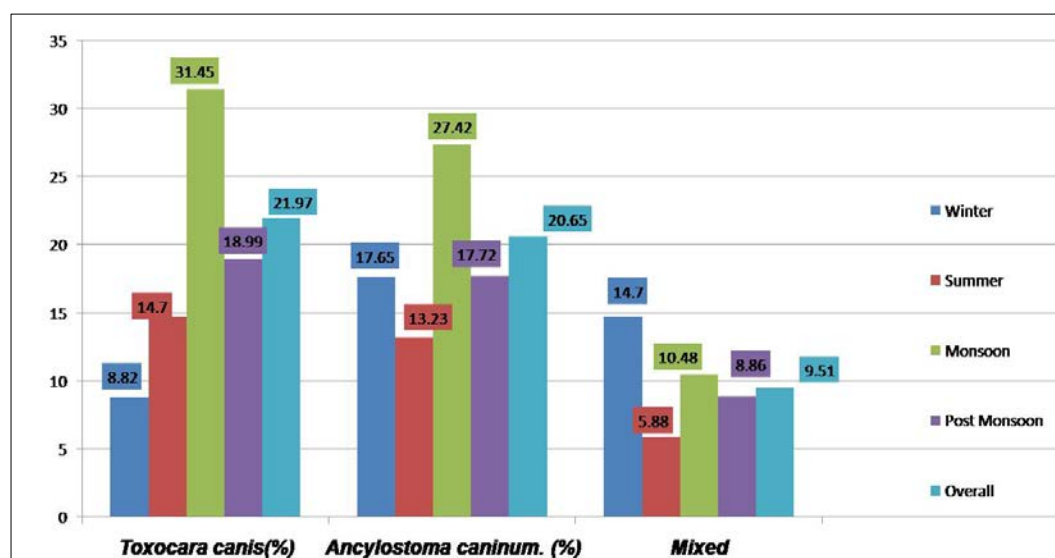
The present study revealed an overall prevalence of GI parasitic infection from 305 faecal samples of dogs aged ≤ 3 months in Jammu area was 52.13%. Among the individual

parasites detected, *Toxocara canis* (21.97%) infection showed the highest prevalence followed closely by *Ancylostoma caninum* (20.65%) while 9.51% of the dogs harboured mixed infections (Table 4 and Figure 1). The relatively high prevalence of *T.canis* in young dogs may be attributed to their increased susceptibility to infection and the efficient transmission of this parasite through multiple routes, particularly transplacental and transmammary transmission (Soulsby, 1982) [33]. The predominance of *T. canis* in the present study therefore highlights its epidemiological importance in young dog and its potential zoonotic significance.

Table 4: Overall and seasonal prevalence pattern of gastrointestinal parasites in dogs (≤ 3month age) of Jammu based on faecal floatation examination

Season	Overall infection		Different gastrointestinal parasitic infection		
	Examined	Infected (%)	<i>Toxocara canis</i> Infected (%)	<i>Ancylostoma caninum</i> Infected (%)	Mixed Infected (%)
Winter (December-February)	34	14 (41.18 ^b)	3 (8.82 ^b)	6 (17.65 ^a)	5 (14.7 ^a)
Summer (March-June)	68	23 (33.82 ^b)	10 (14.70 ^b)	9 (13.23 ^a)	4 (5.88 ^a)
Monsoon (July-September)	124	86 (69.35 ^a)	39 (31.45 ^a)	34(27.42 ^a)	13 (10.48 ^a)
Post Monsoon (October-November)	79	36 (45.57 ^{ab})	15 (18.99 ^{ab})	14 (17.72 ^a)	7 (8.86 ^a)
Overall	305	159 (52.13)	67 (21.97)	63 (20.65)	29 (9.51)

Different superscripts indicate significant difference (*p*<0.05) in season wise prevalence in dogs (a, b)

**Fig 1:** Overall and seasonal prevalence pattern of different gastrointestinal parasites in dogs (≤3 months) of Jammu

A distinct seasonal variation was observed in the occurrence of GIT parasite infections. Among the four seasons, Monsoon (69.35%) had the highest occurrence of overall parasitic infection followed by post-monsoon (45.57%), winter (41.18%), and summer (33.82%). The quantitative analysis of infection showed highest EPG (egg per gram) in monsoon period followed by the post-monsoon season, summer and winter (Table 5 and figure 2). The likelihood of

contracting *T. canis* infection during the monsoon was 4.74 and 2.66 times greater significantly (*p*<0.05) than it was during the winter and summer, respectively. The higher prevalence of endoparasitic infections in dogs recorded during the months from July to September might be associated with the role of rainfall in the translocation and acquisition of L3 infective larvae in the environment as opined by (Soulsby, 1994) [32]. The seasonal pattern

observed in the present study, is however not consistent with all the previous reports. Different investigators have documented peak prevalence in winters (Agnihotri *et al.*, 2008; Kumar *et al.*, 2014) [2, 17], autumn (Totkova *et al.*, 2006) [37], monsoon (Utaaker *et al.*, 2018) [41] and summer (Moudgil *et al.*, 2016) [21] while some workers have reported influence of season on infection as insignificant (Gugsa *et al.*, 2015) [14]. The discrepancies among studies in the seasonal prevalence pattern of *Toxocara canis* infection in different areas may be attributed due to reasons like variation in sampling, geo-climatic conditions, degree of contamination of environment with infective stages of parasite, etc. Therefore, the observed seasonal variations in *T. canis* prevalence appears to be region-specific and influenced by the interaction of environmental,

epidemiological and host related factors.

Table 5: Season wise prevalence and mean egg count of *Toxocara canis* in dogs (≤ 3 month age) of Jammu.

Season	Examined	Infected (%)	Mean epg $\times 10^3$ ($\pm$ SEM)	Range epg $\times 10^3$
Winter	34	3 (8.82 ^b)	0.43 ^w $\pm$ 0.09	0.20-0.80
Summer	68	10 (14.70 ^b)	1.37 ^v $\pm$ 0.14	0.80-1.80
Monsoon	124	39 (31.45 ^a)	2.87 ^u $\pm$ 0.14	2.40-3.40
Post monsoon	79	15 (18.99 ^{ab})	2.68 ^{ux} $\pm$ 0.16	2.2-3.20
Total	305	67 (21.97)	-	-

Different superscripts indicate significant difference ($p < 0.05$) in season wise prevalence in dogs (a, b) and in mean epg in dogs (u,v,w,x) between different seasons

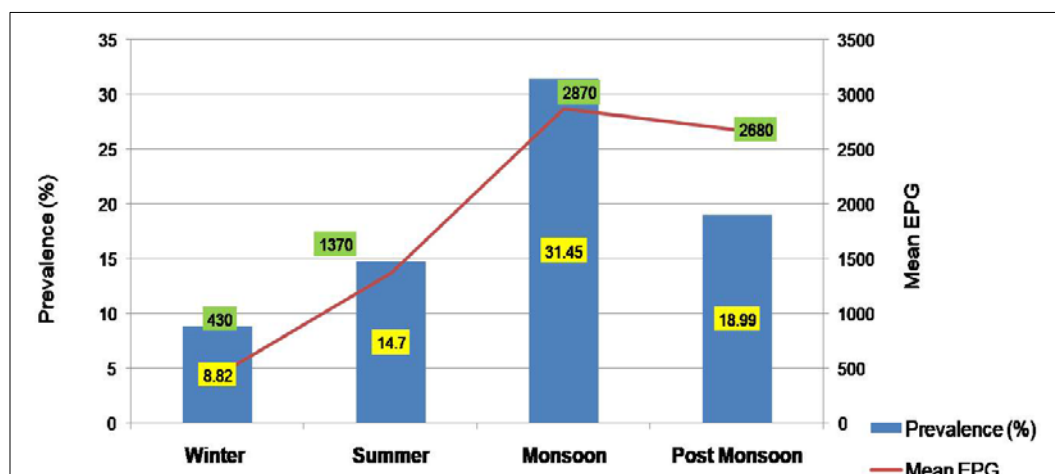


Fig 2: Season wise prevalence and mean egg count of *Toxocara canis* in dogs (≤ 3 month age) of Jammu

Age wise analysis revealed the highest EPG recorded in age group between 31 and 60 days followed by 61–90 days and up to 30 days (Table 6, Figure 3). The probability of *T. canis* infection was shown to be significantly ($p < 0.05$) greater in the age groups up to 30 days and 31–60 days by 3.67 and 3.12 times, respectively, when compared with 61–90 days age group. Similar findings of low prevalence of infection with advancing age in dogs have been reported by several workers (Daryani *et al.*, 2009; Abrere *et al.*, 2013; Rostami *et al.*, 2020) [8, 25]. The higher prevalence of toxocarosis in very young puppies might be attributed to the age susceptibility and lack of premunity. Moreover, puppies

acquire the infections at the early age through prenatal and transmammary routes while the adult dogs develop immunity which decreases the establishment as well as fecundity of the parasites (Soulsby, 1982; Urquhart *et al.*, 1996) [33, 40]. The comparatively high prevalence observed in puppies up to 30 days age may be attributed to their dependence on dam for feeding which facilitates the acquisition of infective larvae through milk. In contrast the pet puppies are commonly weaned by approximately one month of age and are often dewormed at this stage. This practice could be the reason for subsequent decline in the prevalence with the advancement of age.

Table 6: Age wise prevalence pattern of *Toxocara canis* in dogs (≤ 3 month age) of Jammu based on faecal examination

Age	Samples Examined	Infected (%)	Mean EPG $\times 10^3$ ($\pm$ SEM)	Range EPG $\times 10^3$
Upto 30 days	75	24 (32 ^a)	1.32 ^y $\pm$ 0.12	0.90-1.70
31 - 60 days	98	28 (28.57 ^a)	2.55 ^x $\pm$ 0.19	1.80-3.20
61 - 90 days	132	15 (11.36 ^b)	1.72 ^{xy} $\pm$ 0.11	0.20-3.40
Total	305	67 (21.97)	-	-

Different superscripts indicate significant difference ($p < 0.05$) in age wise prevalence in dogs (a, b) and in mean epg in dogs (x,y) between different age group

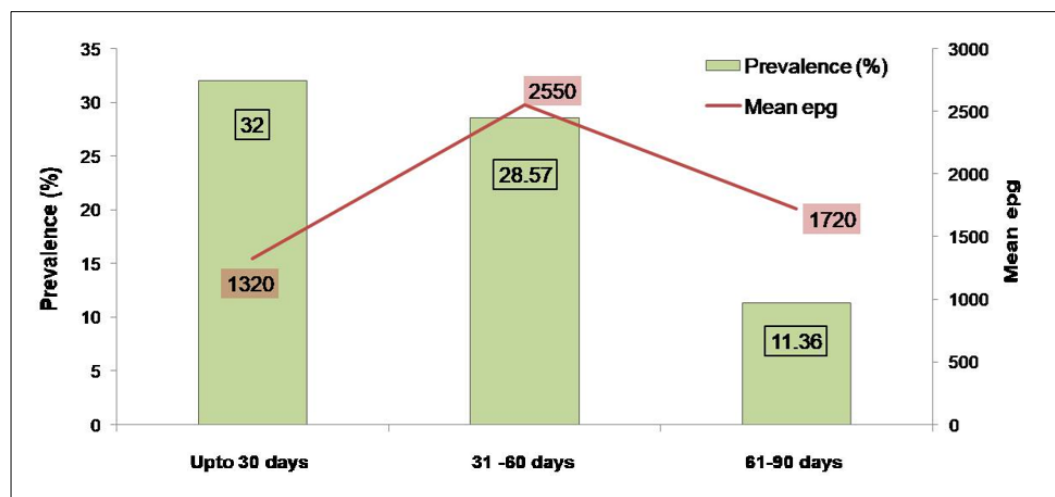


Fig 3: Age wise prevalence pattern of *Toxocara canis* in dogs (≤ 3 month age) of Jammu based on faecal examination

The sex-wise analysis revealed that female dogs exhibited a higher prevalence of infection (23%) than male dogs (21.23%) (Table 7, Figure 4). However, no significant difference ($p > 0.05$) was observed in the mean EPG values of *Toxocara canis* between the two sex groups. Previous studies have reported variable findings regarding sex-related prevalence of *Toxocara* spp. infection in dogs. A higher prevalence in female dogs was documented by Satyal *et al.*, (2013) [29] and Sahu *et al.*, (2010) [28], whereas Swai *et al.*,

(2010) [35] reported a greater prevalence in male dogs. In contrast, several studies have reported no significant association between sex and the occurrence of parasitic infections in dogs (Swai *et al.*, 2010; Bhangale and Khillare, 2018) [35, 4]. The marginally higher prevalence recorded in female dogs in the present study may be attributed to the greater number of faecal samples collected from female animals.

Table 7: Sex wise prevalence and mean egg count of *Toxocara canis* infection in dogs (≤ 3 month age) of Jammu

Sex	No. of samples Examined	No. of positive samples (%)	Mean epg $\times 10^3$ ($\pm$ SEM)	Range epg $\times 10^3$
Male	179	38 (21.23 ^a)	1.64 [*] $\pm$ 0.12	0.20-3.40
Female	126	29 (23 ^a)	1.57 [*] $\pm$ 0.10	0.40-2.80
Total	305	67 (21.97)	-	-

Different superscripts indicate significant difference ($p < 0.05$) in sex wise prevalence in dogs (a) and in mean epg in dogs (x) between different sex

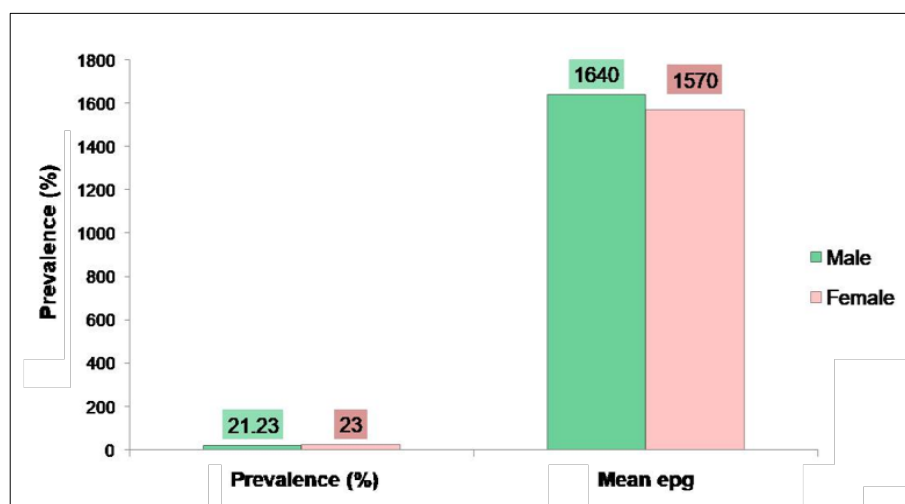


Fig 4: Sex wise prevalence and mean egg count of *Toxocara canis* infection in dogs (≤ 3 month age) of Jammu

An association between clinical manifestations and *T. canis* infection was identified in this study. Among the infected dogs, the most frequently clinical manifestations were pasty faeces (47.76%), rough hair coat (77.61%), and pale mucous membranes (85.07%) (Table 8). These observations are in agreement with the findings of Lefkaditis *et al.* (2006) [18] and Adolph *et al.* (2017) [1]. The occurrence of pale mucous membranes may be attributed to blood loss resulting from extensive intestinal tissue damage and the blood-feeding

activity of infective *Toxocara* larvae in puppies. Similarly, pasty faeces could be due to increased intestinal secretion and reduced absorption caused by the presence of adult parasites, as suggested by Rao *et al.* (1995) [24], Bowman (2021) [5], and Sharma *et al.* (2010) [30]. The rough hair coat observed may reflect poor nutritional status resulting from impaired nutrient absorption, along with the physiological stress associated with parasitic infection.

Table 8: Frequency of clinical signs associated with *Toxocara canis* infection in dogs (≤ 3 month age) of Jammu

Age	<i>Toxocara canis</i> positive dogs/ Samples (n) examined	Faecal Consistency of <i>Toxocara canis</i> positive dogs (Grade 1-5)				Body coat of <i>Toxocara canis</i> positive dogs		Mucous membrane color of <i>Toxocara canis</i> positive dogs	
		Normal (1-2.5) (%)	Pasty (3-3.5) (%)	Loose (4-4.5) (%)	Watery (5) (%)	Normal (%)	Rough (%)	Pale (%)	Roseate (%)
Upto 30 days	24/75	3 (12.5)	12 (50)	7 (29.17)	2 (8.33)	5 (20.83)	19 (79.17)	17 (70.83)	7 (29.17)
31-60 days	28/98	8 (28.57)	16 (57.14)	4 (14.28)	0	7 (25)	21 (75)	26 (92.86)	2 (7.14)
61-90 days	15/132	9 (60)	4 (26.67)	1 (6.67)	1 (6.67)	3 (20)	12 (80)	14 (93.33)	1 (6.67)
Total	67/305	20 (29.85)	32 (47.76)	12 (17.91)	3 (4.48)	15 (22.39)	52 (77.61)	57 (85.07)	10 (14.93)

Molecular characterization and subsequent phylogenetic analysis of *Toxocara canis* of dogs of Jammu:

Molecular characterization of *Toxocara canis* obtained from dogs of Jammu region revealed amplification of ITS-2 gene (plate 1a) and *Toxocara canis* COI gene (plate 1b).

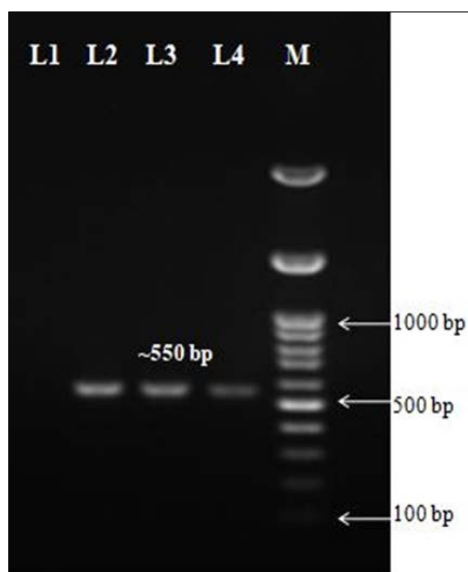


Plate 1a: Lane M: 100 bp DNA ladder, Lane 1: No template control. Lane 2: Amplified ITS-2 gene of *Toxocara canis* at annealing temp. 58°C (~550 bp), Lane 3: Amplified ITS-2 gene of *Toxocara canis* at annealing temp. 54°C (~550 bp), Lane 4: Amplified ITS-2 gene of *Toxocara canis* at annealing temp. 50°C (~550 bp)

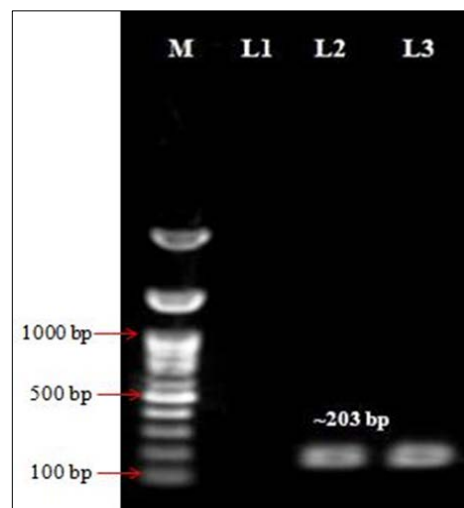


Plate 1b: Lane M: 100 bp DNA ladder, Lane 1: No template control. Lane 2: Amplified COI gene of *Toxocara canis* at annealing temp. 53°C (~203 bp), Lane 3: Amplified COI gene of *Toxocara canis* at annealing temp. 58°C (~203 bp).

Gene sequence of *Toxocara canis* infecting dogs of Jammu:

In the present study, only one sequence was submitted to NCBI Gene Bank as shown in Table 9. The generated sequence was assigned the accession number as *Toxocara canis* COI gene (ON911829). The representative chromatogram of forward sequence has been shown in Plate 2.

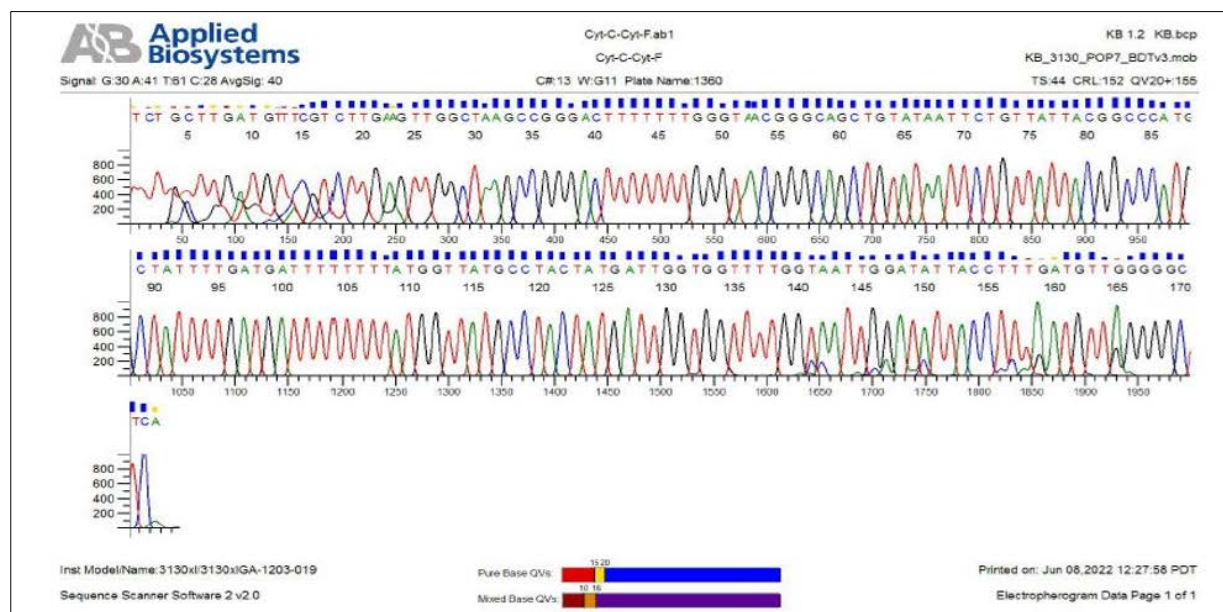


Plate 2: Chromatogram of *Toxocara canis* COI sequence

Table 9: Gene sequence of *Toxocara canis* infecting dogs of Jammu.

Submission title	Place of origin of isolate	NCBI Gene bank Accession number
<i>Toxocara canis</i> isolate Jammu Cytochrome C Oxidase subunit 1 (COI) gene, partial sequence	Jammu	ON911829

Phylogenetic analysis of *Toxocara canis* infecting dogs of Jammu

In the present study, only the *T. canis* COI sequence was retrieved and it exhibited strong similarity with sequences from other *Toxocara* species in the Gene Bank. *Toxocara canis* COI gene (ON911829) as Jammu isolate showed identity with other isolates of *Toxocara* and other species: 98.25% with *Toxocara canis* of Russia (KX365898); 98.25% with *Toxocara canis* of Heilongjiang, China (KC998807); 97.66% with *Toxocara canis* of Poland (MH937708); 97.66% with *Toxocara canis* of Japan (AP017701); 97.66% with *Toxocara canis* of Sri Lanka (JN593098); 97.66% with *Toxocara canis* of Australia (EU730761); 97.66% with *Toxocara canis* of Tianhe district, China (AM411108); 97.08% with *Toxocara canis* of Germany (KX962383); 92.90% with *Toxocara malaysiensis* of Guangzhou, China (AM412316); 91.23% with *Baylisascaris schroederi* of Sichuan, China (KJ587808); 91.23% with *Toxascaris leolina* of Sichuan, China (MW560284); 91.12% with *Baylisascaris transfuga* of Sichuan, China (HQ671079); 90.57% with *Anisakis ziphidarum* of Japan (KF214811); 90.53% with *Hexametra angusticaecoides* of Austria (MW387515) and 84.47% with *Contracaecum osculatum* of Poland (JN786330). The observed phylogenetic relatedness has been shown in Table

10. Similar findings were reported by Li *et al.* (2015) [19] who observed specific amplification of ITS-1 and ITS-2 using species-specific primers for *Toxocara canis*. Additionally, PCR amplification of the ITS-2 gene was carried out by Khademvatan *et al.* (2014) [16] after comparing the obtained sequence with several isolates of *T. canis* observed significant homology. Phylogenetic classifications are a helpful tool for understanding the links between different species during evolution (Mahdy *et al.* 2020) [20]. In addition to ITS-2, the COI region might also help in the accurate identification of *Toxocara* species using PCR.

The phylogenetic tree was built using Maximum Likelihood method using Tamura 3 parameter model based COI sequence generated in the present study with the available database of COI sequence of *Toxocara canis* from Japan, Poland, China, Russia, Sri Lanka, Germany and Australia (Plate). All the *Toxocara canis* COI sequences clustered in separate clades, regardless of their geographical location (Plate 3). The clustering pattern further supports the molecular identification of the Jammu isolate as *T. canis* and demonstrates the utility of the COI region for assessing the phylogenetic relationships among geographically diverse *T. canis* populations.

Table 10: Phylogenetic relatedness of *Toxocara canis* infecting dogs of Jammu with Gene Bank (NCBI) sequences.

Genus	Accession no. of <i>Toxocara canis</i> isolate identified from Jammu	Observed phylogenetic relatedness with other isolates	% Identity
<i>Toxocara canis</i> COI Jammu (ON911829)		Russia (KX365898)	98.25%
		Heilongjiang, China (KC998807)	98.25%
		Poland (MH937708)	97.66%
		Japan (AP017701)	97.66%
		Sri Lanka (JN593098)	97.66%
		Australia (EU730761)	97.66%
		Tianhe district, China (AM411108)	97.66%
		Germany (KX962383)	97.08%
		Guangzhou, China (AM412316)	92.90%
		Sichuan, China (KJ587808)	91.23%
		Sichuan, China (MW560284)	91.23%
		Sichuan, China (HQ671079)	91.12%
		Japan (KF214811)	90.57%
		Austria (MW387515)	90.53%
		Poland (JN786330)	89.47%

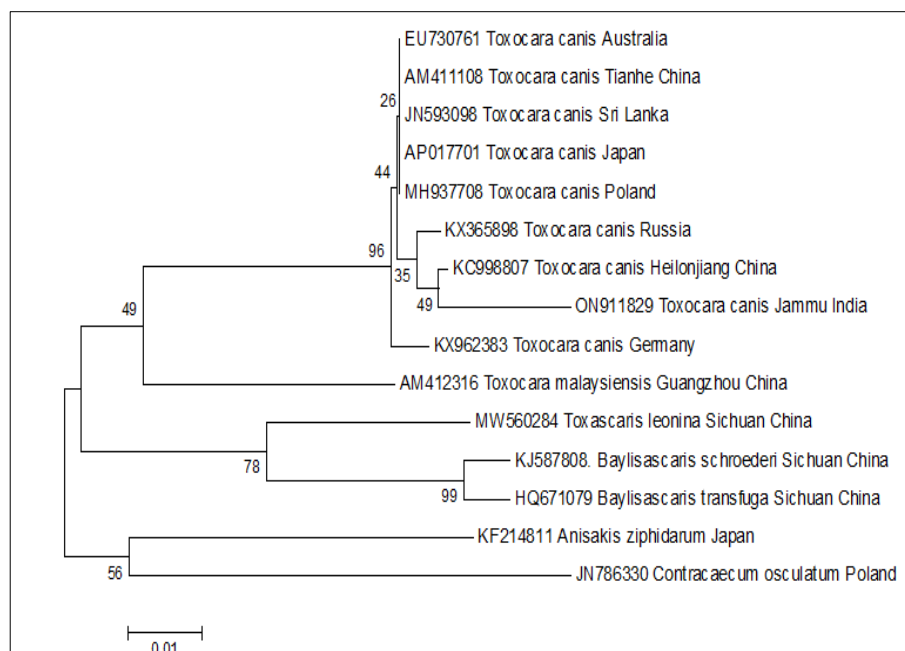


Plate 3: Phylogenetic tree based on COI region of *Toxocara* spp. using Maximum likelihood method. The associated species clustered together in the bootstrap method (1000 replicates) as shown in cladogram

Dogs in the Jammu region of North India are commonly reared under intensive and semi-intensive management systems and, like other mammalian species, are susceptible to a variety of infectious diseases of bacterial, viral and parasitic origin. Among these, *Toxocara canis* infection can adversely affect the hematological and biochemical parameters of dogs. The present study recorded an overall prevalence of 21.9% for *T. canis* infection among young puppies. The highest prevalence was observed in puppies aged less than 30 days, particularly during the monsoon season, and was associated with prominent clinical manifestations such as pasty faeces, rough hair coat, and pale mucous membranes. Molecular characterization of *T. canis* isolates from Jammu region was performed using the ITS-2 and COI genes. The COI sequence generated in the present study was submitted to Gen Bank and assigned accession number ON911829, representing the Jammu isolate. The sequence exhibited high nucleotide identity with other *Toxocara* isolates, showing 98.25% identity with *T. canis* infection from Russia (KX365898) and 98.25% with *T. canis* from Heilongjiang, China (KC998807). These findings further support the molecular identification of the Jammu isolate as *T. canis*.

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