Original Article

Occurrence and Morpho-molecular Investigation of Equine Large Redworms (*Strongylus* spp.) in Iraq

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ABSTRACT

Background: Members of the subfamily Strongylinae are among the most pathogenic gastrointestinal nematodes infecting equids. They can cause severe complications, such as aneurysm formation and thrombosis within the circulatory system, particularly in the cranial mesenteric artery, which may result in fatal outcomes.

Objectives: Due to the limited number of post-mortem investigations, this study aimed to detect natural infection and identify species of the subfamily Strongylinae infecting local donkeys and horses in Baghdad, Iraq.

Methods: Adult worms recovered from infected animals were examined using morphological characterisation and scanning electron microscopy (SEM). Molecular identification was performed through amplification and sequencing of the internal transcribed spacer 2 (ITS-2) region of rDNA, followed by phylogenetic analysis.

Results: The overall prevalence rate of infection was 32.3%. Morphological examination identified three species: *Strongylus vulgaris*, *Strongylus equinus*, and *Triodontophorus serratus*, with *S. vulgaris* being the predominant species. Phylogenetic analysis of ITS-2 sequences revealed that *S. vulgaris* isolates from the present study clustered within the same genetic lineage as isolates previously reported from Egypt and Germany, while sequence comparison demonstrated a high degree of similarity to isolates from Iran and Turkey. Partial sequences of *S. vulgaris* generated in this study were submitted to GenBank under accession numbers PX442251–PX442254.

Conclusion: This study represents the first report from Baghdad, Iraq, describing the diagnosis, morphological characterisation, SEM-based identification of adult worms, and genetic characterisation of *S. vulgaris* recovered from naturally infected equids.

Keywords: Horses, *Equus asinus*, Strongylinae, SEM, ITS2

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Introduction

More than 72% of the global horse population are mostly used as working animals in many nations. The family Equidae contains horses, donkeys (including hybrids, such as the mule), and zebras are known as "equines". These animals are challenged with diverse parasite species in terms of life cycles, pathogenicity, epidemiology, and drugs used for the prevention of parasites' infestations (Abo-Aziza, 2017; Mathewos et al., 2021; Alani & Yousif, 2023). Within the phylum Nematoda, the family Strongylidae includes the subfamily Strongylinae, which comprises five different genera (Lichtenfels et al., 1998). Infections with *Strongylus vulgaris* are generally recognized as the leading cause of morbidity and mortality among parasitic nematode infections in horses (Gonzales-Viera et al., 2019). Other *Strongylus* spp., such as *Strongylus edentatus*, also have a greater impact on horses then species of the genus *Triodontophorus* (Raue et al., 2017; Camino et al., 2019).

In Iraq, horse and donkey populations are projected to decrease (Alali et al., 2022). Epidemiological surveys have shown that horses in different parts of the world are exposed to many parasitic outbreaks, particularly those affecting the digestive system (Chapman et al., 2001, 2003) Helminthes include a large number of species. Although the effects of these worms vary according to their sex, type, and size, they are generally considered a risk to animal health and vitality and may lead to death (Hutchens et al., 1999). The morphological stability of these parasites across such large geological epochs suggests that they have highly conserved genetic characteristics. This implies that molecular studies of these parasite species can provide basic insights into their biology and evolutionary relationships, thereby providing a solid basis for diagnostic and control measures. Their conserved morphology over thousands of years is indicative of a successful parasitic strategy and an ongoing threat to equine health (Lyons et al., 2006).

Locally, most studies have relied on fecal examinations because anatomical investigation of the digestive tract is difficult to conduct in the absence of horse slaughterhouses, unlike in many European countries (Alani et al., 1992, Alali et al., 2022). Given the limited number of postmortem studies, this study aimed to morphologically identify *Strongylus* spp. recovered from the large intestines of postmortem donkeys and horses and to confirm the identified species through molecular analysis.

Materials and Methods

Ethical approval

This research and sampling were conducted in accordance with the local Animal Care and Use Committee (ACUC) requirements. The ACUC at the University of Baghdad's College of Veterinary Medicine authorized this study on March 20, 2025. No experiments were conducted on live animals.

Parasites collection

A postmortem study was conducted from December 1, 2024, through the end of June 2025. The study examined 65 slaughtered animals at Alzawra Zoo (Baghdad, Iraq): 55 donkeys and 10 horses. Of these animals, 34 were male and 31 were female. After postmortem examination, the intestines were thoroughly examined, and worms were collected, washed with phosphate-buffered saline (PBS; pH 7.3), and preserved in 70% alcohol before being transported to the parasitology laboratory.

Morphological study

Sample preparation

The collected nematode worms cleared using a modified lactophenol stain (Attia et al., 2018). Worms were identified according to their morphological characteristics (Lichtenfels et al., 2008).

Scanning electron microscope (SEM) identification

The collected *Strongylus* spp. worms were washed several times with saline. Living nematodes were killed in modified lactophenol. Adult worms were dehydrated in an ascending ethanol series. The specimens were mounted on stubs or holders using conductive adhesive and fully dried before SEM analysis. The SEM (SEM; INSPECT™ S50) was equipped with a high-vacuum chamber, an electron column, a specimen stage, a detector, and a computer-controlled imaging unit with default software. Vacuum generation was supported by an Edward PV25Mk (Crawley, West Sussex, UK), which maintained the required low-pressure environment during sample examination. Magnifications ranged from 200× to 500× (AbdElKader et al., 2021; El-Gameel et al., 2022).

Molecular characterizations of *S. vulgaris*

DNA extraction

Five adult worms preserved at - 20 ˚C were used for DNA extraction with the gSYNCTM kit (Geneaid Biotech Ltd., Germany), following the manufacturer's instructions.

DNA amplification

For polymerase chain reaction (PCR) amplification of the ITS2 gene of *S. vulgaris*, two primers were used: the forward primer 5′-GTATACATTAAATTGTGTCCCCCATTCTAG-3′and the reverse primer 5′-TTGCAAATATCATTAG-ATTTGATTCTTCCG-3′ (Tydén et al., 2019). A thermal cycler (Nexus Gradient; Eppendorf, Germany) was used to conduct the amplification reaction. The PCR procedure consisted of 15 minutes at 95 ˚C, followed by 40 cycles of 94 ˚C for 15 seconds, 55 ˚C for 30 seconds, and 72 ˚C for 30 seconds. The PCR procedure consisted of. Melt-curve analysis was also performed to ensure the specificity of the PCR results. The resulting amplicons were confirmed on a 1.5% agarose gel using a SiZer 100-bp DNA marker as a molecular-weight marker. Following electroelution, the gels were stained with 1 µL of RedSafe (AgaPure, Spain) and UV-transilluminated to reveal the DNA bands.

Sequencing of PCR products and phylogenetic analysis

Five PCR products were sent for sequencing. The resulting sequences were uploaded to NCBI GenBank to obtain accession numbers. Using BLAST (Basic Local Alignment Search Tool), the isolates' nucleotide sequences were matched with reference sequences in the NCBI GenBank databases (Altschul et al., 1990). The numbers in parentheses indicate the evolutionary distances calculated using the maximum composite likelihood method. The phylogenetic tree was built to scale, and MEGA 12 was used to quantify branch lengths in terms of the number of substitutions per site (Kumar et al., 2024).

Results

Adult worms of three strongyle species were collected from the large intestines of the study animals, which had a total infection rate of 32.3% (21/65). Male slaughtered had a higher infection rate than female animals, at 35.29% and 29.03%, respectively, with no significant differences (P≥0.05) (Table 1). The sample size reflects the number of available field cases during the study period. It was recognized that the imbalance between groups may influence statistical inference. Approximately 80 adult strongylid worms were collected from the examined cadavers. Both single- and mixed-species infections were observed. Mixed infections with *S. vulgaris*, *Strongylus equinus*, and *Triodontophorus serratus* occurred in some animals, whereas others were infected with only one species (*S. vulgaris*).

The results differed from those of a study in Egypt, which reported an infection rate of 15.85% (AbouLaila et al., 2020). Diagnosis of Strongyle worm infection usually depends on identifying eggs using traditional methods; however, because the eggs are morphologically similar, species identification is based on larval culture (Ismail et al., 2016). Studies in Turkey and Egypt have also reported parasite infection prevalence ranging from 2.1% to 95.9% in draught and workhorses and from 77.3% to 100% in donkeys (Uslu & Guçlu, 2007; Attia et al., 2018).

In Asian countries, including China, infection rates of common roundworms reached 88.2%, while the genus *Triodontophorus* had an infection rate of 8.8% (Bu et al., 2009). Most studies in Ethiopia were conducted using microscopic examination of fecal samples (Ayele, 2010; Asefa et al., 2011). Similarly, during this period, infec-

Table 1. Total Infection rate of *Strongylus* spp. in slaughtered donkeys and horses

Sex	No.	No. (%)
	Total	Infected Animals
Male	34	12(35.29)
Female	31	9(29.03)
Total	65	21(32.3)
Chi-square (P)	NS (0.5737) 0.8863	

NS: Non-significant.

tions were also identified through microscopy in India, Iran, Sudan, and Yemen (Matto et al., 2015; Tavassoli et al., 2016; Braima et al., 2018).

Regarding animal age, the infection rate in the first age group (less than one year) was 41.67%, while the rate in older animals was 30.19%, with a significant difference (P≤ 0.05) (Table 2). Previous necropsy studies have reported adult worm infections in donkeys in several countries and regions, including Iran, South Africa, Giza, and Upper Egypt (Borji et al., 2014; Abo-Aziza et al., 2017; Muktar et al., 2017; Attia et al., 2018).

Regarding the distribution of infection during the six-month investigation, December 2024 had the highest infection rate, at 50% (5/10). Lower infection rates were reported in January and February, at 36.36% (4/11) and 33.33% (4/12), respectively. The lowest rates were reported in March and April, at 30% (2/10) and 25% (3/12), respectively, with no statistically significant differences (P≤0.05) (Table 3).

The larvae of these worms need a prepatent period exceeding six months to mature into adults inside an animal's intestines. For this reason, infective larvae ingested by the animals during the last rainy season would not be expected to appear in large numbers in the cecum and colon (the large intestine) before May. Furthermore, the decreasing number of adult worms observed in the gut from January to April indicates that older worms were dying and being expelled from the body faster than new larvae could mature and replace them.

Morphological results

The collected *Strongylinae* worms were attached to the intestinal mucosa of the large intestines of the examined animals (Figure 1).

During the initial microscopic examination, the collected species of the subfamily Strongylinae were identified as *S. vulgaris* and *S. equinus* Müller, 1784 based on the morphology of the buccal capsule (Figures 2, 3, 4, and 5).

Table 2. Infection rates of *Strongylus* spp. in slaughtered donkeys and horses by age

Age (y)	No.	No. (%)
	Total	Infected Animals
<1	12	5(41.67)
1-3	53	16(30.19)
Total	65	21(32.31)
Chi-square (P)	(0.04177) 5.029*	

*(P≤0.05).

Table 3. Infection rates of *Strongylus* spp. in slaughtered donkeys and horses during the study months

Month	No.	No. (%)
	Total	Infected Animals
December 2024	10	5(50)
January 2025	11	4(36.36)
February 2025	12	4(33.33)
March 2025	10	3(30)
April 2025	10	2(20)
May 2025	12	3(25)
Chi-square (P)	(0.3551) 6.942*	

*(P≤0.05).



Figure 1. Macroscopic view of the large intestines, which contained relatively long, round, dark reddish worms attached to the mucosa

Another collected *Strongylidae* species, identified as *T. serratus*) (Looss, 1900), was also found in this study. It is a medium-sized strongyle with a smaller buccal capsule. The capsule is spheroid and has four divided teeth at its base (Figure 6).

The female tail is elongated and pointed. It should be noted that the male's dorsal bursal ray is very short (Figures 7 and 8).

Ultramorphological identification of the adult worms

A sample of *S. vulgaris* and *Tridentophuris serratus* underwent SEM processing. Micrographs at magnifications of 250–500× show the anterior end, including the large, coarse buccal capsule, and the female's slender, pointed posterior end. A scanning electron micrograph of the posterior end of the female

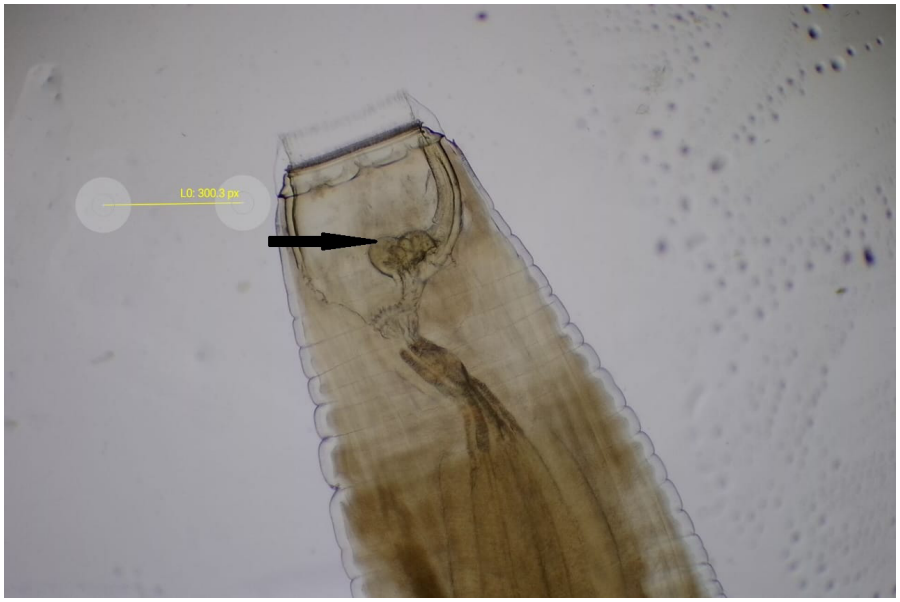


Figure 2. *S. vulgaris*: A bilobed dorsal tooth is visible (black arrow): The tooth's lobes are smooth, rounded, and tall; they extend over half the depth of the buccal cavity and are located anteriorly, near the center of the esophagus (magnification: ×10).

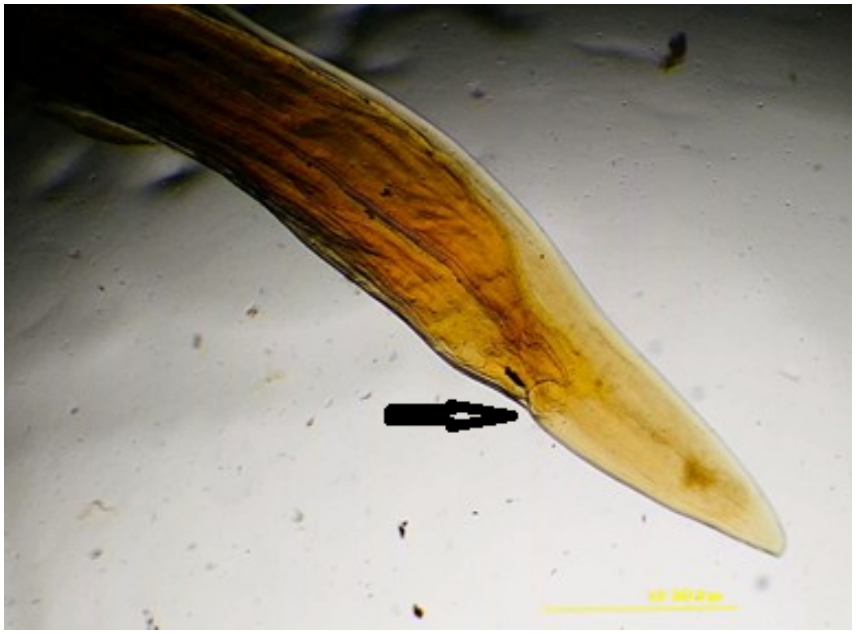


Figure 3. Posterior end of a female *S. vulgaris*, with the anal pore marked by black arrow, viewed under a light microscope (µm=px; [X10])

S. vulgaris also reveals the anal pore and the pointed, smooth genital cones. The posterior end of the copulatory bursa and the dorsal lobe of the *T. serratus* holotype male were triangular, with noticeable dorsal appendages (Figures 9, 10, and 11).

Molecular results

Five PCR products were successfully generated. However, only four specimens yielded clear, readable sequencing results. Their partial ITS2 sequences aligned with corresponding data available in GenBank. The

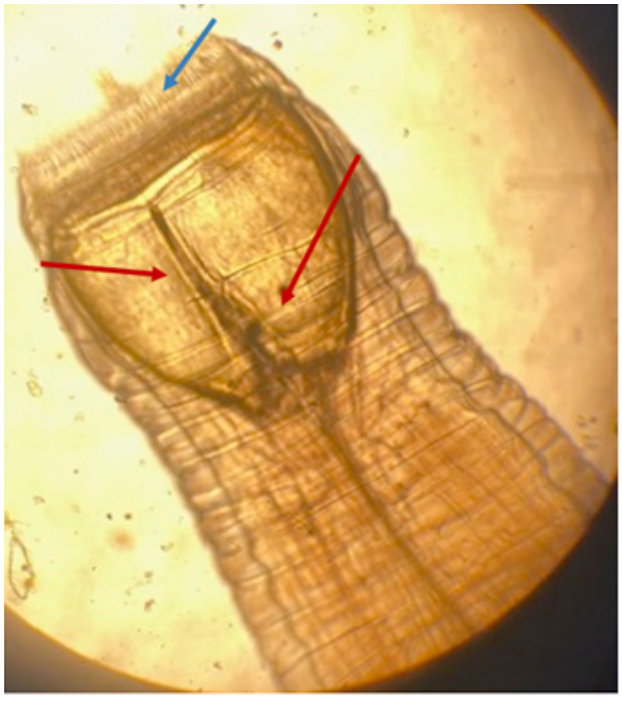


Figure 4. *S. equinus*: The anterior end shows the external leaf crown (blue arrow). The ventral view of the buccal–esophageal region shows the tooth structure and dorsal gutter, indicated by red arrows (magnification: ×10)

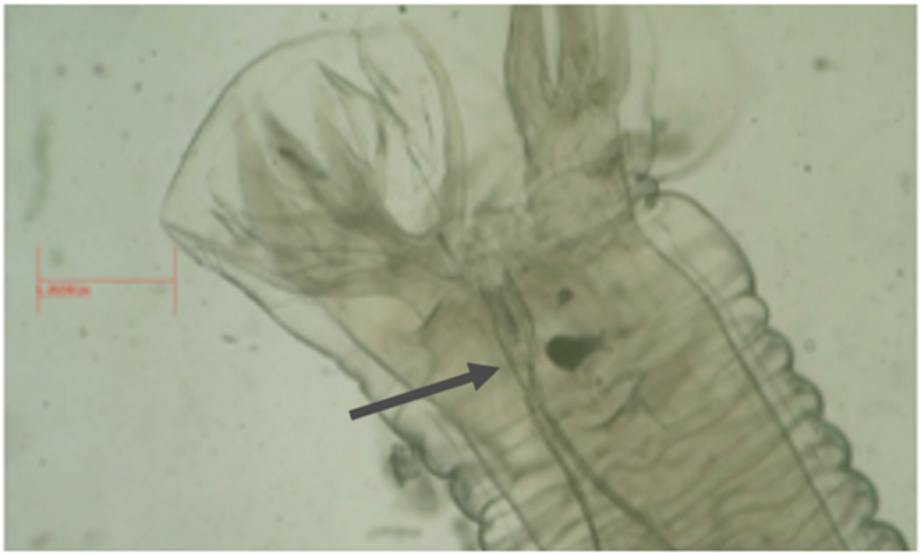


Figure 5. Lateral view of the male tail of *S. equinus*, showing the fused spicule tips (black arrow) (magnification: ×40)

nucleotide sequences of the isolates were compared with reference sequences in the NCBI GenBank database. The BLAST results showed high identity and similarity to the corresponding available data. The partial ITS2 sequence data for *S. vulgaris* were submitted to GenBank, and accession numbers were obtained (PX442251, PX442252, PX442253, PX442254) The partial ITS2 sequence data for *S. vulgaris* were submitted to GenBank, and accession numbers were obtained (PX442251, PX442252, PX442253, PX442254). No data for *S. vulgaris* from Iraq were available for comparison. Only three isolates of *S. equinus* were found in GenBank, and these partial sequences were obtained from a horse fecal sample. The isolates (PQ900954-PQ900956) showed high identity to isolates from Australia (97% to 100%) and to a Chinese isolate (94%) (Jasim et al., 2026).

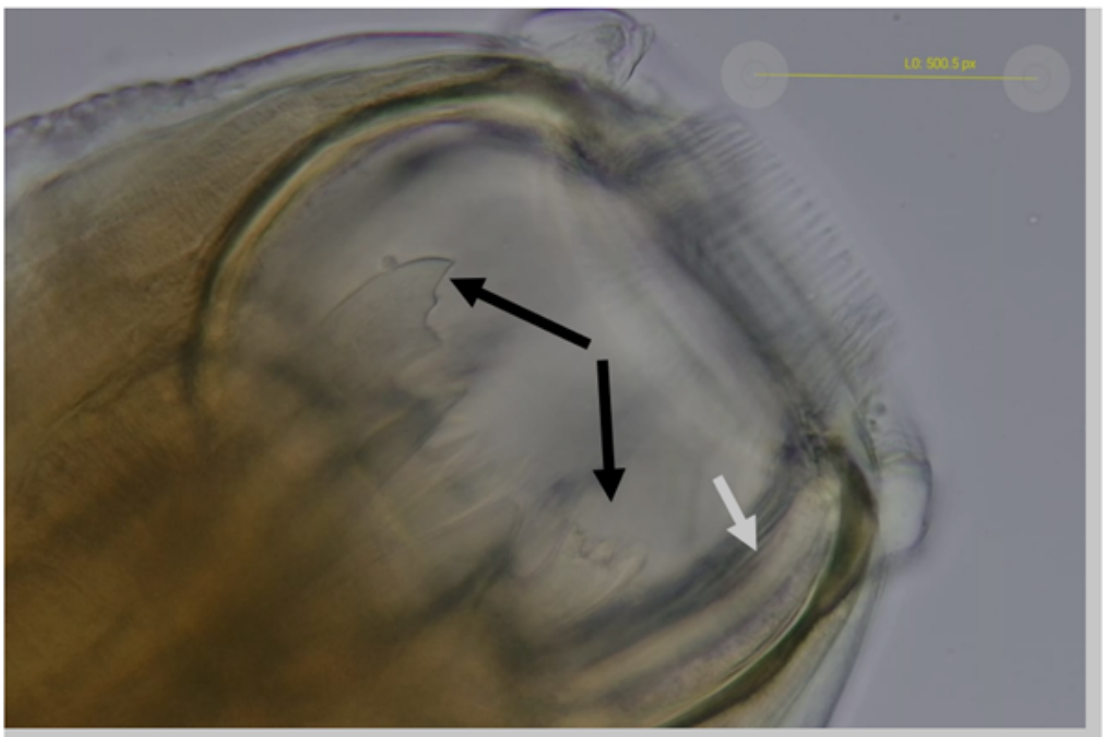


Figure 6. Anterior end of *T. serratus*, showing the inverted teeth (black arrow) in the buccal capsule and the dorsal gutter (white arrow) (magnification: ×40)

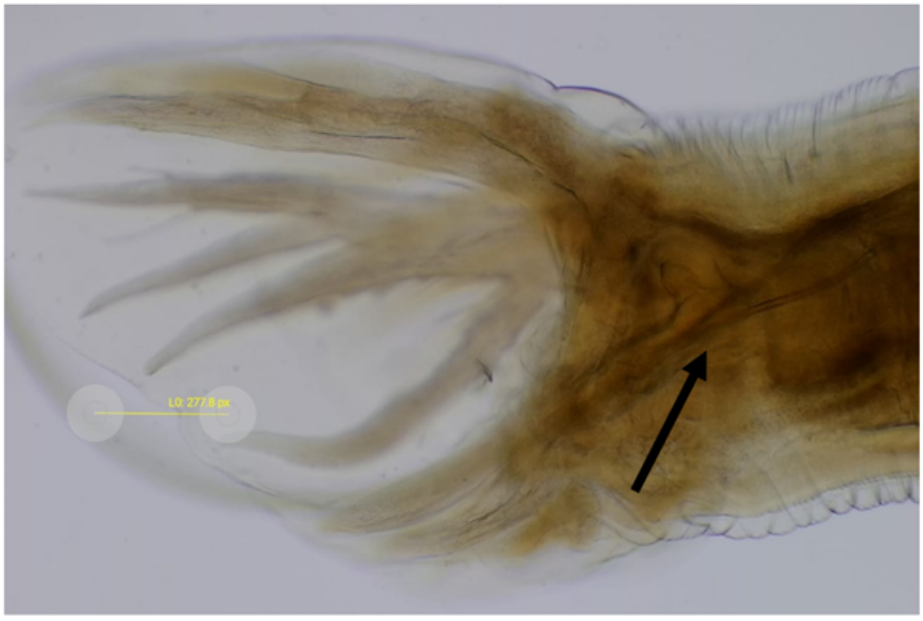


Figure 7. Male tail and spicule tips of *T. serratus* (black arrow) (magnification: ×40)

The phylogenetic tree was constructed using a data set containing 20 isolates. The distances between branches (indicated by parentheses in the tree) were estimated using the maximum composite likelihood method. The four Iraqi isolates clustered together in a closely related monophyletic clade with isolates from Turky, Iran, Germany, and Egypt. The study isolations were highly identical to one another. The clades were distinctly out outgrouped by *Haemonchus contortus* isolates reported in Thailand (Figure 12).

Equine strongylids form a multispecies complex that has not been fully characterized, and information regarding its taxonomic classification and species diversity remains limited. Sequencing the ITS2 region has traditionally been considered a useful starting point, although it does not necessarily provide a complete molecular resolution. Typically, either ITS1 or ITS2 can discriminate among species, but using both regions would significantly increase discriminatory power. This molecular marker is commonly used as a diagnostic tool to identify *S. vulgaris* and related species in fecal samples through

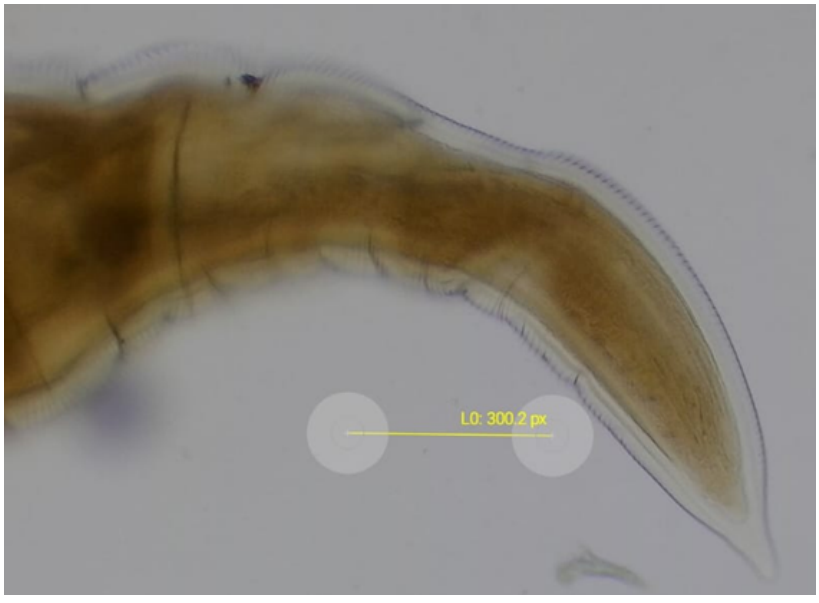


Figure 8. *T. serratus*: Female tail with a sharp point (magnification: ×40)

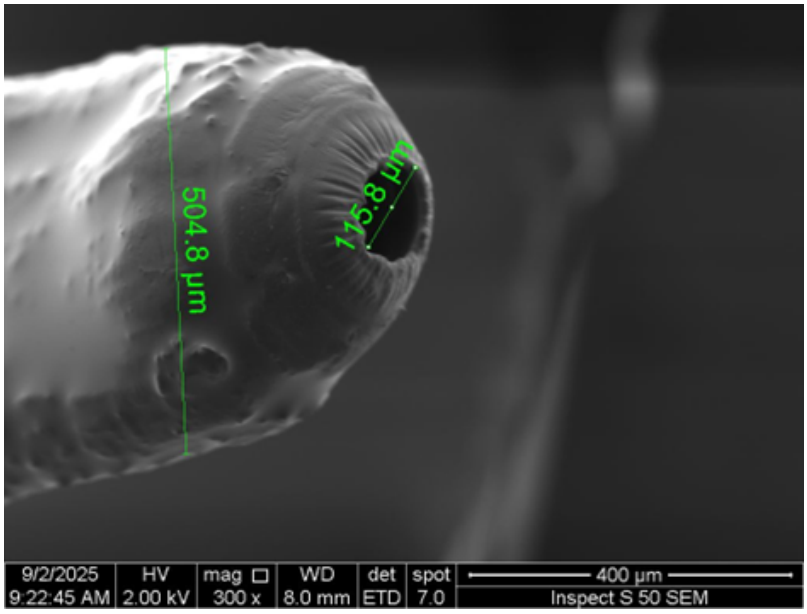


Figure 9. Scanning electron micrograph of the anterior end of *S. vulgaris*, showing the thick, wide buccal capsule and leaf-crown, tooth-like structures

PCR and sequencing. Strongylus infection is usually diagnosed by identifying eggs passed in the feces of infected equines. Other advanced diagnostic methods include larval culture followed by microscopic examination or DNA-based methods (Hasson, 2014; AbouLaila et al., 2019; Poissant et al., 2021).

The present study was limited to postmortem and was not designed as a cross-sectional or epidemiological survey; rather, it was intended as a preliminary investigation to document limited occurrence.

Discussion

In a previous Iraqi study conducted in Baquba City, *Strongylus* spp. infection was reported at a rate of 44.2%. The results also showed that March had the highest monthly prevalence, at 29.9%, while December had the lowest monthly prevalence, at 14.3% (Hasson, 2014). Infection rates are lower during the cold winter months than during dry summers, and researchers have noted severe outbreaks of parasitic diseases in years with mild winters and more humid summers (English, 1979).

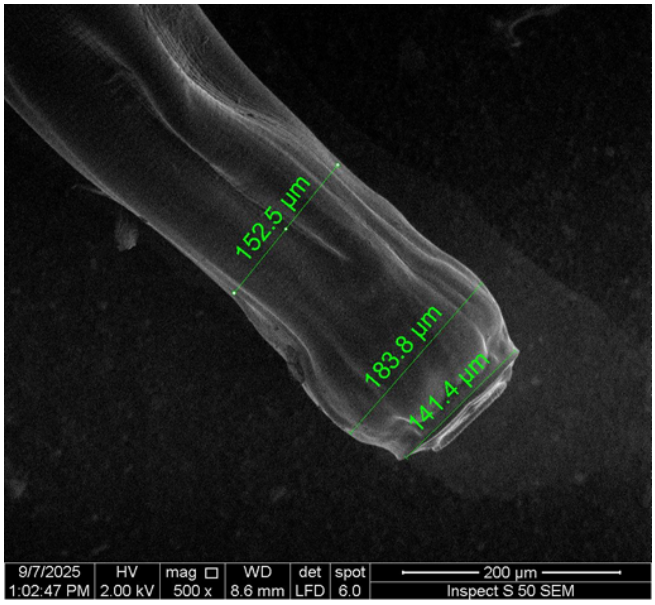


Figure 10. Scanning electron micrograph of the anterior end of *T. serratus*, showing the buccal capsule structures



Figure 11. Scanning electron micrograph of the posterior end of *T. serratus*, showing the bursal structure

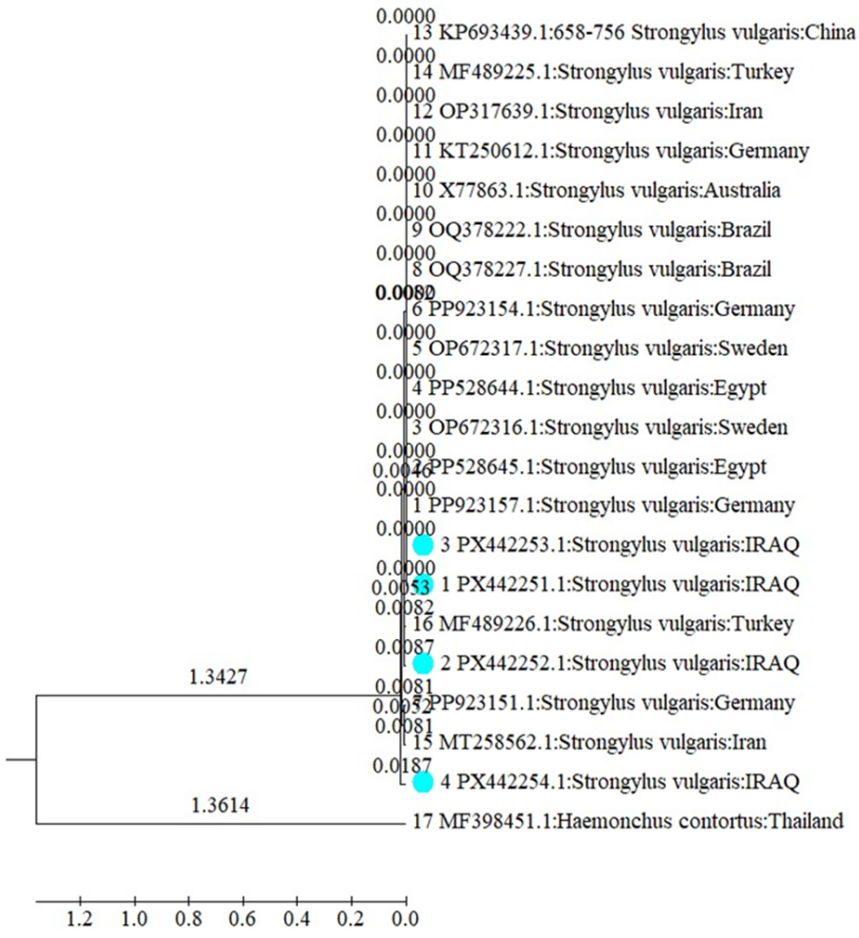


Figure 12. Phylogenetic tree of *S. vulgaris*: Evolutionary distances were measured using the maximum composite likelihood method. Isolates from the present study are denoted by blue spheres.

In donkeys (*Equus asinus*) from Zimbabwe, worm infection was highest in November and lowest in April (Pandey & Eysker, 1989). An earlier Iraqi study found that the eggs of these worms remained viable for over 11 months in the laboratory and for 9 months in external environments (Al-Saqur et al., 1987).

Infections with *S. vulgaris* are generally recognized as the predominant parasite nematode infections responsible for morbidity and mortality in horses (Gonzales-Viera et al., 2019). However, other *Strongylus* species, such as *S. edentatus* and *S. equinus*, also have a greater impact on horses than genera such as *Triodontophorus* (Raue et al., 2017; Camino et al., 2019).

Although SEM is not a new technique, this was the first attempt to photograph these nematodes in Iraq. The most important morphological characteristics distinguishing these species are their size, the structures of the buccal capsule (teeth and dorsal gutter), and the male copulatory bursa. In addition to these microscopic features, SEM reveals external morphological features that confirm the identity of the different species. The ultrastructure shows clear differences in the size and arrangement of the elements of the external leaf crown, the excretory pores, and the other structures of the genital cones (El-Gameel et al., 2022).

Conclusion

The present study provided information regarding worm infection in donkeys and horses, along with morphological descriptions of the three large red worms, including SEM photographs. It also included a genetic analysis of *S. vulgaris* isolates for the first time in Iraq.

Ethical Considerations

Compliance with ethical guidelines

This research and sampling were conducted in accordance with the local Animal Care and Use Committee (ACUC) of the University of Baghdad's College of Veterinary Medicine requirements. The University of Baghdad's College of Veterinary Medicine authorized this study (Code: P.G700). No experiments were conducted on living animals.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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