BMC Veterinary Research

RESEARCH

Open Access

Morphological and molecular identification of *Toxocara* isolated from road-killed golden jackals in Northern Iran

Amirhosain Roohi Koshalshah[1], Meysam Sharifdini[2], Mohammad Saleh Bahreini[1], Amir Masoud Salemi[1], Syed Mohammad Kifayatullah Andrabi[1], Syeda Sadaf Mehdi[1] and Fattaneh Mikaeili[1*]

Abstract

Background Toxocariasis is caused by infection with *Toxocara canis* and *Toxocara cati*, common nematodes of canids and felids, respectively. Humans become infected after the accidental ingestion of embryonated eggs of *Toxocara* from the soil or the consumption of raw and undercooked meat containing *Toxocara* larvae. The aim of this cross-sectional study was to identify ascarid nematodes isolated from jackals in Guilan and Mazandaran provinces, based on morphological and molecular approaches.

Methods This cross-sectional study was conducted on 41 road-killed golden jackals collected from Guilan and Mazandaran provinces in northern Iran. At first, species identification was carried out based on morphological characterization. Genomic DNA was extracted from the isolates of *Toxocara* collected from jackals. PCR-RFLP of Ribosomal DNA regions (ITS) using *Rsa*I endonuclease enzyme and PCR-sequencing were carried out to identify *Toxocara* species. The sequence data were aligned using Bioedit software and compared with published sequences in GenBank using the BLAST system. Phylogenetic analysis was performed using MEGA 5.0 software.

Results Eleven out of 41 road-killed golden jackals (26.8%) were infected with *Toxocara* nematodes. All the isolates were confirmed as *T. canis* based on morphological and molecular methods. A pairwise comparison of the sequences did not show any differences in nucleotide sequences within *T. canis* isolates, and the sequences were identical and exhibited 100% homology.

Conclusions Considering the almost high prevalence of *T. canis* in golden jackals and its critical role in human toxocariasis, the identification of parasite species by molecular methods can be used to plan prevention and control programs in human and animal communities. Since, the ITS sequences of *T. canis* isolated from jackals in Iran were utterly similar to the ITS sequences of *T. canis* isolated from other hosts from different areas of the world, it is hypothesized that the type of host and geographical region do not affect the genetic diversity of the ITS region sequences of *T. canis*.

Keywords Morphology, Molecular identification, *Toxocara*, Jackal, North of Iran

*Correspondence:
Fattaneh Mikaeili
mikaeelf@sums.ac.ir; fmikaeili@yahoo.com

[1]Department of Medical Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Department of Medical Microbiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Background

Toxocariasis, one of the most common zoonotic diseases, is caused by *Toxocara canis* and *Toxocara cati*, two common nematodes of canids and felids, respectively [1, 2]. The parasites of the genus *Toxocara* are the helminth nematodes living in the small intestine of the host. The *Toxocara* eggs are released into the environment through feces and be infective after a few weeks in the outdoor environment. Humans are infected through the ingestion of embryonated eggs of *Toxocara* from contaminated soil and food, or the consumption of raw and undercooked meat containing *Toxocara* larvae [3].

Risk factors such as age, poverty, ethnicity, gender, geographic region, and contact with animal pets have been identified to cause Toxocariasis in humans [4, 5]. Epidemiological studies have shown a variable seroprevalence of human infection with this parasite in different parts of Iran [6–8].

In recent years, environmental changes, including the destruction of natural habitats and increased urbanization, have caused canids and felids live freely in urban and rural areas, and discharge *Toxocara* eggs in the environment, which can increase the transmission of *Toxocara* to humans. Although domestic cats and dogs are likely to be the main source of environmental contamination with *Toxocara* eggs in urban environments, the importance of wild carnivores such as jackals as definitive hosts of *T. canis* should also be considered. The golden jackal eat mainly small rodents, birds, and in some instances even invertebrates, which likely increases their exposure to parasites such as *Toxocara* [9].

Canine and feline infection with *Toxocara* has been reported from different parts of the world [10, 11]. Studies conducted in other parts of Iran have shown that the infection rate of dogs, cats, foxes, and jackals with *Toxocara* is 12.34, 26.24, 35.01, and 22.32%, respectively [12]. The prevalence of *Toxocara* in jackals ranges from 4.4 to 27.5% in different parts of Iran [13–19].

Accurate identification of *Toxocara* species and their differentiation from other ascarid nematodes is essential to investigate the life cycle, epidemiology, biology, and diagnosis of toxocariasis. Traditionally, the nematodes of the *Toxocara* genus are identified based on morphological characteristics. Since morphological methods cannot distinguish eggs or larvae of *Toxocara* species from each other, molecular techniques are used [20, 21]. Since there is no report on the genetic characteristics of *Toxocara* species isolated from jackals in Iran, this cross-sectional study was performed on the identification of ascarid nematodes isolated from jackals in Guilan and Mazandaran provinces, based on morphological and molecular approaches.

Methods

Study area

This cross-sectional study was conducted in Guilan and Mazanderan provinces, north of Iran. Guilan province (37° 26′ 0″ N, 49° 33′ 0″ E), one of the 31 provinces of Iran, is located in the north of Iran. It lies along the Caspian Sea. Guilan has a humid subtropical climate with the heaviest rainfall in Iran. Rasht, the province's capital, is known as the City of Rain (Fig. 1).

Mazandaran province (36° 33′ 56.16″ N, 53° 3′ 31.68″ E) is located in the northern part of Iran, along the southern coast of the Caspian Sea and in the adjacent Central Alborz mountain. Sari is the largest city and the capital of Mazandaran province. Mazandaran has a moderate, subtropical climate with an average temperature of 25 °C in summer and about 8 °C in winter (Fig. 1).

Sample collection

Overall, 41 road-killed golden jackals were obtained in Guilan and Mazandaran provinces, northern Iran. The digestive tract of the animal was cut from the pyloric region to the anus. After making a longitudinal cut from the digestive tract, the intestinal contents were examined for the presence of ascarid nematodes. The adult *Toxocara* worms were separated, after washing several times with physiological saline, kept in 70% ethanol for further investigation (Fig. 2).

Morphological studies

Primary species identification of adult *Toxocara* worms was carried out based on morphological characteristics. To examine the morphology of adult worms, they were placed in a plate containing distilled water, and adult male and female nematodes were separated based on the size and morphological characteristics. The curved posterior end of the worm with a prominent point was used to distinguish male *Toxocara* nematodes from *Toxascaris*. The morphological characteristics of eggs in the uterus were used to differentiate female *Toxocara* nematodes from *Toxascaris* [22].

Molecular studies

The genomic DNA was extracted from the isolates of *Toxocara* collected from jackals using a commercial DNA extraction kit (Yekta Tajhiz Azma, Tissue Genomic DNA Extraction Mini kit, Cat. No. FATGK001) according to manufacturer's instructions.

The ITS region in Ribosomal DNA (partial sequence of internal transcribed spacer 1 and internal transcribed spacer 2) was PCR-amplified by forward (FM1: 5′-TTG AGGGGAAATGGGTGAC-3′) and reverse (FM2: 5′-TG CTGGAGGCCATATCGT-3′) primers. The ITS regions are usually conserved and used as effective genetic markers for the identification of helminths [23]. The PCR

Fig. 1 Map of Iran, Guilan and Mazandaran Provinces

reaction was performed in a final volume of 25 µl. Each reaction contained 12.5 µl of Taq 2X Master mix (Ampliqon, Denmark), 12.5 pmol of each primer, and 5 µl of template DNA in an automated thermocycler. The temperature profile was one cycle of 94 °C for 12 min (primary denaturation), followed by 35 cycles of 94 °C for 30 s (denaturation), 60 °C for 30 s (annealing), and 72 °C for 1 min (extension), and a final extension 72 °C for 5 min [23]. The PCR products were visualized by electrophoresis on a 1.5% TBE (Tris 0.09 M, Borate 0.09 M, EDTA 0.02 M) agarose gel, and stained with Gel Red (GelRed™ Nucleic Acid Gel Stain, 10,000X in water, cat. No. 41003).

PCR-RFLP of Ribosomal DNA regions (ITS) using *Rsa*I endonuclease enzyme was carried out to identify *Toxocara* species. The PCR products were digested with restriction endonuclease *Rsa*I (New England BioLab Inc., New England Biolabs (NEB), R0167S) at 37 °C for 3 h. The digested PCR amplicons were electrophoresed on a 2% agarose gel at 80 V for 1 h. Digestion with the *Rsa*I enzyme produces 108, 267, and 332 bp fragments for *T. canis*, and 103, 105, and 528 bp fragments for *T. cati* [23].

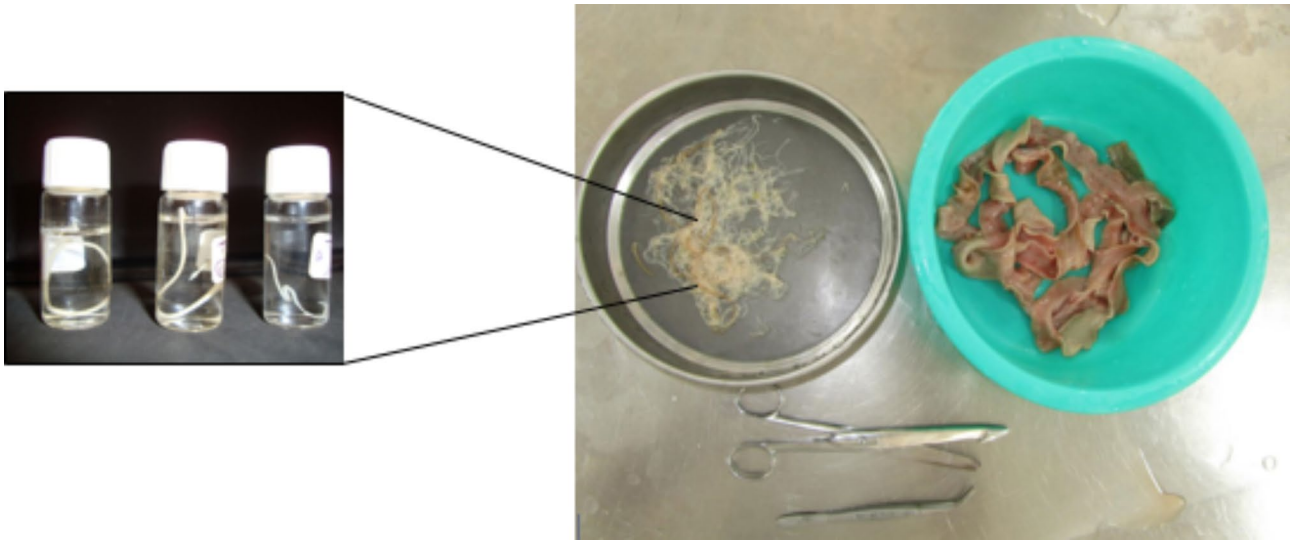

Fig. 2 Isolation of adult *Toxocara* worms from digestive tract of road-killed golden jackals

Four PCR products were submitted to sequencing in two directions using the same forward and reverse primers used in the PCR by the Sanger sequencing method. The sequence results were edited by the Geneious software and compared with published sequences in GenBank using the BLAST (Basic Local Alignment Search Tool) system, and the genus and species of nematode were identified. The homology of the obtained consensus sequences was compared with each other and with the reference sequences in the GenBank using Bioedit software. A phylogenetic tree was constructed with sequences obtained in the present study and the reference sequences available in GenBank using the Molecular evolutionary genetic analysis software (MEGA 5.0), Maximum Likelihood method. Bootstrap analyses with 1000 replicates were carried out to determine the robustness of the findings.

Data analysis

Prevalence rate of *Toxocara* in jackals was determined by applying the following formula:

$$\text{Prevalence rate} = (\text{number of infected animals}/\text{total number of animals}) \times 100$$

Results

A total of 11 (26.8%) of 41 road-killed golden jackals in Guilan and Mazandaran provinces were positive for *Toxocara* nematodes (Table 1). The intensity of infection ranged from one to three worms per animal, with a mean of 1.6 ± 0.77. One isolate of *Toxocara* from each jackal were characterized by morphological and molecular methods.

Toxocara nematodes have cephalic alae, *T. canis* showed a lance-shaped cephalic alae (Fig. 3A), while *T. cati* has a very broad cephalic alae. Male and female worms were separated from each other; the size of the female worms was larger, and the male worms had a curved posterior end with a finger-shaped appendage (Fig. 3B). In the female worms, the *Toxocara* eggshell was uneven and porous (Fig. 3C). All the isolates were confirmed as *T. canis* based on morphological features.

An approximately 700 bp band was amplified from 11 *Toxocara* samples isolated from jackals after the PCR for ITS fragment (Fig. 4), and digestion of the ITS products of the *T. canis* with restriction endonuclease *Rsa*I produced three fragments, 108, 267, and 332 bp (Fig. 5).

Four *Toxocara* samples isolated from jackals were sequenced, and results based on PCR-RFLP analyses were confirmed by sequence analyses of representative ITS amplicons. After editing the sequences using the Geneious software, the consensus sequence of 655 nucleotides for the ITS region of *Toxocara* isolates was obtained. BLAST analysis of sequenced isolates indicated that the nematodes isolated from jackals were identified as *T. canis*. The ITS sequences of four *Toxocara* samples isolated from jackals were deposited in the

Table 1 Infection rate of road-killed golden jackals with *Toxocara* nematodes in Guilan and Mazandaran provinces, northern Iran

Geographical origin	No. of road-killed golden jackals	No. of jackals infected with *Toxocara*	Percent	95% confidence interval	
				Lower	Upper
Guilan province	15	2	13.3	1.7	40.5
Mazandaran province	26	9	34.6	17.2	55.7
Total	41	11	26.8	14.2	42.9

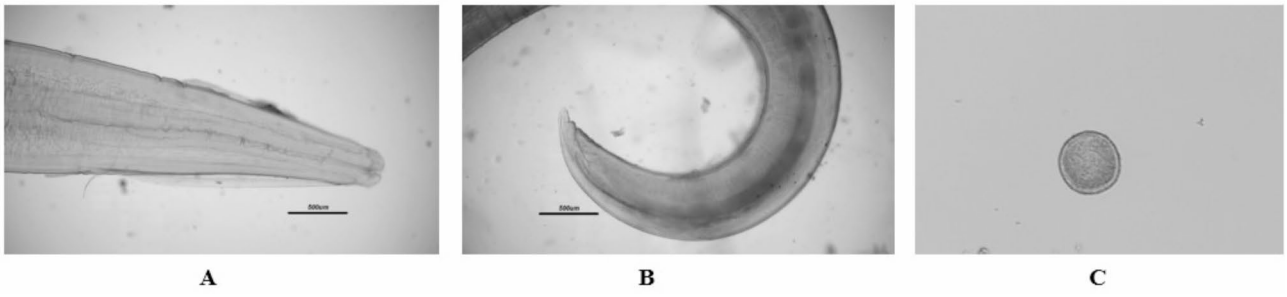

Fig. 3 Cephalic alae of *Toxocara canis* (**A**), curved posterior end with a finger-shaped appendage of male of *Toxocara canis* (**B**), Egg of *Toxocara canis* (**C**)

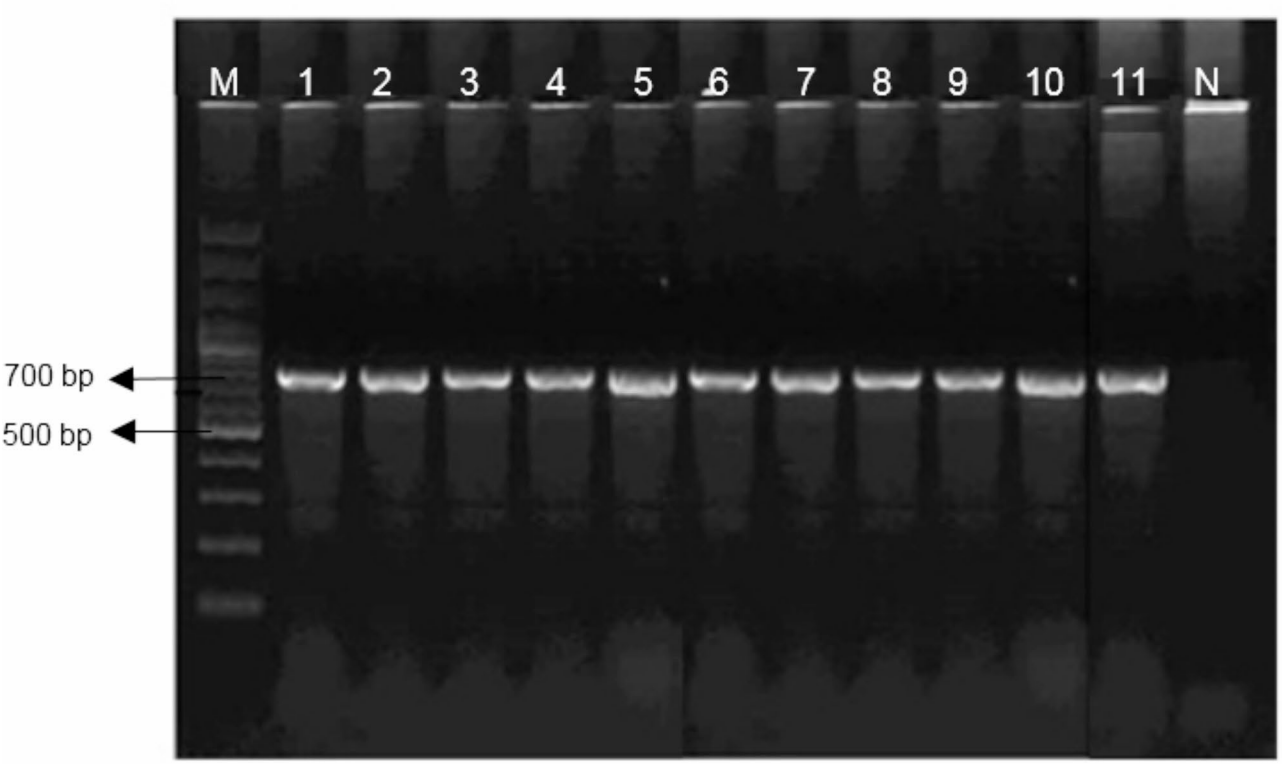

Fig. 4 Agarose gel electrophoresis of ITS-PCR products. M: 100 bp DNA Marker, 1–11: *T. canis* samples isolated from jackals, *N* negative control

GenBank database with accession numbers MW590694-MW590697. A phylogenetic tree was constructed with sequences obtained in this study and the reference sequences available in the GenBank using MEGA 5.0 software. The phylogenetic tree indicated that four isolates of *T. canis* obtained from jackals in Iran based on ITS sequences were similar and therefore one haplotype was identified (Fig. 6). Intra-species variation was reported as 0–0.2%.

Discussion

Toxocariasis is one of the common zoonotic parasitic diseases in humans and animals. Canids and felids as the main hosts are infected with *Toxocara* worldwide [6]. So far, the infection of dogs and cats with *T. canis* and *T. cati*, respectively, has been reported from different parts of Iran [7, 24]. Wild carnivores such as golden jackals are considered as potential reservoirs for the transmission of intestinal helminths such as *T. canis* to humans. Due to close contact between humans and golden jackals, these animals are dangerous for people [19]. In this study, the infection rate of jackals with *Toxocara* was investigated in Guilan and Mazandaran provinces, and morphological and molecular investigation of *Toxocara* isolates was carried out.

The results of the present study showed that 11 out of 41 (26.8%) road-killed golden jackals were infected with *T. canis* in Guilan and Mazandaran provinces. Similar to our study, the infection rate of *T. canis* in jackals in Guilan province was 27.2% [18]. Different studies conducted on the infection rate jackals with *Toxocara* have reported a lower prevalence rate than that in our study

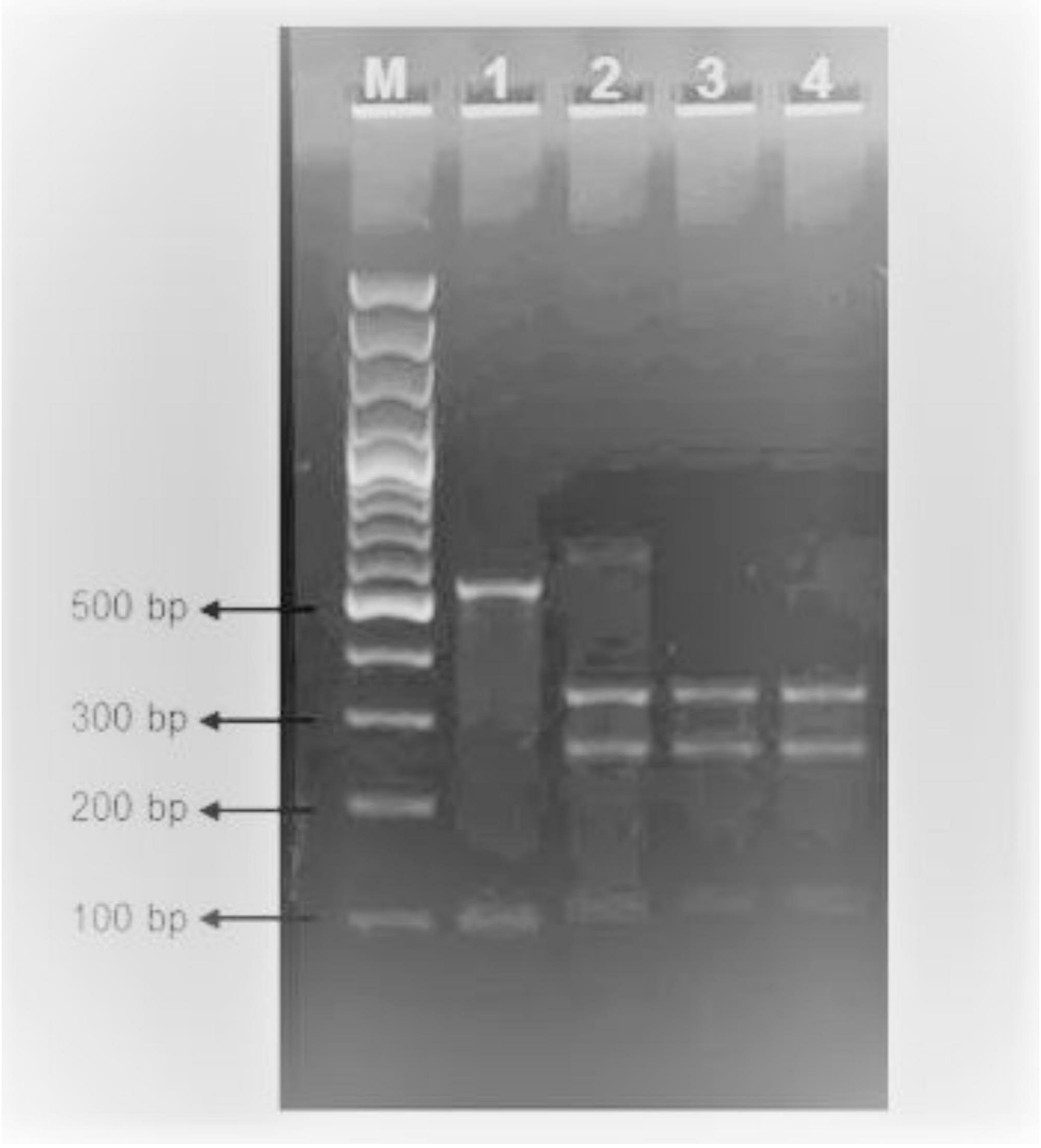

Fig. 5 Agarose gel electrophoresis of PCR-RFLP after digestion with *Rsa*I restriction enzyme. M: 100 bp DNA Marker, 1: *T. cati* as control, 2–4: *T. canis* samples isolated from jackals

[13, 15, 18, 19]. Survey of Siyadatpanah et al. in northern Iran showed that 2 out of 43 (4.5%) road-killed golden jackals were infected with *T. canis* in Mazandaran province [25]. The prevalence rate of *Toxocara* in jackals was investigated in three different climatic zones of Iran, 1st zone: Guilan, Mazandaran and Golestan provinces, 2nd zone: East Azerbaijan, West Azerbaijan, Ardabil, Isfahan and Khorasan provinces, and 3rd zone: Khuzestan and Hormozgan provinces. Twenty-seven jackals from the 1st zone, 33 jackals from the 2nd zone, and 19 jackals from the 3rd zone were examined. Four jackals (5%) collected from the second zone were infected with *T. canis*.

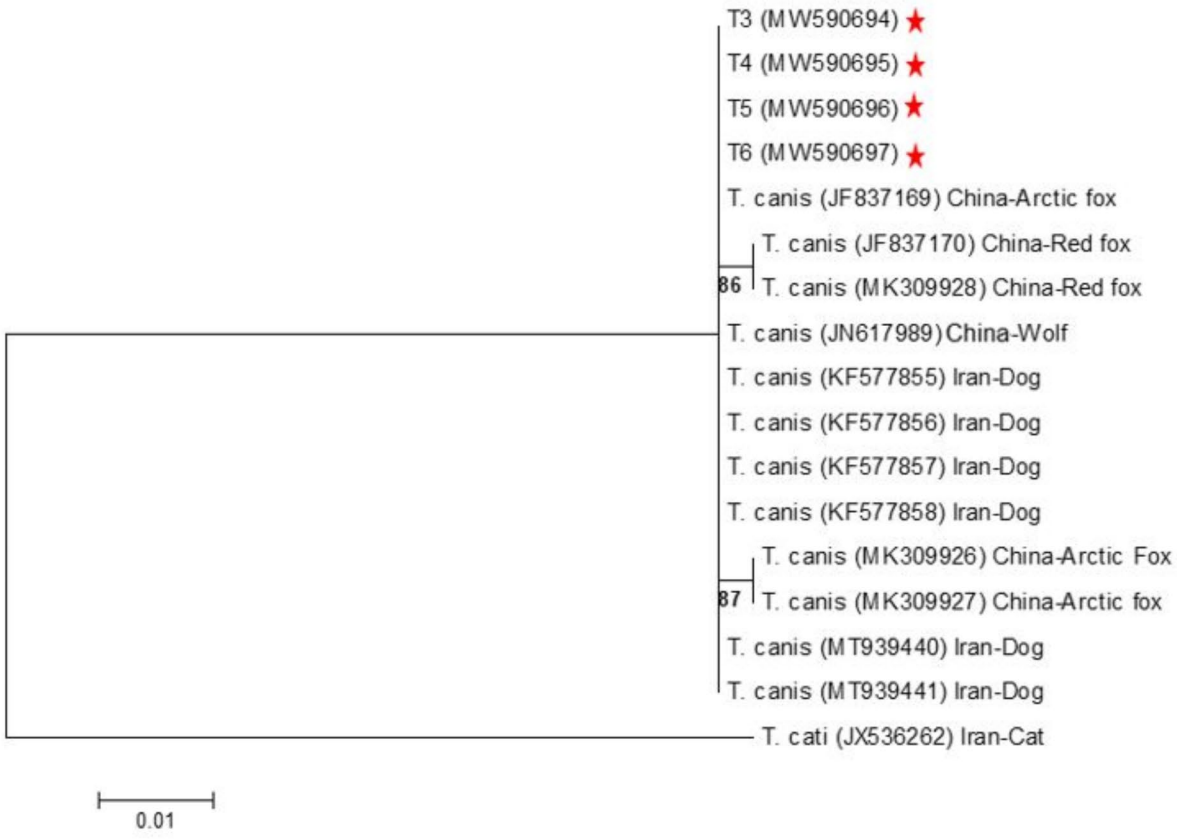

Fig. 6 Phylogenetic tree of ITS sequences of *Toxocara canis* isolates obtained in this study and reference sequences available in GenBank using Maximum Likelihood method. *Toxocara cati* (Accession number: JX536262) is considered as the outgroup

In contrast with our study, none of the jackals collected from Guilan and Mazandaran provinces were infected with *Toxocara* [17]. The sample size, sample type, and diagnostic method could affect the prevalence rate of *Toxocara* in the host.

Molecular methods are used to detect *Toxocara* infection in canids and felids, identify species, and investigate genetic diversity within the population. Since the morphological identification of some *Toxocara* species, especially in the egg and larval stages, is difficult, the use of molecular methods using suitable genetic markers is an excellent method for species identification. In this study, the genus and species of *Toxocara* nematodes isolated from jackals were identified using morphological and molecular methods. Eleven *Toxocara* nematodes isolated from jackals identified as *T. canis* based on morphological features, and molecular methods confirmed these results. In 1993, morphologically, the ascaridoid nematode isolated from cats in Malaysia was identified as *T. canis* [26]. In 1998, this the ascaridoid nematode was characterized as *Toxocara sp. cf. canis* using the sequence of the ITS1 and ITS2 regions [27]. In 2001, more detailed morphological and molecular studies of this nematode showed that this nematode was a new species named *Toxocara malaysiensis* [28]. Therefore, molecular methods are reliable alternatives to morphological methods for the specific identification of nematodes. The study conducted by Mikaeili et al. showed that *Toxocara* nematodes isolated from stray cats in Shiraz were confirmed as *T. cati* based on morphological and molecular methods [29].

Phylogenetic studies based on the differences in the DNA sequence are suitable means to investigate the evolutionary relationship of organisms. There are reports on genetic diversity among ascarid nematodes [30, 31]. In the present study, the isolates of *T. canis* based on ITS sequence showed one haplotype and had a 100% homology with each other. The sequences of the ITS region of *T. canis* isolated from jackals in this study were utterly similar to the ITS region sequences of *T. canis* separated from dogs in Iran (Accession numbers: KF577855-58, MT939440-41), arctic fox in China (Accession number: JF837169) and wolf in China (Accession number: JN617989), so it is hypothesized that the host type and

geographical area do not affect the genetic diversity of the ITS region sequence of *T. canis* isolates. Fogt et al. also reported no difference in the ITS-1 and ITS-2 region sequences of *T. canis* isolated from dogs and foxes in different geographical regions of the world [32].

Conclusions

T. canis is a common intestinal nematode in jackals; therefore, identification of parasite species by molecular methods can be used to plan prevention and control programs in human and animal communities. Since, the ITS sequences of *T. canis* isolated from jackals in Iran were utterly similar to the ITS sequences of *T. canis* isolated from other hosts from different areas of the world, it is hypothesized that the type of host and geographical region do not affect the genetic diversity of the ITS region sequences. For characterization, phylogenetic analysis and genetic diversity of *Toxocara*, comprehensive molecular epidemiological studies on larger sample sizes and isolates from different hosts and more different geographical areas of Iran are recommended.

Abbreviations

ITS	Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

Acknowledgements

Not applicable.

Author contributions

FM and MSH designed the study. MSH collected the samples. MSB, AMS and ARK carried out the morphological and molecular methods. FM performed the molecular and phylogenetic analysis. FM and MSH analyzed the data. SMKA and SSM drafted the manuscript. FM and MSH reviewed and edited the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Office of the Vice-Chancellors for Research of Shiraz University of Medical Sciences (Grant Number 98-01-01-20030).

Data availability

All data generated or analyzed during this study are included in this published article. The sequence data were deposited in the GenBank database with the accession numbers: KF577859, MW590694-MW590697.

Declarations

Ethics approval and consent to participate

The Ethical Committee of Shiraz University of Medical Sciences, Shiraz, Iran, approved this study (ethical code: IR.SUMS.MED.REC.1399.009). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 25 April 2023 / Accepted: 4 October 2024
Published online: 24 February 2025

References

1. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev. 2003;16:265–72.
2. Lee RM, Moore LB, Bottazzi ME, Hotez PJ. Toxocariasis in North America: a systematic review. PLOS Negl Trop Dis. 2014;8:e3116.
3. Zibaei M, Sadjjadi SM, Maraghi S. The occurrence of *Toxocara* species in naturally infected broiler chickens revealed by molecular approaches. J Helminthol. 2017;91:633–36.
4. Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann Trop Med Parasitol. 2010;104:3–23.
5. Raissi V, Sohrabi Z, Getso M, Raiesi O, Hashemi Hafshejani S, Shabandoust H, et al. Risk factors and prevalence of toxocariasis in pregnant women and diabetic patients compared to healthy adults in Ilam province, western Iran. EXCLI J. 2018;17:983–88.
6. Zibaei M, Sadjjadi S. Trend of toxocariasis in Iran: a review on human and animal dimensions. Iran J Vet Res. 2017;18:233.
7. Abbaszadeh Afshar MJ, Zahabiun F, Heydarian P, Mozafar Saadati H, Mohtasebi S, Khodamoradi F, et al. A systematic review and meta-analysis of toxocariasis in Iran: is it time to take it seriously? Acta Parasitol. 2020;65:569–84.
8. Shafiei R, Rahimi MT, Emameh RZ, Mirzaei M, Perez-Cordon G, Ahmadpour E. Status of human toxocariasis, a neglected parasitic zoonosis in Iran: a systematic review from past to current. Trop Doct. 2020;50:285–91.
9. Holland CV. A walk on the wild side: a review of the epidemiology of *Toxocara canis* and *Toxocara cati* in wild hosts. Int J Parasitol Parasites Wildl. 2023;22:216–28.
10. Rostami A, Riahi SM, Hofmann A, Ma G, Wang T, Behniafar H, et al. Global prevalence of *Toxocara* infection in dogs. Adv Parasitol. 2020;109:561–83.
11. Rostami A, Sepidarkish M, Ma G, Wang T, Ebrahimi M, Fakhri Y, et al. Global prevalence of *Toxocara* infection in cats. Adv Parasitol. 2020;109:615–39.
12. Sarvi S, Daryani A, Sharif M, Rahimi MT, Kohansal MH, Mirshafiee S, et al. Zoonotic intestinal parasites of carnivores: a systematic review in Iran. Vet World. 2018;11:58–65.
13. Sadighian A. Helminth parasites of stray dogs and jackals in Shahsavar area, caspian region. Iran J Parasitol. 1969;55:372–4.
14. Arfaa F. Status of infections with larva migrans in Iran. Iran J Public Health. 1972;2:168–74.
15. Gholami S, Mobedi E, Ziaee H, Sharif M. Intestinal helminth parasites in dog and jackal in inferent areas of Sari in the years 1371-72. J Mazandaran Univ Med Sci. 1999;9:1–12.
16. Dalimi A, Sattari A, Motamedi G. A study on intestinal helminthes of dogs, foxes and jackals in the western part of Iran. Vet Parasitol. 2006;142:129–33.
17. Meshgi B, Eslami A, Bahonar AR, Kharrazian-Moghadam M, Gerami-Sadeghian A. Prevalence of parasitic infections in the red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) in Iran. Iran J Vet Res. 2009;10:387–91.
18. Vafae Eslahi A, Kia EB, Mobedi I, Sharifdini M, Badri M, Mowlavi G. Road killed carnivores illustrate the Status of Zoonotic helminthes in Caspian Sea Littoral of Iran. Iran J Parasitol. 2017;12:230–5.
19. Amouei A, Jahandar H, Daryani A, Sharif M, Sarvi S, Mizani A, et al. Carnivores as Important Reservoirs of Intestinal Helminthic Infections in Mazandaran Province, Northern Iran. Iran J Parasitol. 2018;13:251–7.
20. Gasser RB, Chilton NB. Applications of single-strand conformation polymorphism (SSCP) to taxonomy, diagnosis, population genetics and molecular evolution of parasitic nematodes. Vet Parasitol. 2001;101:201–13.
21. Borecka A. The spread of nematodes from *Toxocara* Genus in the world. Wiad Parazytol. 2010;56:117–24.
22. Soulsby EJL, Helminths. Arthropods and Protozoa of Domesticated animals. Bailliere Tindall London, UK; 1982.
23. Mikaeili F, Mathis A, Deplazes P, Mirhendi H, Barazesh A, Ebrahimi S, et al. Differentiation of *Toxocara canis* and *Toxocara cati* based on PCR-RFLP analyses of rDNA-ITS and mitochondrial COX1 and NAD1 regions. Acta Parasitol. 2017;62:549–56.
24. Siyadatpanah A, Pagheh AS, Daryani A, Sarvi S, Hosseini SA, Norouzi R, et al. Parasitic helminth infections of dogs, wolves, foxes, and golden jackals in Mazandaran Province, North of Iran. Vet World. 2020;13:2643–648.
25. Siyadatpanah A, Gholami S, Daryani A, Sarvi S, Sharif M, Seguel M, et al. The prevalence of intestinal helminths in Free-ranging canids of Mazandaran, Northern Iran. Iran J Parasitol. 2019;14:563–71.
26. Lee CC, Cheng NABY, Bohari Y. *Toxocara canis* from domestic cats in Kuala Lumpur. Trop Biomed. 1993;10:79–80.

27. Zhu XQ, Jacobs DE, Chilton NB, Sani RA, Cheng NA, Gasser RB. Molecular characterization of a *Toxocara* variant from cats in Kuala Lumpur, Malaysia. Parasitol. 1998;117:155–64.
28. Gibbons LM, Jacobs DE, Sani RA. *Toxocara malaysiensis* n. sp. (Nematoda: Ascaridoidea) from the domestic cat (*Felis catus* Linnaeus, 1758). J Parasitol. 2001;87:660–5.
29. Mikaeili F, Mirhendi H, Hosseini M, Asgari Q, Kia EB. *Toxocara* nematodes in stray cats from Shiraz, soulrann iran: intensity of infection and molecular identification of the isolates. Iran J Parasitol. 2013;8:593–600.
30. Zhu XQ, Gasser RB. Single-strand conformation polymorphism (SSCP)-based mutation scanning approaches to fingerprint sequence variation in ribosomal DNA of ascaridoid nematodes. Electrophore. 1998;19:1366–73.
31. Wickramasinghe S, Yatawara L, Rajapakse RP, Agatsuma T. *Toxocara canis* and *ToxVitulorumulorum*: molecular characterization, discrimination, and phylogenetic analysis based on mitochondrial (ATP synthase subunit 6 and 12S) and nuclear ribosomal (ITS-2 and 28S) genes. Parasitol Res. 2009;104:1425–30.
32. Fogt-Wyrwas R, Jarosz W, Rząd I, Pilarczyk B, Mizgajska-Wiktor H. Variability in sequences of mitochondrial cox1 and nad1 genes in *Toxocara canis*, *Toxocara cati*, and *Toxascaris leonine* (Nematoda: Toxocaridae) from different hosts. Ann Parasitol. 2016;62:48.

Publisher's note