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Morphological characterization and partial mitochondrial *cox1* sequencing of gastrointestinal nematodes recovered from dromedary camels in Kuwait

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Abstract

Information on gastrointestinal nematodes infecting dromedary camels in Kuwait remains limited. During January–December 2020, the gastrointestinal tracts of 290 slaughtered camels were examined. *Nematodirella cameli* was detected in 11 camels (3.79%; exact 95% confidence interval [CI], 1.91–6.69%), *Nematodirus spathiger* in two (0.69%; 95% CI, 0.08–2.47%), and *Parabronema skrjabini* in one (0.34%; 95% CI, 0.01–1.91%). *Nematodirella cameli* prevalence was 9.23% in females (6/65) and 2.22% in males (5/225; two-sided Fisher's exact P = 0.018). No statistically significant associations with age group or season were detected. These exploratory comparisons require caution because only 11 animals were positive. Morphological observations included cuticular ridge counts in *N. cameli*. Partial mitochondrial cox1 sequences were obtained from six *N. cameli* specimens and one specimen morphologically identified as *N. spathiger*. The *N. cameli* sequences showed the highest reported BLAST matches to Iranian *N. cameli* reference sequences. The specimen morphologically identified as *N. spathiger* showed only 91.01% sequence identity to the corresponding reported reference sequence, suggesting the need for further molecular verification. The findings provide baseline prevalence, morphological observations, and comparative sequence data for camels slaughtered in Kuwait.

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1. Introduction

The health and productivity of ruminants, including camels, is significantly affected by the gastrointestinal nematode infections (Sharifiyazdi et al. 2015; Alhaboubi et al. 2021). The presence of gastrointestinal helminths and other parasites have been documented earlier in the farm animals in Kuwait (Abdul-Salam and Farah 1988; Majeed et al. 2015), but the studies providing comprehensive information by integrating adult-worm morphology and molecular characterization in camels remains limited. Among the gastrointestinal nematodes of camels, *Nematodirella* species are the significant ones which are identified using features of the male bursa and spicules, the female reproductive system, and cuticular-ridge patterns. However, the species identification is generally complicated by the overlapping morphometric characteristics, which highlights the importance of standardized anatomical comparisons (Hoberg et al. 2005). Although, the configuration of the male spicule tips is useful for differentiation *Nematodirus* species also share morphological features with other species (Taylor et al. 2016). Therefore, the identification at molecular may complement the differentiation based on morphology, although interpretation depends on sequence quality and the availability of authenticated reference sequences.

Another important camel nematode is *Parabronema skrjabini*, an abomasal nematode characterized by anterior cuticular ornamentation. In addition to camels, it has been reported in other ruminants as well, and earlier morphological and molecular studies have provided a basis for its comparative identification (Yazd et al. 2025; Hasheminasab et al. 2016). Therefore, documenting these nematodes in camels slaughtered in Kuwait may contribute comparative information for subsequent surveillance, without demonstrating new parasite biology or the geographical source of infection. Therefore, the present study aimed to estimate the prevalence and describe the morphology of *Nematodirella cameli*, *Nematodirus spathiger*, and *Parabronema skrjabini* recovered from dromedary camels slaughtered in Kuwait. Furthermore, the morphological identification of *N. cameli* and *N. spathiger* was supplemented with partial mitochondrial *cox1* sequencing. The study was intended to provide baseline observations rather than establishing host preferences, seasonal transmission, or parasite origins.

2. Materials and Methods

2.1 Study design and sampling

During January–December 2020, the gastrointestinal tracts of 290 dromedary camels (*Camelus dromedarius*) slaughtered at Al-Jahra Slaughterhouse in northern Kuwait (Al-Jahra Governorate) and Al-Dhahar Slaughterhouse in southern Kuwait (Mubarak Al-Kabeer Governorate), were examined. The sample comprised 225 males and 65 females. Animals were categorized as 0–8 months (n = 104), >8 months–3 years (n = 118), and >3 years (n = 68). Age of the animals was estimated based on the information provided by the animal owners and the dentition assessment (Bello et al. 2013). Sampling months were classified as dry (April–November; n = 188) and wet (December–March; n = 102) seasons (Uddin et al. 2010). The camels used in this study were local-breed animals reared under an extensive grazing system. No individual dietary and husbandry histories were available. Body condition was assessed using the five-category scoring system of Kamili et al. (2006). The recorded numbers of animals with scores of 1, 2, 3, 4, and 5 were 1, 47, 99, 90, and 53, respectively. The study was approved by the Animal Ethics Committee of the Public Authority of Agriculture Affairs and Fish Resources (PAAF), Kuwait, and permission was obtained from the relevant authorities.

2.2 Worm recovery and light microscopy

After slaughter, the abomasum, small intestine, and large intestine were collected. The abomasal and intestinal contents were washed, the mucosa was scraped. The recovered contents were examined under a stereomicroscope for the presence of helminths. Specimens were preserved in 70% ethanol for molecular examination, cleared in lactophenol for light microscopy, or preserved in 80% ethanol for scanning electron microscopy. Prevalence was calculated at the host level, and worm counts per animal were used to describe infection intensity among infected hosts. Nematodes were identified using published morphological descriptions and identification keys (Sabatini et al. 2023; Melnychuk et al. 2021; Ivashkin 1978; Hoberg et al. 2005; Sazmand et al. 2019; Taylor et al. 2016). Specimens were photographed using a Leica EC3 camera connected to an Olympus BX50 microscope. Measurements retained in the morphometric summary are expressed in millimeters.

2.3 Scanning electron microscopy

Selected Nematodirella specimens preserved in 80% ethanol were examined by scanning electron microscopy at the Iran Polymer and Petrochemical Research Institute, Tehran, Iran. Specimens were mounted on SEM stubs and sputter-coated with gold for 30 s at approximately 70 mTorr. Scanning electron micrographs were obtained using a TESCAN VEGA scanning electron microscope to examine surface morphological features.

2.4 Molecular examination

2.4.1 DNA extraction and PCR amplification

Seven worms were sent to the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand for molecular examination. Six specimens were morphologically identified as *Nematodirella cameli* (N1F1, N2F1, N3F1, N4F1, N4F2, and N6F1) and one was identified as *Nematodirus spathiger* (N5F1). Specimens were washed with sterile distilled water and examined using an Olympus SZ51 stereomicroscope. Genomic DNA was extracted individually using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Partial mitochondrial cox1 was amplified using the JB3 and JB4.5 primers (Bowles et al. 1992; Table 1) in a T100 thermocycler (Bio-Rad, USA). The stock primer concentration was 50 μM and the concentration of each primer used was 2.5 μM.

Thermocycling consisted of initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 5 min. Final reaction volume of 30 μL, containing 15 μL of 2× i-Taq master mix (iNtRON Biotechnology, South Korea), 1.5 μL of of each primers, 2 μL of template DNA, and 10 μL of nuclease-free water. PCR products were visualized on a 1% agarose gel stained with SYBR Safe (Life Technologies, USA). The amplified product was purified using a Geneaid PCR Purification kit and sent to Macrogen (Seoul, South Korea) for Sanger sequencing with the same amplification primers. No amplification was reported in the negative controls. Quantitative DNA concentration and purity measurements and genomic-DNA integrity records were unavailable for verification. Inclusion and identity of a positive control could not be confirmed from the available records; visualization of PCR products does not establish the quality of the original genomic DNA.

2.4.2 Sequence comparison and phylogenetic analysis

Sanger sequencing electropherograms were checked in BioEdit 7.0, and sequences were aligned using Clustal X version 2.1 with Nematodirella, Nematodirus, and Trichostrongylus reference sequences obtained from GenBank. Maximum-likelihood phylogenetic analysis used IQ-TREE version 2.4.0 with the TPM2u+F+G4 substitution model and the analysis was based on an alignment of 14 sequences and 367 nucleotide positions (Fig. 13). Branch support was assessed using 1,000 SH-aLRT replicates and 1,000 ultrafast bootstrap replicates. The tree was rooted on *Trichostrongylus axei* (GQ888719.1) for display; *Trichostrongylus vitrinus* (GQ888711.1) was retained as an additional reference sequence. Pairwise uncorrected p-distances are reported as the percentage of differing nucleotides over the 367-base alignment. Haplotype summaries distinguish study specimens from reference sequences; haplotypes are distinct aligned sequence patterns rather than groups defined by the phylogenetic tree. These specimen-level summaries do not assume independent-host sampling.

2.5 Statistical analysis

Prevalence was calculated as the number of infected camels out of the total number examined and was expressed as a percentage. Exact binomial 95% confidence intervals were calculated using the Clopper–Pearson method. Associations of *Nematodirella cameli* infection with sex and season were examined using two-sided Fisher's exact tests. The Fisher–Freeman–Halton exact test was used for the three age groups. P-

Table 1. Primers used for partial *cox1* amplification

Gene	Primer	Primer sequence (5′ – 3′)	Reported product (bp)	Reference
cox1	JB3	TTTTTTGGGCATCCTGAGGTTTAT	443	Bowles et al. (1992)
	JB4.5	TAAAGAAAGAACATAATGAAAATG		

Table 2. ***Nematodirella cameli*** **prevalence by sex, age group, and season**

Parameter	Category	Negative (n)	Positive (n)	Examined (n)	Prevalence (%)	95% CI (%)	Exact-test P-value
Sex	Male	220	5	225	2.22	0.73–5.11	0.018
	Female	59	6	65	9.23	3.46–19.02	
Age group	0–8 months	101	3	104	2.88	0.60–8.20	0.730
	>8 months–3 years	112	6	118	5.08	1.89–10.74	
	>3 years	66	2	68	2.94	0.36–10.22	
Season	Dry	180	8	188	4.26	1.85–8.21	0.752
	Wet	99	3	102	2.94	0.61–8.36	
Overall		279	11	290	3.79	1.91–6.69	

Confidence intervals (CI) are exact binomial intervals calculated using the Clopper–Pearson method. P-values are from two-sided Fisher's exact tests for sex and season and the Fisher–Freeman–Halton exact test for age group

values <0.05 were considered statistically significant. Mean intensity was defined as the total number of recovered worms divided by the number of infected hosts; the range describes the minimum and maximum counts among infected hosts.

3. Results

The numbers of infected camels with body condition scores of 2, 3, 4, and 5 were 6, 4, 2, and 3, respectively. No infected camel with a score of 1 was observed.

3.1 *Nematodirella cameli*

Nematodirella cameli was detected in 11/290 camels (3.79%; 95% CI, 1.91–6.69%). The prevalence was higher in females (6/65, 9.23%) than in males (5/225, 2.22%; Fisher's exact test, P = 0.018). No statistically significant association was detected between age group and *N. cameli* infection (exact P = 0.730) or season (Fisher's exact test, P = 0.752; Table 2). The sex comparison is exploratory only because it was based on only 11 positive hosts. The reported overall mean intensity was 81.7 worms per infected host, with a range of 1–342 (Table 3). Cuticular-ridge counts varied with sex and anatomical region (Table 4).

3.1.1 Morphology

Males specimens were smaller and thinner than female ones. Body length ranged from 8 to 18 mm, body width from 0.217 to 0.298 mm, and head width from 0.045 to 0.064 mm. Esophagus length ranged from 0.332 to 0.702 mm. The caudal bursa measured 0.248–0.480 mm in width and had a double dorsal ray. Each distal stem bifurcated into two unequal branches, with the outer branch approximately twice as long as the inner branch. Prebursal papillae were also present. The spicules were brown, thin, and very long (11.1–13 mm), enclosed in a transparent sheath, and had spoon-shaped tips (Fig. 1–2).

Female specimen body length ranged from 15 to 33 mm. Body width measured at the posterior end of the esophagus ranged from 0.395 to 0.704 mm and head width ranged from 0.045 to 0.076 mm. Esophagus length ranged from 0.656 to 0.864 mm. The reported distance from the anterior end to the excretory pore was 0.0009–0.0018 mm, and the distance from the anterior end to the vulva was 6.378–13.582 mm. Tail length ranged from 0.159 to 0.372 mm. The tail had a

Table 3. Overall prevalence and worm intensity among infected camels

Species	Infected / examined	Prevalence % (95% CI)	Mean intensity	Range
N. cameli	11/290	3.79 (1.91–6.69)	81.7	1–342
*N. spathiger**	2/290	0.69 (0.08–2.47)	2.5	2–3
P. skrjabini	1/290	0.34 (0.01–1.91)	8	8

** Nematodirus spathiger was identified morphologically; the molecular assignment of specimen OQ632529 requires further verification*

Table 4. Cuticular-ridge counts at different anatomical levels in ***Nematodirella cameli***

Sex	Head region	Excretory pore	Mid body	Pre- vulva
Male	4	6-7	9	-
Female	4	7-10	10	16-21

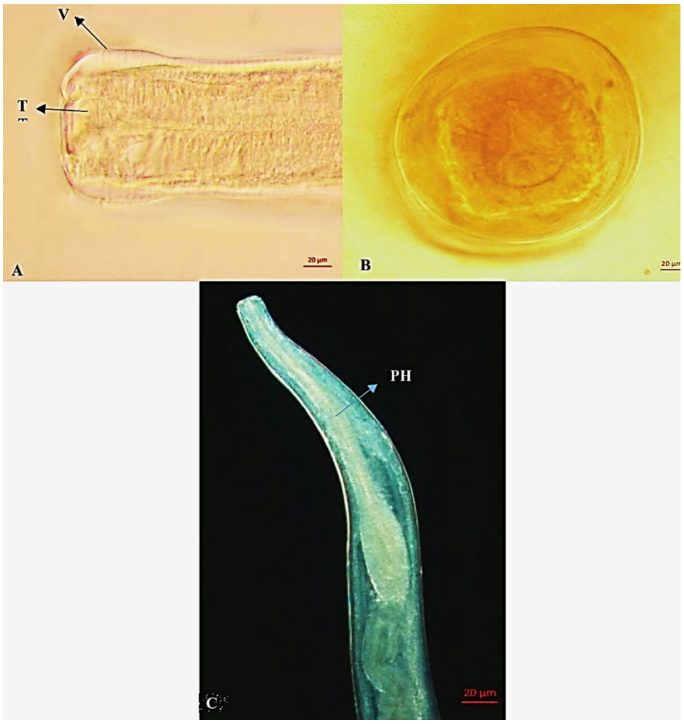


Fig. 1. Anterior region of Nematodirella cameli. (A) Anterior vesicular bulge. (B) Cross section through the head region. (C) Pharynx. V: vesicular bulge; Th: tooth; PH: pharynx

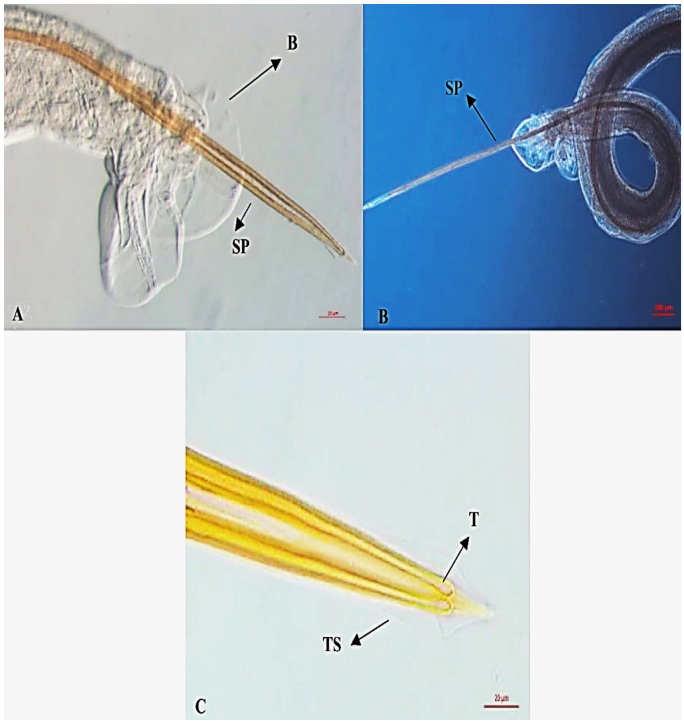


Fig. 2. Male Nematodirella cameli. (A) Bursa and spicules. (B) Long spicule. (C) Spicule tip and transparent sheath. B: bursa; SP: spicule; T: spicule tip; TS: transparent sheath

blunt end bearing a caudal spine. The eggs are large with a diameter equals to 0.193-0.239 (0.215 ± 0.023) mm x 0.095-0.123 (0.106 ± 0.14) mm. (Table 5; Figures 3–4). Cuticular-ridge counts differed between sexes and among anatomical regions, as observed using light and scanning electron microscopy (Table 4; Fig. 5).

3.2 *Nematodirus spathiger*

Two male camels aged 18 months and 3 years were infected (2/290; 0.69%; 95% CI, 0.08–2.47%). The reported mean intensity was 2.5 worms per infected host (range 2–3; Table 3).

3.2.1 Morphology

The worms had a relatively thin anterior region, a cephalic vesicle, and small cuticular alae. The male bursa had two prominent lateral lobes and a smaller divided dorsal lobe. The distal spicule tips were joined within a thin transparent membrane, forming a spatulate expansion. Female specimens showed a slit-like vulva with surrounding lips and a blunt posterior tail (Fig. 6–9). Male specimen body length ranged from 10 to 14 mm, body width from 0.180 to 0.221 mm, and spicule length from 1.150 to 1.205 mm. Female specimen body length ranged from 10 to 17 mm.

Table 5. Selected *Nematodirella cameli* morphometric ranges compared with published reference ranges

Character (mm)	Supplied reference ranges *	Current study ranges
Male body length	16–26	8–18
Male body width	0.10–0.16	0.217–0.298
Male esophagus length	0.60–1.06	0.332–0.702
Male spicule length	7.83–15.6	11.1–13
Female body length	23–24	15–33
Female body width	0.45–0.56	0.395–0.704
Female esophagus length	0.48–0.98	0.656–0.864
Female tail length	Not provided	0.159–0.372
Egg length × width	0.22–0.28 × 0.10–0.13	0.193–0.239 × 0.095–0.123

** Reference ranges are attributed to Ivashkin (1978)*

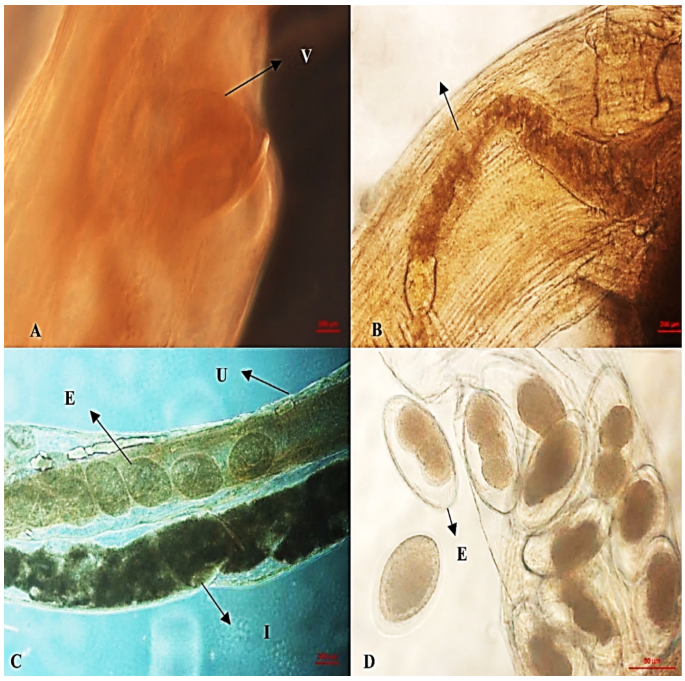


Fig. 3. *Female Nematodirella cameli. (A) Vulva. (B) Ovary. (C) Eggs within the uterus and the intestine. (D) Oval eggs. E: eggs; I: intestine; O: ovary; U: uterus; Vu: vulva*

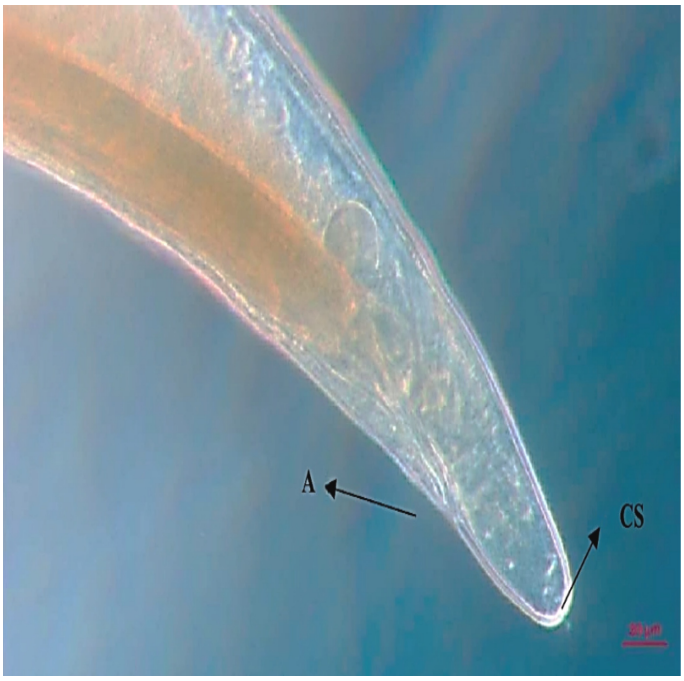


Fig. 4. *Female Nematodirella cameli tail showing the anus (A) and caudal spine (CS)*

3.3 *Parabronema skrjabini*

Eight adults female *Parabronema skrjabini* were recovered from the abomasum of one 4-year-old female camel examined in late June. Prevalence was 1/290 (0.34%; 95% CI, 0.01–1.91%), and intensity in the infected host was eight worms. No male *P. skrjabini* were recovered (Table 3).

3.3.1 Morphology

The body tapered at both ends and had transverse cuticular striations. The anterior end displayed six prominent horseshoe-shaped cuticular cordons. A narrow pharynx led to a short muscular and a longer glandular esophagus. Female body length ranged from 13 to 36 mm. The anus and anal papillae were visible near the caudal end, and eggs were oval, 0.7 × 0.018 mm (Fig. 10–11).

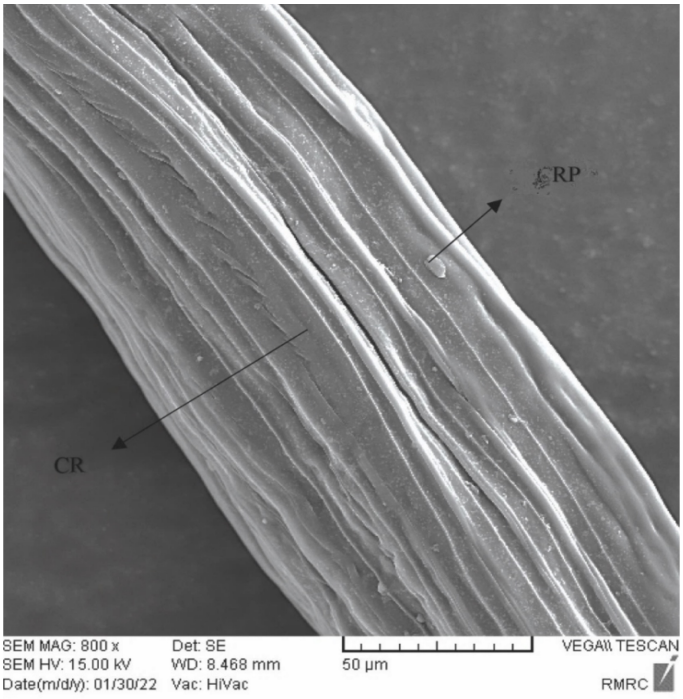


Fig. 5. *Scanning electron micrograph of the cervical region of Nematodirella cameli showing cuticular ridges (CR) and cervical papillae (CRP)*

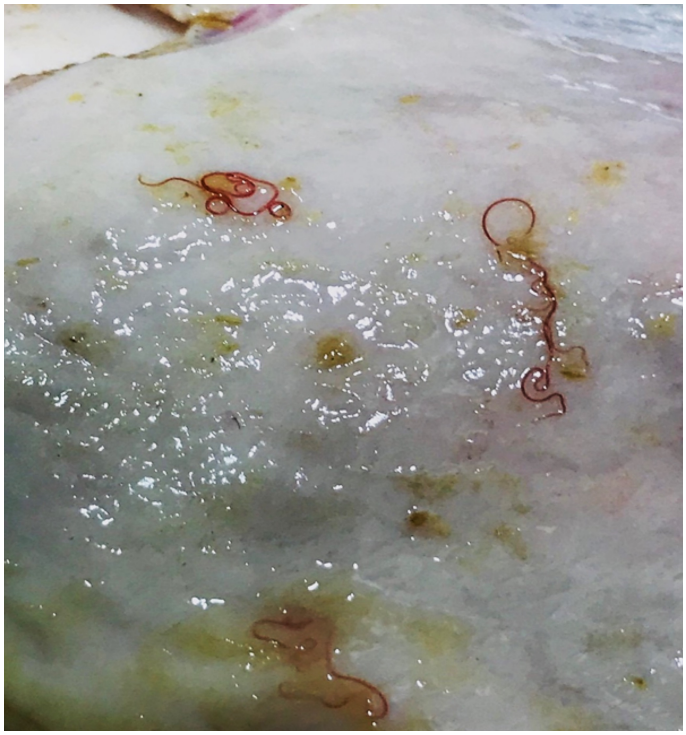


Fig. 6. *Nematodirus specimens attached to the mucosa of the small intestine*

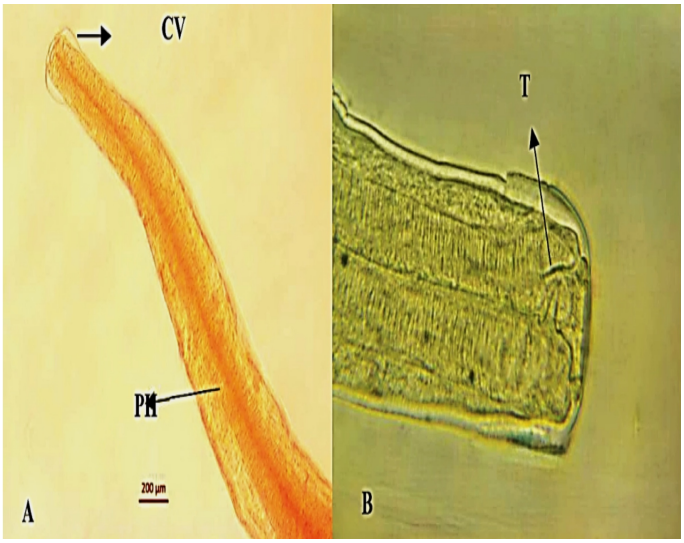


Fig. 7. *Anterior region of Nematodirus spathiger. (A) Cephalic vesicle (CV) and pharynx (PH). (B) Tooth (Th)*

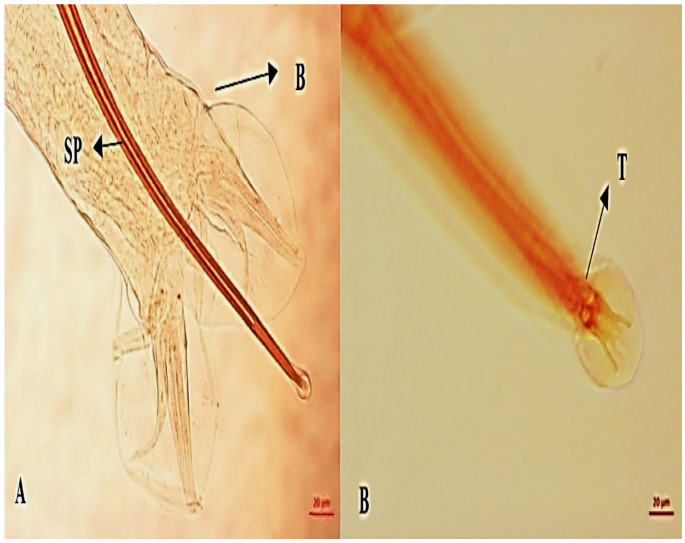


Fig. 8. *Male Nematodirus spathiger. (A) Bursa and spicules. (B) Spoon-shaped spicule tip. B: bursa; SP: spicule; T: spicule tip*

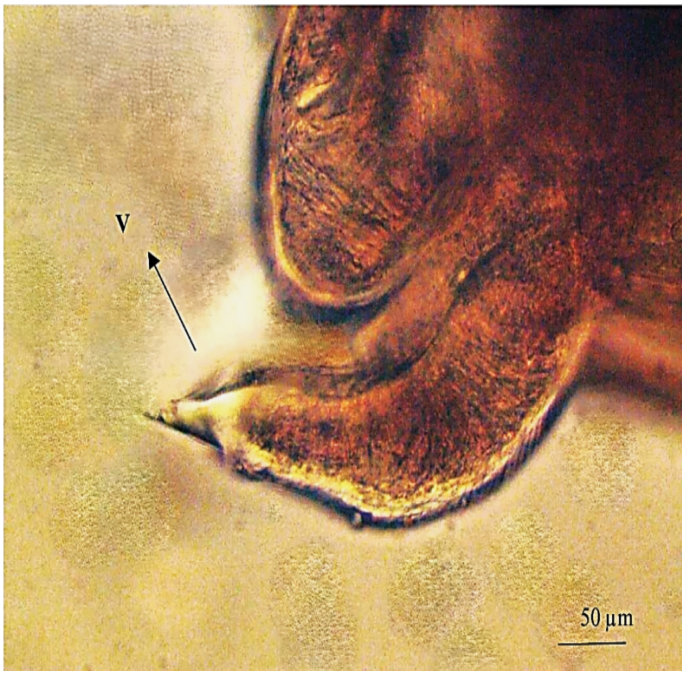


Fig. 9. *Female Nematodirus spathiger showing the vulva (Vu)*

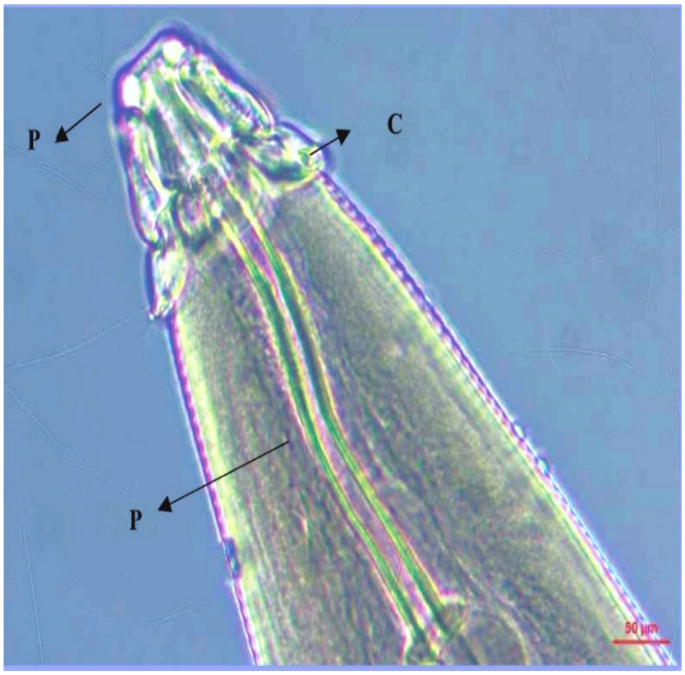


Fig. 10. *Anterior end of Parabronema skrjabini with a narrow pharynx. CO: cordons. PA: papillae. PH: pharynx*

3.4 Molecular observations

Partial *cox1* sequences were obtained from seven specimens: six identified as *Nematodirella cameli* (GenBank OQ632524–OQ632528 and OQ632530) and one identified morphologically as *Nematodirus spathiger* (OQ632529). Comparative sequence analysis used 367 unambiguous aligned base positions. The accession numbers of all reference sequences used in the phylogenetic analysis are given in Table 6. The reported BLAST comparisons showed that six Kuwait *N. cameli* sequences matched Iranian *N. cameli* reference sequences JX305976, JX305966, and JX305969 with 93% query coverage and 97.55–98.91% identity. Specimen OQ632529 matched the *N. spathiger* reference KF573749 at 100% query coverage and 91.01% identity (Table 7). The comparatively low identity observed for OQ632529 warrants further examination of its taxonomic assignment.

Among the six Kuwait sequences labelled *N. cameli*, six distinct sequence haplotypes and 16 segregating sites were observed over the 367-bp sequence fragment. Haplotype diversity (Hd) was 1.000, mean pairwise difference (k) was 6.200, and nucleotide diversity (π) was 0.01689 (1.689%). Pairwise nucleotide differences ranged from 3 to 9 bases among the six Kuwait sequences (uncorrected p-distance 0.82–2.45%; Table 8). These statistics describe six specimens but their independence at the host level has not been established. Comparison of the six Kuwait *N. cameli* sequences with the three Iranian reference sequences displayed 4–9 nucleotide differences (1.09–2.45%). All nine sequences were distinct haplotypes within the analyzed fragment (Table 9; Fig. 12). The labels H1–H9 are specific to this dataset and do

Table 6. Nematode species used in the COX1 phylogenetic analysis, with their hosts, localities, and GenBank accession numbers

Nematode species (specimen/strain)	Host	Locality	Accession number
Nematodirella cameli (N1)	*Camelus dromedarius*	Kuwait	OQ632524.1
Nematodirella cameli (N2)	*Camelus dromedarius*	Kuwait	OQ632525.1
Nematodirella cameli (N3)	*Camelus dromedarius*	Kuwait	OQ632526.1
Nematodirella cameli (N4_1F)	*Camelus dromedarius*	Kuwait	OQ632527.1
Nematodirella cameli (N4_2F)	*Camelus dromedarius*	Kuwait	OQ632528.1
Nematodirella cameli (N6)	*Camelus dromedarius*	Kuwait	OQ632530.1
Nematodirus spathiger (N5)	*Camelus dromedarius*	Kuwait	OQ632529.1
Nematodirella cameli (Nem-11)	*Camelus dromedarius*	Iran	JX305976.1
Nematodirella cameli (Nem-1)	*Camelus dromedarius*	Iran	JX305966.1
Nematodirella cameli (Nem-4)	*Camelus dromedarius*	Iran	JX305969.1
Nematodirus spathiger	Sheep	China	KF573749.1
Nematodirus oiratianus	Goat	China	KF573750.1
Trichostrongylus vitrinus	Not specified	Not specified	GQ888711.1
Trichostrongylus axei (outgroup)	Not specified	Not specified	GQ888719.1

not imply the worldwide novelty of these haplotypes. Specimen OQ632529 differed from KF573749 by 33 bases (8.99% uncorrected p-distance) and from KF573750 by 44 bases (11.99%). In the maximum-likelihood tree, the Kuwait *N. cameli* specimens grouped with the Iranian *N. cameli* references, while OQ632529 grouped with KF573749 (Fig. 13). Sequence similarity and tree placement do not establish infection origin.

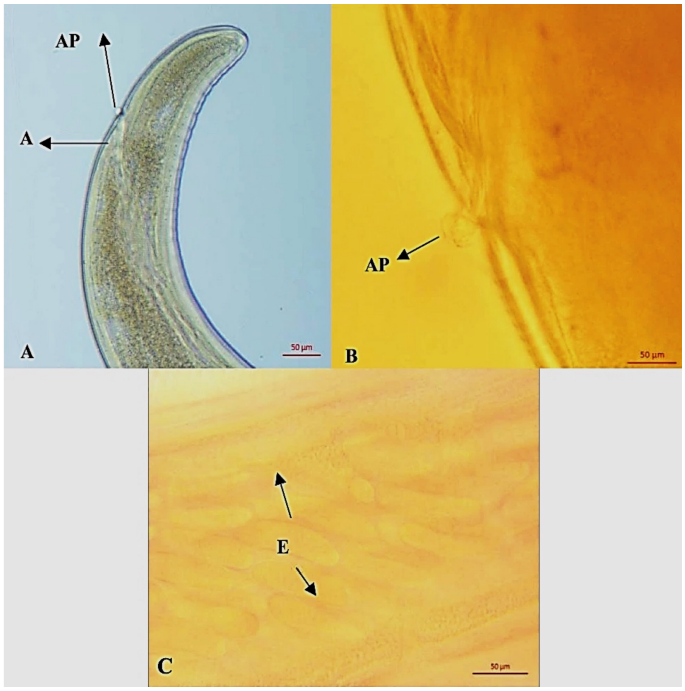


Fig. 11 A & B are showing the posterior part of the female including the anus and the anal papillae. C is showing the oval shape of the eggs. A: anus. AP: anal papillae. E: eggs

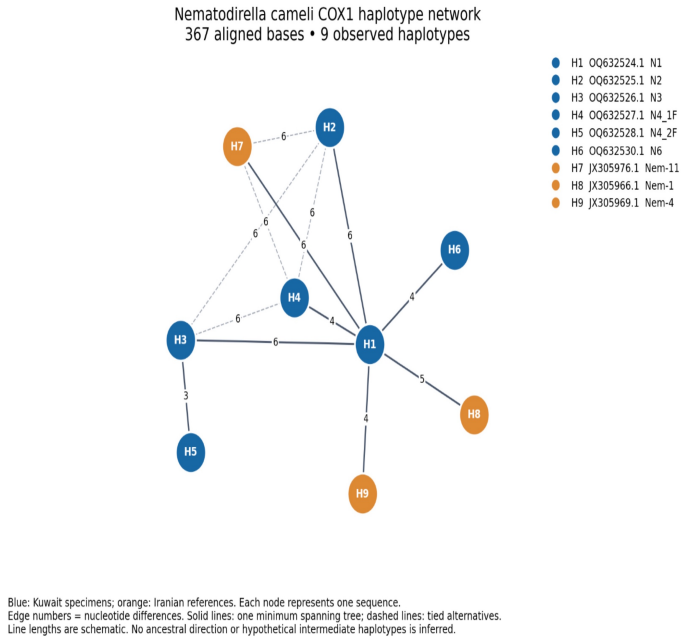


Fig. 12 Haplotype network of the Nematodirella cameli sequences. Nodes represent six Kuwait specimens and three Iranian references; each sequence represents a distinct haplotype. Equal node sizes reflect one sequence per haplotype. Edge labels show observed nucleotide differences; dashed connections represent alternatives rather than additional independent events. Haplotype identities are listed in Table 8

Table 7. Reported BLAST query coverage and identity for study specimens and selected GenBank references

Study accession / specimen	Reference accession / taxon	Coverage %	Identity %
OQ632524 / N1F1	JX305976 / *N. cameli*	93	98.37
OQ632524 / N1F1	JX305966 / *N. cameli*	93	98.64
OQ632524 / N1F1	JX305969 / *N. cameli*	93	98.91
OQ632525 / N2F1	JX305976 / *N. cameli*	93	98.37
OQ632525 / N2F1	JX305966 / *N. cameli*	93	97.55
OQ632525 / N2F1	JX305969 / *N. cameli*	93	97.82
OQ632526 / N3F1	JX305976 / *N. cameli*	93	97.82
OQ632526 / N3F1	JX305966 / *N. cameli*	93	97.55
OQ632526 / N3F1	JX305969 / *N. cameli*	93	97.82
OQ632527 / N4F1	JX305976 / *N. cameli*	93	98.37
OQ632527 / N4F1	JX305966 / *N. cameli*	93	98.09
OQ632527 / N4F1	JX305969 / *N. cameli*	93	98.37
OQ632528 / N4F2	JX305976 / *N. cameli*	93	97.55
OQ632528 / N4F2	JX305966 / *N. cameli*	93	97.82
OQ632528 / N4F2	JX305969 / *N. cameli*	93	97.55
OQ632530 / N6F1	JX305976 / *N. cameli*	93	97.82
OQ632530 / N6F1	JX305966 / *N. cameli*	93	98.37
OQ632530 / N6F1	JX305969 / *N. cameli*	93	98.37
OQ632529 / N5F1	KF573749 / *N. spathiger*	100	91.01

Query coverage and percentage identity are distinct BLAST measures. Values summarize comparisons between study sequences and references assigned to the corresponding species

4. Discussion

This study revealed the presence of *Nematodirella cameli*, *Nematodirus spathiger*, and *Parabronema skrjabini* in camels slaughtered in Kuwait, with observed prevalences of 3.79%, 0.69%, and 0.34%, respectively. Although, earlier studies have reported on the gastrointestinal helminths and parasites of farm animals in Kuwait (Abdul-Salam and Farah 1988; Majeed et al. 2015) present study integrated the host-level prevalence estimates and morphological observations with partial mitochondrial *cox1* sequence data for a more comprehensive description of these parasites. However, the geographical detection of these nematodes alone does not establish biological novelty or local transmission. The prevalence of *N. cameli* was higher than the 1.6% reported by Majidi Rad et al. (2015), while other Iranian investigations also documented this parasite in slaughtered camels (Borji et al. 2010; Anvari-Tafti et al. 2013). Variations in reported prevalence among studies may reflect differences in the populations examined, geographical location, grazing exposure, previous anthelmintic treatment, and sampling periods. Recovery and identification procedures may also influence the detection of parasites low in abundant. However, these possible explanations were not evaluated directly in the present study and should therefore not be regarded as demonstrated causes of the observed differences. The available literature provides relatively few directly comparable investigations of *N. cameli*, particularly studies combining morphological and molecular identification. Consequently, Iranian studies remain especially relevant to the interpretation of the findings of the present study in Kuwait (Sharifiyazdi et al. 2015; Sazmand and Joachim 2017). This limited evidence base should not be interpreted as proof of a restricted geographical distribution. Rather, the present findings emphasize the value of further species-level investigations in camel populations that have received little parasitological attention.

Morphological observations supported identification and provided additional information on the cuticular-ridge pattern of *N. cameli*. Previous studies have demonstrated the taxonomic value of cuticular-ridge patterns for differentiating *Nematodirella* species (Hoberg et al. 2005). The relatively low ridge counts observed in the present specimens therefore warrants further investigation. The molecular observations made in this study require cautious interpretation. In the

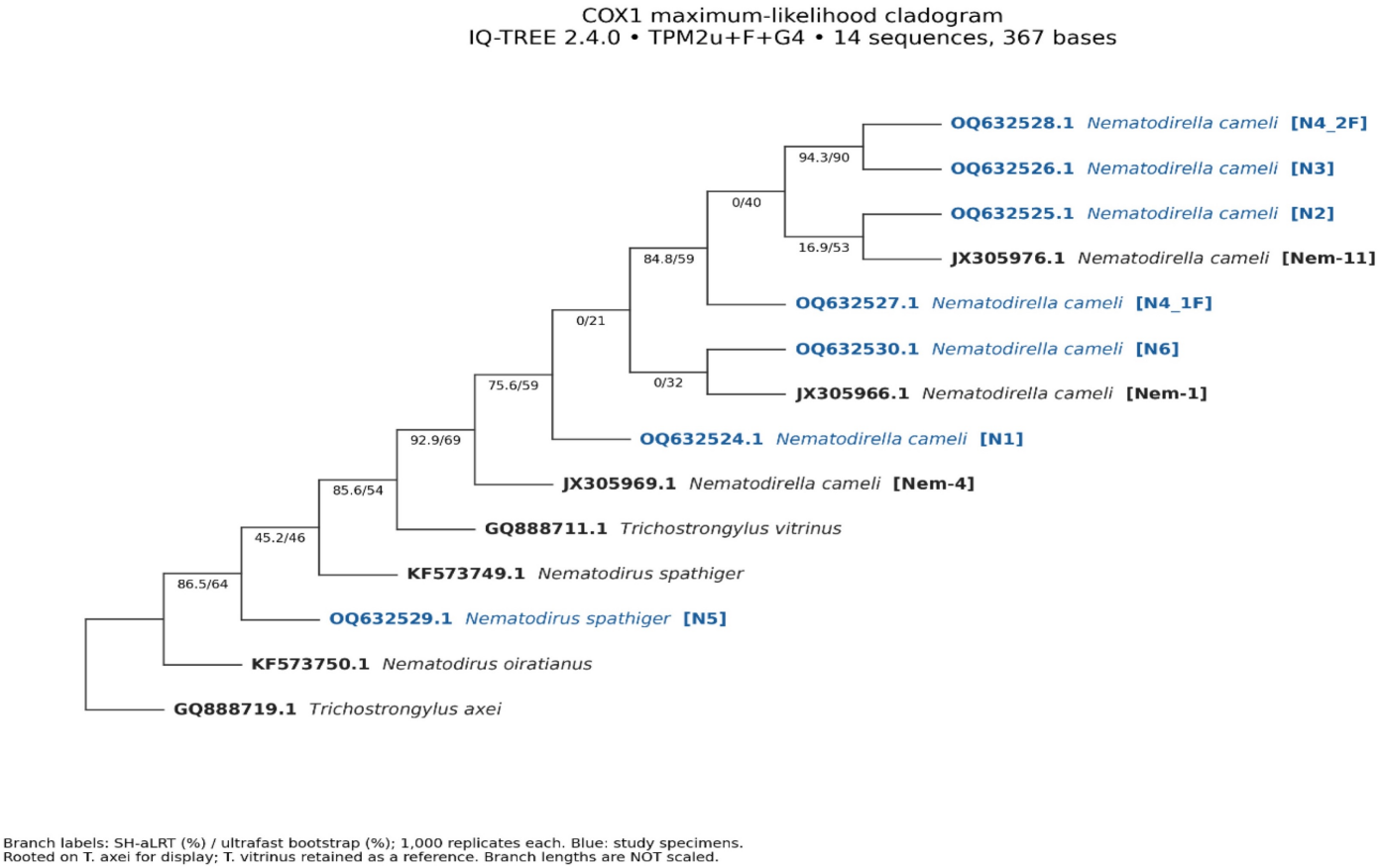


Fig. 13 *Maximum-likelihood cox1 cladogram of Nematodirella and Nematodirus specimens and reference sequences. The displayed analysis used IQ-TREE 2.4.0 and the TPM2u+F+G4 model, with 14 sequences and 367 nucleotide positions. Paired branch labels indicate SH-aLRT support (%) / ultrafast bootstrap support (%), with 1,000 replicates for each measure. Study specimens are shown in blue. The tree is rooted on Trichostrongylus axei (GQ888719.1) for display, with T. vitrinus (GQ888711.1) retained as a reference. Branch lengths are not scaled. Study-label mapping: N1, OQ632524; N2, OQ632525; N3, OQ632526; N4_1F, OQ632527; N4_2F, OQ632528; N5, OQ632529; N6, OQ632530*

maximum-likelihood phylogenetic tree, the six Kuwait *N. cameli* sequences grouped with the Iranian reference sequences, while several internal relationships had low branch support. The relatively short mitochondrial sequence fragment and limited reference sequence availability limits phylogenetic resolution. These observations are compatible with similarity to the available *N. cameli* references but cannot establish a geographical source of infection. However, the presence of multiple distinct *cox1* sequence patterns in this study within the small Kuwait sample provides valuable comparative material for future studies. Direct numerical comparison with previous studies requires consistent sequence regions, alignment lengths, sampling units, and analytical methods (Sharifiyazdi et al. 2015). Related

Table 8. Pairwise uncorrected p-distances among the seven study sequences

Study sequence	N1	N2	N3	N4 1F	N4 2F	N5	N6
OQ632524.1 *Nematodirella cameli* N1	0.00	1.63	1.63	1.09	1.91	12.81	1.09
OQ632525.1 *Nematodirella cameli* N2	1.63	0.00	1.63	1.63	1.91	12.53	2.18
OQ632526.1 *Nematodirella cameli* N3	1.63	1.63	0.00	1.63	0.82	12.81	2.18
OQ632527.1 *Nematodirella cameli* N4 1F	1.09	1.63	1.63	0.00	1.91	12.26	1.63
OQ632528.1 *Nematodirella cameli* N4 2F	1.91	1.91	0.82	1.91	0.00	12.53	2.45
OQ632529.1 *Nematodirus spathiger* N5	12.81	12.53	12.81	12.26	12.53	0.00	12.81
OQ632530.1 *Nematodirella cameli* N6	1.09	2.18	2.18	1.63	2.45	12.81	0.00

Values are percentages over the 367-base fragment; 1.63% corresponds to six differing positions. Specimen N5 was identified morphologically as Nematodirus spathiger and is included for comparison, not as an N. cameli haplotype

Table 9. Haplotype identities in the *Nematodirella cameli* comparison

Haplotype	Sequence	Group	Frequency
H1	OQ632524.1 *Nematodirella cameli* N1	Kuwait study specimen	1
H2	OQ632525.1 *Nematodirella cameli* N2	Kuwait study specimen	1
H3	OQ632526.1 *Nematodirella cameli* N3	Kuwait study specimen	1
H4	OQ632527.1 *Nematodirella cameli* N4 1F	Kuwait study specimen	1
H5	OQ632528.1 *Nematodirella cameli* N4 2F	Kuwait study specimen	1
H6	OQ632530.1 *Nematodirella cameli* N6	Kuwait study specimen	1
H7	JX305976.1 *Nematodirella cameli* Nem-11	Iranian reference	1
H8	JX305966.1 *Nematodirella cameli* Nem-1	Iranian reference	1
H9	JX305969.1 *Nematodirella cameli* Nem-4	Iranian reference	1

mitochondrial sequences could reflect shared ancestry or animal movement from several sources; they do not demonstrate introduction from Iran. Evaluating transmission routes would require broader geographical sampling and reliable animal-movement histories.

Only two camels were identified as infected with *Nematodirus spathiger*, giving a prevalence of 0.69%. Previous studies documented this species in young camels in Bahrain and investigated gastrointestinal nematodes in Nigerian camels (Abubakr et al. 2000; Abdullahi et al. 2024). An Egyptian study also identified *N. spathiger* among several Nematodirus species in camels (Abd El-Wahed 2005). Such comparisons should distinguish species-specific prevalence from the overall prevalence of the genus and recognize differences between fecal examinations, larval identification, and adult-worm recovery. Both infected camels were males of aged group >8 months – 3 years. The detection of only two infected camels is insufficient to establish sex or age predispositions, and the wide confidence interval illustrates the uncertainty in the prevalence estimate. Slaughterhouse sampling also limits extrapolation to the wider camel population. The specimen identified morphologically as *Nematodirus spathiger* showed only 91.01% BLAST identity to reference KF573749, with an uncorrected p-distance of 8.99%. Zhao et al. (2014) provided mitochondrial reference data for *N. spathiger* and *N. oiratianus*, but restricted reference sampling limits interpretation here. Evaluation of the original sequence chromatograms, comparison with additional authenticated reference sequences, and a longer mitochondrial region or an independent nuclear marker would strengthen taxonomic assessment. No internal transcribed spacer (ITS) sequence data were generated in this study.

The detection of *P. skrjabini* in only one sample out of 290 samples indicates a low observed prevalence. In contrast, the Iranian camels revealed the prevalence of 2.1% (Majidi Rad et al. 2015) and the other studies from Saudi Arabia and Iraq reported comparatively higher prevalences (Banaja and Ghandour 1994; Fadhil and Al-Zubaidi 2018). Such differences in prevalence rates cannot inevitably be attributed to climate or management because the studies examined different animal populations and exposure conditions. Unlike the other common intestinal nematodes, *P. skrjabini* has an indirect life cycle involving an insect intermediate host (Anderson 2000). A study from Inner Mongolia, China, investigated the infection and development of *P. skrjabini* in *Haematobia irritans*, illustrating the importance of vector ecology in transmission (Liu et al. 2021). Nevertheless, no intermediate hosts were examined in the present study. The single infection detected in late June therefore cannot establish seasonal transmission or identify the location and timing of acquisition. The presented of *P. skrjabini* in sheep and goats as reported from Iran and Indo-Pakistan indicates a broader host preference of the parasite (Yazd et al. 2025; Hasheminasab et al. 2016). Therefore, shared environments could provide opportunities for cross-transmission, but the presence of camels and other ruminants in the same farming areas does not establish this cross-transmission. Demonstration of cross-transmission would require investigation of the different hosts and intermediate hosts, supported by comparative morphological and molecular parasite identification.

5. Conclusions

Nematodirella cameli, *Nematodirus spathiger*, and *Parabronema skrjabini* were recovered from camels slaughtered in Kuwait. The prevalence estimates and morphological observations provide baseline information, supplemented by partial mitochondrial *cox1* sequence data for the first two taxa. The low number of infected hosts limits epidemiological inference, and the sequence assignment of the specimen identified as *N. spathiger* requires additional verification. Larger studies with documented animal histories and independent molecular markers are needed to assess distribution and transmission.

Declarations

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Conflict of interest: The authors declare no conflicts of interest

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Ethics approval: The study was approved by the Animal Ethics Committee of the Public Authority of Agriculture Affairs and Fish Resources (PAAF), Kuwait, and permission was obtained from the relevant authorities

Data availability statement: The *cox1* sequences are deposited in

GenBank under accession numbers OQ632524–OQ632530. Summary observations are presented in the article

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