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A preliminary study on helminths detected in red foxes (*Vulpes vulpes*) in Bingol Province of Türkiye: morphological and molecular approaches

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Abstract

Background Red foxes (*Vulpes vulpes*) are hosts to a large number of endoparasites, some of which are zoonotic. This study was carried out to identify and molecularly characterise intestinal helminths in foxes in Bingol province, eastern Türkiye. For this purpose, carcasses of 15 red foxes that died as a result of traffic accidents in Bingol province were obtained. Parasite material obtained from the intestines was identified according to its morphological characteristics, and complete gDNA was isolated from each individual parasite samples. A 446 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (mt-*CO1*) gene was amplified using PCR. Subsequently, a unidirectional sequence analysis was conducted.

Results The mt-*CO1* gene region of a total of 8 helminth species was successfully sequenced and identified by BLAST searches. One trematode (*Alaria alata*), five cestodes (*Dipylidium caninum*, *Joyeuxiella* sp., *Taenia hydatigena*, *Mesocestoides litteratus*, *Mesocestoides* sp.), one nematode (*Toxascaris leonina*) and one acanthocephalan (*Macracanthorhynchus hirudinaceus*) species were identified. Phylogenetic and haplotype analyses were performed on the obtained *Mesocestoides* spp. sequences. Haplotype analysis of *Mesocestoides litteratus* isolates revealed 22 haplotypes *Mesocestoides*.

Conclusion These findings are important to draw attention to the wild circulation of some zoonotic helminths.

Keywords Red Fox, Helminth, Sequence, Haplotype, Türkiye

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Introduction

The red fox (*Vulpes vulpes* Linnaeus, 1758) is the most common member of the genus *Vulpes*. The species primarily inhabits the temperate zone, steppes, and mixed woodlands of Eurasia [1]. The red fox has demonstrated the ability to thrive in a variety of habitats and climates, including both urban and rural environments, resulting in significant changes in both behaviour and diet [2]. Red fox (*Vulpes vulpes*) is the dominant carnivore species within the geographical boundaries of Türkiye. Based on the findings of Ambarlı et al. [3], the estimated population of red foxes in Türkiye ranges from 16,000 to 20,000 individuals [4]. Red foxes are prevalent throughout all geographical zones of Türkiye. Due to their exceptional adaptability, they can adjust to many climatic and topographical circumstances [5]. Despite the knowledge that red foxes maintain viable populations in Türkiye, there is an absence of precise census data or a long-term monitoring program [3]. While red foxes mostly live in forested areas, open fields and agricultural regions, they have made significant progress in adapting to and coexisting with human populations in both rural and urban environments. This indicates that the environment contains zoonotic helminth eggs, which have the potential to cause disease in humans. Dogs and cats are the primary hosts for certain parasites, such as *Echinococcus multilocularis* and *Toxocara canis*, which are common in red foxes. As a result, pets can acquire these parasites and then become a direct source of infection for humans [6–8]. Among these, *E. multilocularis* is the most dangerous for humans. The eggs are shed in the feces of definitive hosts (wild canids, occasionally dogs) and contaminate the environment. Rodents ingest eggs, developing alveolar hydatid cysts. Definitive hosts consume infected rodents, allowing larvae to mature into adult tapeworms. Foxes are the main reservoirs, while dogs can contribute to human transmission. Humans can acquire infection through egg ingestion, leading to alveolar echinococcosis. Dogs may shed eggs, while cats rarely act as hosts but can carry eggs in their fur [9].

Obtaining data on parasites in wildlife is of great importance as it allows for the deployment of appropriate approaches to mitigate the adverse effects of parasites on dynamic ecosystems [10]. Wild animals are widely acknowledged to serve as important reservoirs of human parasites, hence posing a serious risk to human health and well-being [11, 12]. Canids are recognised as the primary source of human parasite infections in wildlife [12, 13]. Red foxes (*Vulpes vulpes*) have been found to be hosts for various endoparasite species, including zoonotic trematodes, cestodes, nematodes and protozoa [14]. Several species from the trematode families Echinostomatidae and Opisthorchiidae, as well as *Alaria alata*, have been documented in red foxes. Multiple *Taenia* species within the cestode class, including *Taenia hydatigena*, *Taenia crassiceps*, *Taenia pisiformis*, *Taenia polyacantha*, *Taenia serialis*, and *Taenia taeniaeformis*, as well as *E. multilocularis*, *Dipylidium caninum*, and *Joyeuxiella echinorhynchoides*, have been documented [8, 15, 16]. Additionally, several nematode species, including *T. canis*, *Toxocara cati*, *Toxascaris leonina*, *Uncinaria stenocephala*, *Ancylostoma caninum*, and *Trichuris vulpis*, have been documented in red foxes [15, 17–22]. In addition, Acanthocephala parasites such as *Macracanthorhynchus* sp., *Pachysentis* sp. and *Centrorhynchus* sp. [15, 23, 24] and *Mesocestoides* species have also been detected in red fox populations [15, 18].

There are limited studies on the identification of endohelminths in red foxes (*Vulpes vulpes*) in Türkiye. In previous studies, helminth infections in red foxes were mostly identified by traditional methods such as fecal examination and microscopic identification of species. For example, in 2019, 409 red fox fecal samples from Central Anatolia and Thrace were examined and different types of helminth eggs were found [25]. Between 2004 and 2007, 20 red fox carcasses were examined for helminth species in Kars province and microscopically identified [26]. Molecular studies in red foxes are very limited. In a study conducted in Sivas province, 103 red fox carcasses were examined and PCR and DNA sequencing methods were used for definitive species identification of taeniid cestode species after microscopic identification [15]. In Erzurum province, 50 red foxes were examined and fecal samples were genetically identified by PCR method to determine how common *E. multilocularis* is in red foxes [27].

Mitochondrial *cytochrome c oxidase subunit 1* (*CO1*) gene is extensively utilized in PCR for the identification of *Taenia* species because of its elevated copy number, conserved areas, and unique sequences among species [28]. The mt-*CO1* gene region exhibits significant variability among *Taenia* spp., however remains reasonably conserved within each species. Moreover, it serves as an ideal marker for the construction of phylogenetic trees, showing genetic differences among species and clarifying evolutionary connections between both near and distantly related species, owing to its excellent nucleotide exchange rates [29, 30].

The aim of this study was to investigate the parasitological and molecular characteristics of helminth species found in red foxes (*Vulpes vulpes*) from rural areas of Bingol province, Türkiye, with a focus on identifying potential zoonotic species and other associated helminth parasites.

Materials and methods

Study area and sample collection

The material of this study consisted of 15 red foxes collected from rural areas of Bingol province (38°27'-40°27'N, 41°20'-39°54'E) between September 2023 and January 2024. All were found dead after being hit by a car on the motorway and brought to the Veterinary Faculty of Bingol University by villagers living in rural areas. The carcasses were kept at -80 °C for two weeks to deactivate any potential *Echinococcus* species and mitigate the danger of contamination [31].

Parasitological examination

The red foxes were removed from the freezer and kept at room temperature overnight, and a general necropsy was performed the following day in the presence of a pathologist. The contents of the small intestine were transferred to a beaker after its longitudinal dissection. We used a stereo-microscope to look for helminths after scraping the mucosa and decanting it multiple times. The sedimentation counting technique (SCT) was performed according to the methodology of Hofer et al. [32]. Initially, the small intestine was sliced lengthwise and subsequently partitioned into five identical segments. The fragments were inserted into a glass beaker that was filled with 0.9% NaCl solution. After a short and vigorous shaking of the beaker, the intestines were removed and the mucosa scraped off. Following a 15-minute period of sedimentation, the liquid component was judiciously drained off. This procedure was iterated five times in total, until the liquid above the sediment achieved transparency. Small 10 ml petri dishes containing the sediment were utilized to analyze it under a stereomicroscope (Nikon, SMZ745T). The helminths were numbered and collected in separate falcon tubes and stored in 70% ethanol at -20℃ for molecular identification.

Morphologic identification

The parasites collected from infected red foxes were washed several times in warm phosphate buffer saline solution to remove fecal particles and other matter and then classified as trematodes, cestodes and nematodes. Parasites were classified according to their morphological characteristics at the superfamily level according to Soulsby [33] and Yamaguti [34] and helminths were photographed using calibrated software (Nikon's NIS-Elements software).

Molecular identification

After morphological identification of adult parasites collected from intestinal sediment, one adult parasite from each species was used for genomic DNA isolation. A small fragment excised from each individual adult parasite specimen was subjected to a minimum of five washes using sterile PBS buffer in order to eliminate any remnants of ethanol. The genomic DNA from adult parasites was isolated using the PureLink™ Genomic DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, Missouri, TX, USA) following the manufacturer's instructions. The DNA was then kept at -20 °C until PCR. Primer pairs JB3 and JB4.5 amplify a 446 bp fragment of the mt-*CO1* gene, which was used to amplify the relevant gene region by PCR. The PCR was conducted using a 50 µL reaction mix comprising 5 µL 10X PCR buffer, 5 µL 25 mM $MgCl_2$, 400 µM of each dNTP's, 20 pmol of each primer, 0.2 µL Taq DNA polymerase enzyme (1.25 IU) (Hibrigen, Türkiye), 28.8 µL PCR-grade water, and 5 µL of the template gDNA. The products underwent separation through agarose gel electrophoresis with a concentration of 1.5%. Visualizing the gel with RedSafe (iNtRON Biotech, South Korea) and purifying the PCR products, a unidirectional sequence analysis was performed using the sense primer set (BM Labosis, Ankara, Türkiye). The obtained sequences were deposited in the GenBank database.

Alignment

Analysis of the sequence results was conducted using FinchTV 1.4.0 software (Geospiza Inc., based in Seattle, Washington, USA). After obtaining the sequence results, a BLAST search was conducted to identify sequence similarities and determine species classifications using the National Center for Biotechnology Information genome database (http://www.ncbi.nlm.nih.gov). Following that, the sequence data underwent alignment using MEGA X [35]. We utilized the Clustal W module for aligning nucleotide sequences. A previously identified *E. multilocularis* sequence (accession no. JF747251) was included in the phylogenetic analysis as an outgroup. We conducted distance-based analyses in MEGA X utilizing the General Time Reversible (GTR) distribution, (G + I) model distance estimates, and built the phylogenetic tree with the maximum likelihood algorithm. 1000 replicates were used to conduct bootstrap analysis.

In this study, the extended mt-*CO1* data set was used for the phylogenetic analysis of sequences obtained from *M. litteratus* and *Mesocestoides* sp. The sequences of the red fox isolates obtained were aligned with sequences of domestic and wild carnivore isolates obtained from the adult and larval stage of the parasite and previously deposited in the GenBank database. While constructing the phylogenetic tree, representatives of *Mesocestoides* species identified in GenBank (11 sequences) and three sequences identified as *Mesocestoides* sp. identified in this study were used. In addition, one representative from each haplotype was selected for the 22 haplotypes consisting of 53 sequences used in the *M. litteratus* haplotype analysis.

Haplotype network and nucleotide sequence variation, diversity and neutrality indices

For haplotype network analysis, the data sets used in phylogenetic analyses were used. Haplotype number (h), nucleotide diversity (π) and haplotype diversity (Hd), population diversity indices and neutrality indices were calculated using DnaSP6 software [36]. A haplotype network was constructed using the isolates and GenBank sequences obtained in this study. The haplotype network was constructed using PopART-1.7 software and the Minimum Spanning Networks (MSN) method [37].

Results

In this study, 15 red fox carcasses were examined parasitologically and all red fox samples were determined to be infected. The gDNA of isolates obtained from infected red foxes were amplified by PCR and a total of 8 helminth species were identified. Among the identified species, one species belonging to the trematoda class (*A. alata*), five species belonging to the cestoda class (*D. caninum* (could not be sequenced), *Joyeuxiella* sp., *T. hydatigena*, *M. litteratus* and *Mesocestoides* sp.,), one species belonging to the nematoda class (*T. leonina*) and one acanthocephala (*M. hirudinaceus*) (could not be sequenced) were identified (Table 1).

Morphological features

At least one parasite species was detected in all 15 red fox carcasses examined and the overall prevalence of helminth infection was 100%. Parasites were identified morphologically and classified under a stereomicroscope (Nikon, SMZ745T) (Fig. 1).

Molecular identification

A total of eight helminth species were identified and one morphologically distinct representative from each was selected and successfully sequenced for the mt-*CO1* gene. One trematode; *A. alata* (Acc. No. PP431387), five cestodes; *D. caninum* (could not be sequenced), *Joyeuxiella* sp., (Acc. No. PP434592), *T. hydatigena* (Acc. No. PP434593), *M. litteratus* (Acc. No. PP406895-899) and *Mesocestoides* sp. (Acc. No. PP406868-870), one nematode; *T. leonina* (Acc. No. PP434590) and one acanthocephala; *M. hirudinaceus* (could not be sequenced) were identified. The specimens used in this study were taxonomically identified by a BLAST search against the GenBank nucleotide database and recorded in the GenBank database (Table 1).

Phylogenetic tree

The mt-*CO1* gene data set of *M. litteratus* includes five sequences obtained in this study and 53 sequences obtained from GenBank. The mt-*CO1* gene region tested for phylogeny was restricted to 311 bp to ensure alignment with sequences in GenBank.

The maximum likelihood generated from the mt-*CO1* gene data set displayed an effective phylogenetic signal. Through ML tree analysis (Fig. 2) *Mesocestoides litteratus* samples were in the same cluster as the reference sequences and did not form a separate cluster. The haplotypes (Hap01, Hap02, Hap03, Hap04) formed by the sequences of *M. litteratus* isolates obtained in this study formed a single monophyletic group in the phylogenetic tree. *Mesocestoides* sp. isolates (marked with an asterisk) obtained in this study formed a sister taxa with *M. canislagopodis* and *M. lineatus*, exhibiting significant support values. This result suggests that our specimens are

Table 1 Information on parasites obtained from the intestines of examined red foxes, the number and sex of infected red foxes, PCR product size and GenBank accession numbers

Phylum	Parasite species	Stage	Sex		Number of infected red foxes	PCR product size (bp)	GenBank Accession Numbers
			Female	Male			
Trematoda	*Alaria alata*	Adult	1		1	446	PP431387
Cestoda	*Dipylidium caninum*	Adult	1		1	446	could not be sequenced
	Joyeuxiella sp.	Adult		1	1	446	PP434592
	Taenia hydatigena	Adult		1	1	446	PP434593
	Mesocestoides litteratus	Adult	4	1	5	446	PP406895 PP406896 PP406897 PP406898 PP406899
	Mesocestoides sp.	Adult	1	2	3	446	PP406868 PP406869 PP406870
Nematoda	*Toxascaris leonina*	Adult	2	1	3	446	PP434590
Acanthocephala	*Macracanthorhynchus hirudinaceus*	Adult	1		1	446	could not be sequenced

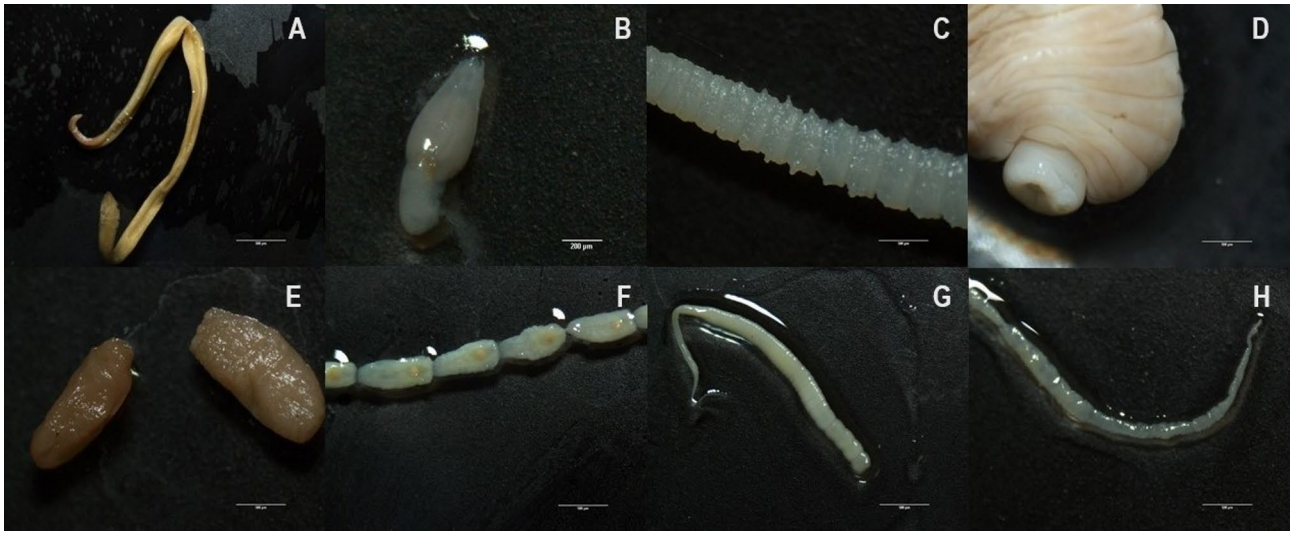

Fig. 1 Stereo microscope images of detected helminth species. **A**: *T. leonina*, **B**: *A. alata*, **C**: *Mesocestoides* sp., **D**: *M. hirudinaceus*, **E**: *D. caninum*, **F**: *M. litteratus*., **G**: *Joyeuxiella* sp. **H**: *T. hydatigena*

likely part of a lineage closely related to *M. canislogopodis* and *M. lineatus.*

Haplotype network for *M. litteratus*

For the haplotype network of *M. litteratus* isolates, 53 gene sequences were used, five of which were from isolates obtained in this study. The final length of the sequences was equalized to 311 bp. In the haplotype network, 22 haplotypes were found. According to haplotype analysis, no main haplotype separated by sharp boundaries was formed, but 3 main haplotypes with similar frequencies and haplogroups separated from them were found including a major haplotype (Hap06, Hap07,Hap10) (Fig. 3). Five isolates obtained in this study formed four different haplogroups (Hap01, Hap02, Hap03, Hap04). These 4 different haplotypes were formed by 2–4 nucleotide differences from the main haplotype (Hap06). Meanwhile, two of the sequences containing these four haplotypes were exclusive to this study and did not exhibit a shared haplotype with GenBank sequences (Hap01, Hap04). The other three sequences constituted a haplogroup in proximity to the red fox isolates submitted to NCBI database from Türkiye. The geographical origin and hosts of the sequences forming the haplotypes are shown in Table 2.

Nucleotide sequence variation, diversity and neutrality indices for *M. litteratus*

The sequences included 13 polymorphic regions with parsimony informative and 10 non-informative region. A conserved fragment (96–151 bp) was found in all sequenced isolates. Mean nucleotide variations were 0,01661 ± 0,00549 and haplotype variations were 0,922 ± 0,020. Tajima's D value was 0,06161 but not significant ($P > 0.10$). This suggests a reduction in population size and/or the impact of purifying selection. Fu's Fs was − 6,279 ($P < 0.02$), indicating allelic redundancy. Both values indicate a population decline following a recent population expansion.

Discussion

In this study, parasitic helminths were detected in all red foxes examined. Simultaneously, it was discovered that red foxes were harboring many helminth species that have the potential to infect humans (*A. alata*, *T. leonina*, *Mesocestoides* sp., *D. caninum* and *M. hirudinaceus*).

The distribution of *A. alata* varies significantly based on the geographical location and the availability of water sources [18]. In a study conducted in Poland, the prevalence in the northern part of the country exceeded 90%, while in the southern part of the country only around 20% was detected [38]. *A. Alaria alata* was not detected in 103 red foxes collected from the Black Sea, Mediterranean, Aegean, Marmara and Central Anatolian regions of Türkiye [15]. A study carried out in eastern Türkiye revealed that among 20 red foxes, the prevalence of *A. alata* was 30% [26]. In this study, *A. alata* was found in only one of 15 red foxes (6.67%). *Alaria alata* is typically located in the duodenum and occasionally in the proximal jejunum [20]. The fact that *A. alata* was not detected in red foxes living in wetlands throughout Türkiye suggests that it is related to the intestinal parts examined rather than habitat conditions.

Red foxes are frequently infected with ascarid nematodes (primarily *T. leonina* and *T. canis*), which are intestinal parasites [39]. The prevalence found in this study 20% (3/15) was lower than the 46.42%, 65% and 31%

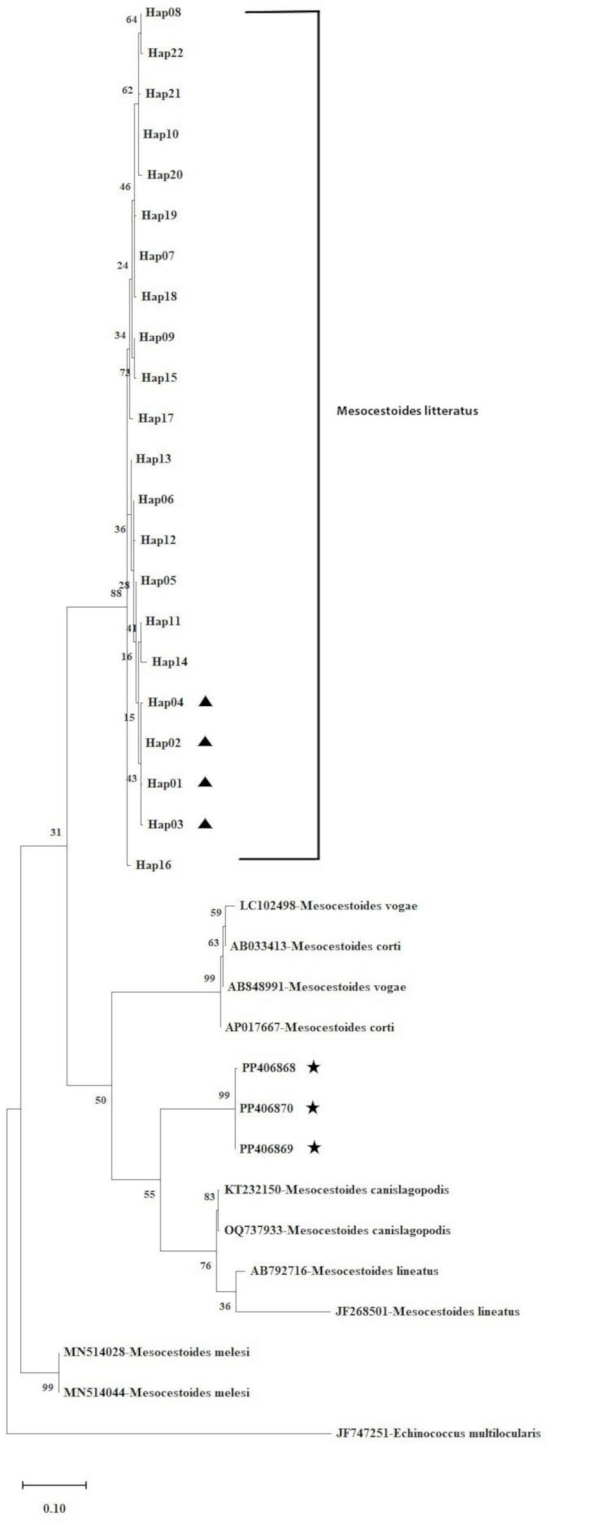

Fig. 2 Maximum likelihood tree showing relationships between *Mesocestoides* spp. Based on the mt-*CO1* dataset. Outgroup: *E. multilocularis*. Details of sample codes are listed in Table 2. ▲: haplogroups formed by the *M. litteratus* isolates in this study. ★: *Mesocestoides* sp. isolates obtained in this study

reported in different studies in red foxes in Türkiye [15, 25, 26].

Conversely, the prevalence of *D. caninum* in red foxes was found to be 0.97% (1/103) in a recent study [15], while the prevalence found in this study was 6,67% (1/15). In our study, it was not possible to interpret the effect of habitat and geographical conditions, as our sample size was smaller than in these studies. Although *D. caninum* gave a strong band at the target size with the relevant primers, DNA sequencing was not read despite repeated reads. It is thought that this may be due to the DNA quality and concentration of the PCR product.

Taenia hydatigena has not been observed in red foxes in Türkiye. Worldwide, a study conducted in Australia examined 499 red foxes and reported *T. hydatigena* in only one, and a study conducted in England reported *T. hydatigena* in only one of 111 red foxes [40, 41]. In this study, *T. hydatigena* was detected in a red fox for the first time in Türkiye. However, when the *T. hydatigena* sequence was aligned with GenBank sequences, it showed a low similarity of 87%. This may result from insufficient conservation of the amplified region (mt-*CO1* gene) among some species, sequencing errors, or low-quality bases during sequencing. In order to eradicate these limitations and discover potential cryptic species, future research should be corroborated by examining not just mt-*CO1* but also the internal transcribed spacer (*ITS*) region or additional mitochondrial/genomic markers.

Another cestode species identified is *Joyeuxiella* sp. In studies conducted in Türkiye, it was found to be the most common species after *Mesocestoides* species [15]. In this study, *Joeuxiella* sp. was detected in only one red fox. In addition to the identified species, one acantocephalan genera, *M. hirudinaceus* was identified. *Macracanthorhynchus hirudinaceus* has been observed in red foxes in both Türkiye and around the world [42].

The *Mesocestoides* species was the most frequently identified species in red foxes examined in this study, with 8/15 (53%). *Mesocestoides* species were reported to be prevalent in 78%, 78%, and 81% of recent studies, in Poland, Lithuania, and Italy, respectively [18, 43, 44]. A comparable high frequency has been recorded in some European countries, including Türkiye (56%) [15]. Our results are consistent with studies reporting a high prevalence of *Mesocestoides* species in red foxes. Literák et al. [45] reported in their study that *M. litteratus* was the most common species in all isolates from the Czech Republic, Slovakia and Spain. In a study conducted on red foxes in China, *M. litteratus* was reported to be the most common species [46]. In previous studies conducted in Türkiye, *M. litteratus* was reported in a wolf [47]. In addition, *M. litteratus*, *M. corti* in a dog and *Mesocestoides* sp. in a cat were reported. The sequences of the isolates have

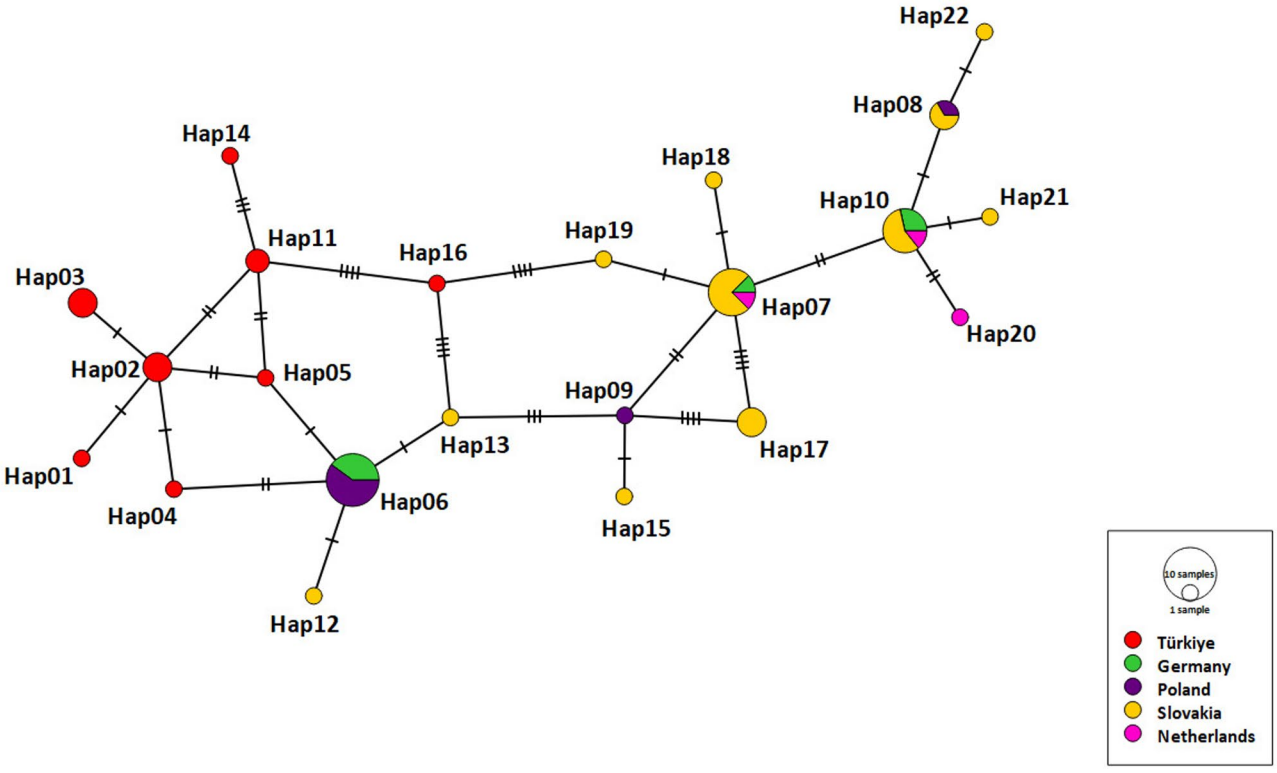

Fig. 3 Haplotype network for the mt-*CO1* gene (311 bp) of *M. litteratus*. The size of the circles is proportional to the frequency of each haplotype. The number of mutations separating the haplotypes is shown by dashes, and the host diversity of the haplotypes is shown in different colours. Hap: Haplotype

been deposited in GenBank, but the published data could not be accessed (Genbank accession numbers: PP407279, JN572111, MT160425). Identifying the isolates in this study was done, with *M. litteratus* accounting for 33% and *Mesocestoides* sp. for 20%. These results were consistent with the results of studies that reported *M. litteratus* to be a common species.

In a previous study conducted on red foxes in the Mediterranean, Black Sea, Marmara, Aegean and Central Anatolia Regions of Türkiye, a prevalence of 56% for *Mesocestoides* sp was reported, but no records of the *M. litteratus* isolate were reported [15]. This situation is thought to be related to the habitat conditions and hunting habits of red foxes, as the red fox isolates obtained in this study were from eastern Türkiye. Another study conducted in Türkiye examined 20 red fox guts and reported that *M. lineatus* was found in 12 (60%) [26]. Nevertheless, the complexity surrounding the taxonomy of *Mesocestoides* species suggests that identifying distinct lineages within the genus cannot rely solely on scolex and other morphological characteristics that exhibit significant variability [48]. Therefore, it is likely that some of the species previously reported as *M. lineatus* from Türkiye could be confused with *M. litteratus*, as they were reported on the basis of morphological characters only. Uncertainties in the taxonomy of *Mesocestoides* species remain [48]. Molecular data in previous studies have shown that *M. litteratus* and *M. lineatus* are two separate species and do not share the most recent common ancestor [48]. In the phylogenetic tree analysis including the Mesocestoides isolates obtained in this study, *M. litteratus* isolates were found to form a separate cluster from *M. lineatus*. Within the defined sequences, there are isolates that are genetically distant from each other.

According to the median-joining network analysis generated using the *M. litteratus* mt-*CO1* gene sequences, 22 different haplotype groups were identified. The five isolates obtained in this study formed four different haplogroups and were not included in the main haplotype (Hap01, Hap02, Hap03 and Hap04). This indicates that the haplotypes probably originated in Türkiye and that an ancestral haplotype is not found in any other geographical area. The fact that these four haplotypes form close haplogroups with the sequence of a gray wolf and red fox isolates reported from Türkiye, which is included in Hap05, and are grouped separately from other haplotypes, suggests that these haplotypes originated in Türkiye. It can be seen that these haplotypes are separated from the most closely related main haplotype (Hap06) and that this haplotype comes from the Palearctic region genotype of wild animals. This situation may be related to the migration of wild animals from the north to Türkiye due to hunting.

Table 2 Haplotype groups obtained from haplotype analysis of *M. litteratus* isolates and GenBank accession numbers of the sequences forming the haplotypes. The final sizes of the sequences were equalized at 311 bp

Haplotype Name	No. of isolate	Accession numbers	Product size	Country	Host
Hap01	1	PP406895	384 bp	Türkiye (this study)	Red fox
Hap02	3	PP406896	384 bp	Türkiye (this study)	Red fox
		PV105577	417 bp	Türkiye	Red fox
		PV105580	419 bp	Türkiye	Red fox
Hap03	3	PP406897	384 bp	Türkiye (this study)	Red fox
		PP406898	384 bp	Türkiye (this study)	Red fox
		PV105583	417 bp	Türkiye	Red fox
Hap04	1	PP406899	384 bp	Türkiye (this study)	Red fox
Hap05	1	MT806194	381 bp	Türkiye	Gray wolf
Hap06	10	MH998119	396 bp	Germany	Wild cat
		KX962367	396 bp	Germany	Gray wolf
		KX962362	396 bp	Germany	Gray wolf
		KX962361	396 bp	Germany	Gray wolf
		MN514039	415 bp	Poland	Red fox
		MN514038	415 bp	Poland	Red fox
		MN514037	415 bp	Poland	Red fox
		MN514036	415 bp	Poland	Red fox
		MN514035	415 bp	Poland	Red fox
		MN514034	415 bp	Poland	Red fox
Hap07	8	MH463514	373 bp	Slovakia	Red fox
		MH463512	373 bp	Slovakia	Red fox
		PP028804	1599 bp	Germany	Red fox
		JF268516	395 bp	Slovakia	Red fox
		JF268519	396 bp	Slovakia	Red fox
		JF268513	396 bp	Slovakia	Red fox
		JF268514	396 bp	Slovakia	Red fox
		KF751230	352 bp	Netherlands	Red fox
Hap08	3	MH463513	373 bp	Slovakia	Red fox
		MN514041	415 bp	Poland	Red fox
		JF268505	396 bp	Slovakia	Red fox
Hap09	1	MN514040	415 bp	Poland	Red fox
Hap10	7	KX962372	396 bp	Germany	Gray wolf
		KX962360	360 bp	Germany	Gray wolf
		JF268506	396 bp	Slovakia	Red fox
		JF268508	396 bp	Slovakia	Red fox
		KF751228	352 bp	Netherlands	Red fox
		JF268502	396 bp	Slovakia	Red fox
		JF268507	396 bp	Slovakia	Red fox
Hap11	2	PV105579	420 bp	Türkiye	Red fox
		PV105575	419 bp	Türkiye	Red fox
Hap12	1	JF268522	396 bp	Slovakia	Red fox
Hap13	1	JF268523	396 bp	Slovakia	Red fox
Hap14	1	PV105574	420 bp	Türkiye	Red fox
Hap15	1	JF268515	396 bp	Slovakia	Red fox
Hap16	1	PV105576	418 bp	Türkiye	Red fox
Hap17	3	JF268520	396 bp	Slovakia	Red fox
		JF268524	396 bp	Slovakia	Red fox
		JF268525	396 bp	Slovakia	Red fox
Hap18	1	JF268504	396 bp	Slovakia	Red fox
Hap19	1	JF268517	396 bp	Slovakia	Red fox
Hap20	1	KF751227	352 bp	Netherlands	Red fox
Hap21	1	JF268509	396 bp	Slovakia	Red fox
Hap22	1	JF268503	396 bp	Slovakia	Red fox

Due to the uncertainties in the taxonomy of *Mesocestoides* spp, several *Mesocestoides* species have been added to the GenBank database as *Mesocestoides* sp. As a result of mt-*CO1* sequence analysis and BLAST search, 3 isolates were identified as *Mesocestoides* sp. If these specimens possess a distinct haplotype group compared to the species constituting the sister taxa, it implies that *Mesocestoides* populations in Türkiye may exhibit genetic differentiation. Nonetheless, extended DNA sequences, *ITS* gene sections, or analyses including additional specimens may be necessary to establish a conclusive differentiation at the species level.

The study of parasites in wild animals and the assessment of their interactions in different habitats is important to reveal the potential risks to humans by determining the risk of transmission between wild and domestic animal populations in different environments and helminths with zoonotic character [10].

Conclusions

In this study, a comprehensive analysis of the intestinal helminths of red foxes in Türkiye was performed and showed that they host a number of intestinal helminth species. The identified helminth species can cause infections in domestic dog populations and humans. Thus, it is important to take into account red fox populations when trying to manage these parasites in dogs, livestock, and humans. Some of the species identified in this study have zoonotic properties, which is important for control strategies to be developed in the region. Additionally, this is the initial record of *M. litteratus* in red foxes within Türkiye. When *Mesocestoides* spp. isolates were examined in the study, haplotypes specific to Türkiye were found. More detailed studies are needed to demonstrate the transmission patterns of red fox-transmitted helminths and their distribution in rural and especially urban areas and the existence of haplotypes.

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Author contributions
S.G.K methodology, wrote the main manuscript text. H.K.K Methodology, Editing. F.C. Methodology, Conceptualization. S.S. Writing, Editing, Supervision.

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Data availability
The datasets generated and/or analysed during the current study are available in the GenBank repository, [https://www.ncbi.nlm.nih.gov/]. (Acc. No. PP431387, PP434592, PP434593, PP434590, PP406895, PP406896, PP406897, PP406898, PP406899, PP406868, PP406869, PP406870)

Declarations

Ethics approval and consent to participate
For this work, ethics committee approval was obtained from Bingol University Animal Experiments Local Ethics Committee, numbered E-85680299-020-149608 and dated 14.03.2024.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have infuenced the work reported in the present study and its outcome.

Consent to participate
Not applicable.

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