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Original Research Article

First Molecular Identification and Phylogenetic Analysis of *Strongylus vulgaris* in Horses from Al-Najaf Province, Iraq

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Abstract: *Strongylus vulgaris* ranks among the most dangerous parasitic nematodes affecting horses worldwide. This large strongyle invades both gastrointestinal and vascular tissues, leading to verminous arteritis, severe colic episodes, and considerable mortality in affected animals. Its continued presence in domestic horse populations poses substantial challenges for veterinary practitioners, especially in geographic areas where equine agriculture remains economically important. The present investigation was designed to determine the prevalence of *Strongylus vulgaris* among equine populations in Al-Najaf Province, Iraq. We employed both conventional microscopy and molecular techniques for parasite detection. Furthermore, phylogenetic relationships were examined, and potential associations with host gender, age category, and collection season were evaluated. Between October 2025 and February 2026, we obtained 200 fresh fecal specimens from horses of varying ages and both sexes across multiple locations in Al-Najaf Province. Initial screening involved direct smear examination and flotation procedures under light microscopy. Subsequently, 100 samples underwent molecular analysis through polymerase chain reaction (PCR) targeting the ITS1 gene, which yielded an amplification product of 290 base pairs. Phylogenetic reconstruction was accomplished using MEGA11 software package. Microscopic analysis revealed an overall infection rate of 34.5% (69 out of 200 samples). Male horses exhibited a slightly elevated infection frequency (38.75%) relative to females (31.67%), though this difference lacked statistical significance ($P > 0.05$). Adult horses demonstrated significantly higher infection rates (42.0%) compared with younger foals (27.0%) ($P \leq 0.05$). Seasonal variation showed peak prevalence during December (45.0%) and lowest rates in November (20.0%). Molecular confirmation through PCR identified *S. vulgaris* in 30% of tested samples (30/100) with high significance ($P < 0.001$). Five local sequences were successfully deposited in NCBI GenBank under accession numbers (PZ012358, PZ012359, PZ012360, PZ012361, and PZ012362.

Keywords: Al-Najaf Province, Horses, ITS1 gene, PCR, Phylogenetic Analysis and *Strongylus Vulgaris*.

INTRODUCTION

Strongylus vulgaris has long been recognized as one of the most pathogenic helminths affecting domestic horses across diverse geographic regions. This large strongyle, classified within family Strongylidae, inflicts particularly severe damage through its migratory larval phases. These larvae preferentially invade arterial walls, especially targeting the cranial mesenteric artery and associated vessels, thereby producing verminous arteritis [1]. The subsequent ischemic injury to intestinal tissues frequently culminates in life-threatening colic, intestinal infarction, and potentially fatal outcomes when therapeutic intervention is delayed [2].

The biological cycle of *S. vulgaris* begins when horses consume infective third-stage larvae from contaminated grazing areas. Once ingested, these larvae embark on an extensive migratory journey through the host's arterial system before eventually returning to the large intestine as mature adults. This entire developmental process typically spans six to seven months [1]. The prolonged vascular migration phase represents the primary source of pathological changes, underscoring the critical importance of prompt and accurate diagnostic procedures for successful therapeutic management and prevention strategies.

Historically, parasitologists have depended on coproscopic approaches for detecting *S. vulgaris* infections. While these conventional techniques including quantitative fecal egg counts, direct smear preparations, and various flotation protocols remain economically practical and suitable for field applications, they suffer from a fundamental limitation: morphological examination alone cannot reliably differentiate strongyle eggs to species level [3, 4]. The advent of molecular diagnostic tools, particularly polymerase chain reaction (PCR) targeting conserved genomic regions such as the internal transcribed spacer 1 (ITS1) of ribosomal RNA, has significantly enhanced diagnostic sensitivity and specificity [5].

Phylogenetic investigations conducted on regionally collected *S. vulgaris* isolates have revealed remarkable genetic stability within the ITS1 region across geographically separated populations spanning Europe, Asia, Africa, and Middle Eastern countries [3-6]. Studies from Iran have documented widespread distribution of equine strongylid parasites, including *S. vulgaris*, with prevalence rates showing considerable provincial variation influenced by local environmental factors and management practices [4].

MATERIALS AND METHODS

Ethical Approval

All procedures involving animal subjects received formal approval from the College of Veterinary Medicine, University of Al-Qadisiyah. The institutional review board granted ethical clearance under reference number 5637 on December 28, 2023.

Collection of Samples

During the five-month period extending from October 2023 through February 2024, we systematically collected 200 fecal specimens from horses residing in various localities throughout Al-Najaf Province. The study population included animals of both sexes and diverse age categories. Fresh samples were obtained either through direct rectal collection or immediately following natural defecation. Personnel wore clean disposable gloves during collection procedures, and each sample was placed into a properly labeled sterile container. Throughout transport to the laboratory facilities, samples remained under refrigerated conditions to preserve parasite viability and prevent degradation.

Microscopic Examination

Direct smear methodology involves spreading a small quantity of fresh fecal material onto a glass slide for immediate microscopic observation. This rapid screening approach facilitates detection of strongyle eggs and larvae, though it generally exhibits lower sensitivity compared with molecular approaches. Nevertheless, it remains valuable for preliminary assessment purposes [7].

Flotation procedures capitalize on density gradients between parasitic elements and fecal debris. Samples are thoroughly mixed with specialized flotation media, then subjected to centrifugation, causing eggs to rise toward the surface where they become accessible for microscopic identification. This technique proves especially effective for recovering strongyle eggs and is frequently employed in conjunction with PCR to enhance diagnostic accuracy, particularly when parasite burdens are minimal [3].

DNA Extraction

DNA was extracted from 100 fecal samples using a stool DNA extraction kit (CWBIO, China) according to the manufacturer's protocol. Briefly, 0.1 g of fecal sample was added to a lysis tube with 750 µL of buffer QSL and 4 µL of RNase, vortexed, and incubated at 70°C for 10 minutes. Following centrifugation at 13,000 rpm for 2 minutes, the supernatant was processed through sequential wash and elution steps. The eluted DNA was stored at −20°C until further analysis. DNA concentration was measured using a Quantus™ Fluorometer (Promega, USA).

PCR (Polymerase Chain Reaction)

The Polymerase Chain Reaction (PCR) is a molecular diagnostic technique employed for the detection of *Strongylus vulgaris* eggs in fecal samples of horses. By amplifying specific DNA sequences unique to S. vulgaris, PCR demonstrates high sensitivity and specificity, even in cases with low egg counts or prepatent infections [2]. Unlike traditional microscopy or flotation methods, PCR enables definitive species-level identification of *S. vulgaris* and clear differentiation from related strongylid species, even in subclinical cases or low-intensity infections [8]. This capability supports earlier diagnosis and consequently improved clinical management of serious complications including verminous arteritis, colic, and intestinal infarction caused by S. vulgaris [1, 2].

Primers targeting the ITS1 gene region of Strongylidae were designed for this study. The degenerate forward primer sequence is R(G/A) CCCTTCGTAAGGTGTCTATGT and the reverse primer sequence is CGCTGAAGCTAGTCGATTGT, targeting the ribosomal ITS1 region. The primers were synthesized by Macrogen, Korea, and provided in lyophilized form. These were dissolved in high-purity water to achieve a final stock concentration of 100 picomoles/µL. The primer stocks were stored at −20°C until further use. A working concentration of 0.5 picomoles per 20 µL total PCR reaction was employed. The resultant amplicon size is 290 base pairs (bp), targeting the partial region of the ITS1 gene. PCR products were analyzed by agarose gel electrophoresis (1.5%). Products from 20 positive samples were submitted to Macrogen, Korea, for Sanger sequencing. The generated sequences were trimmed from poor-quality signals, submitted to NCBI GenBank for accession numbers (PZ012358–PZ012362), and compared with deposited sequences using NCBI BLASTn for species-level identification.

Statistical Analysis

Data were collected, organized into tables, and statistically analyzed using a personal computer equipped with the Statistical Package for the Social Sciences (SPSS) version 26 software (IBM Corporation, Armonk, NY, USA). The chi-square test was applied to evaluate qualitative data and compare between groups. Values were considered statistically significant at a probability level of $P < 0.05$ (Field, 2013).

RESULTS

Microscopic Examination

The overall incidence rate of *Strongylus vulgaris* in fecal specimens of examined horses by microscopic examination was 34.5% (69/200), as shown in Table (1). Characteristic strongyle eggs were identified under microscopic examination, displaying typical oval morphology with thick shell and internal morula stage (Figure 1).

Table 1: Incidence Rate of *Strongylus vulgaris* in Horses from Al-Najaf Province

Horses	No. of examined specimen	Positive Samples	Percentage (%)
Total	**200**	**69**	**34.5%**
Chi-Square (χ2)		—	
P value		—	

***No statistical comparison applicable for single group**

Figure 1: Microscopic visualization of *Strongylus vulgaris* egg from equine fecal sample. The characteristic oval shape with thick shell and internal morula stage is visible under 40× magnification.

Infection Rate as Per Gender

The infection rate was higher in male horses at 38.75% (31/80) compared to female horses at 31.67% (38/120). Table (2) shows no statistically significant variation between genders ($P > 0.05$).

Table 2: Incidence Rate of *Strongylus vulgaris* in Horses from Different Groups Based on Gender

Gender	No. of examined specimen	Positive Samples	Percentage (%)
Female	120	38	31.67%
Male	80	31	38.75%
Total	**200**	**69**	**34.5%**
Chi-Square (χ2)		1.066	
P value		0.302 (NS)	

NS: Not Significant ($P > 0.05$)

Infection Rate as Per Age

Adult horses exhibited a significantly higher infection rate of 42.0% (42/100) compared to foals at 27.0% (27/100) ($P \leq 0.05$), as shown in Table (3).

Table 3: Incidence Rate of *Strongylus vulgaris* in Horses from Different Age Groups

Age Group	Number of Horses Examined	Number of Positive Samples	Percentage (%)
Foals	100	27	27%
Adult	100	42	42%
Total	**200**	**69**	**34.5%**
Chi-Square (χ2)		4.978	
P value		0.026 (S)	

S: Significant at P ≤ 0.05

Infection Rate as Per Month

The highest infection rate was recorded in December at 45.0% (18/40), followed by January at 42.5% (17/40), February at 35.0% (14/40), October at 30.0% (12/40), and the lowest in November at 20.0% (8/40). Table (4) shows no statistically significant difference between months ($P > 0.05$).

Table 4: Incidence Rate of *Strongylus vulgaris* in Horses from Different Months

Month	Number of Samples	Positive Samples	Percentage (%)
December	40	18	45.0%
January	40	17	42.5%
February	40	14	35.0%
October	40	12	30.0%
November	40	8	20.0%
Total	**200**	**69**	**34.5%**
Chi-Square (χ2)		7.169	
P value		0.127 (NS)	

NS: Not Significant (P > 0.05)

PCR (Polymerase Chain Reaction)

The overall incidence rate of *Strongylus vulgaris* confirmed by PCR was 30.0% (30/100), with a highly statistically significant difference ($P < 0.001$), as presented in Table (5). Agarose gel electrophoresis (1.5%) showed amplicons targeting the partial ITS1 gene region (290 bp) at an optimal annealing temperature of 60°C, as shown in Figure (2). NC: negative control; M: molecular marker from Genedirex (100–1500 bp).

Table 5: Incidence Rate of *Strongylus vulgaris* in Fecal Specimens of Examined Horses by PCR

Horses	Number of Samples	Number Positive Samples	Percentage (%)
Total	**100**	**30**	**30%**
Chi-Square (χ2)		16.00	
P value		< 0.001 (HS)	

HS: Highly Significant at P ≤ 0.01

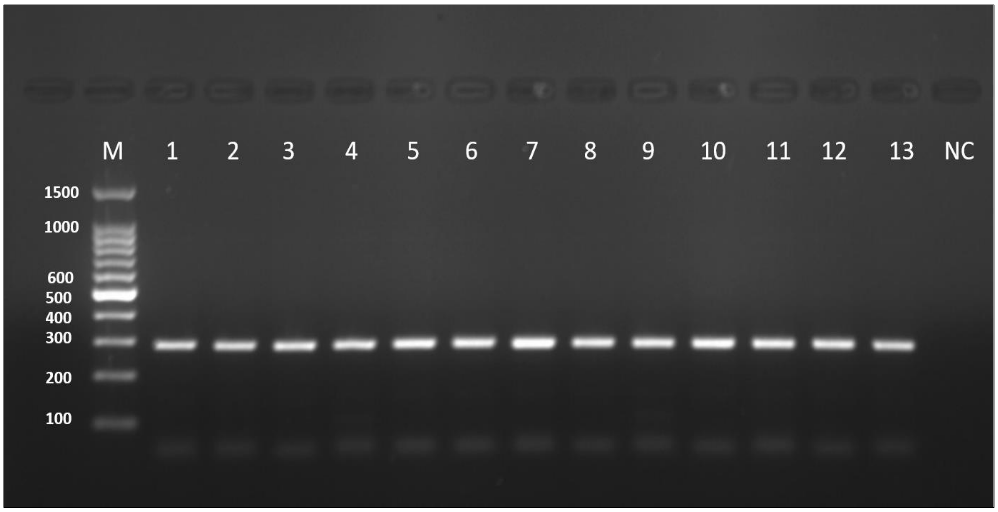

Figure 2: Agarose gel electrophoresis image (1.5 %) shows the amplicons of Strongylidae targeting partial region of ITS1 gene (size= 290 bp) (1-13). NC is negative control in which H_2O was added instead of template DNA. M is molecular marker from Genedirex (100-1500 bp).

Phylogenetic Analysis of Strongylus Vulgaris

The homology sequence identity (%) of local *Strongylus vulgaris* isolates (PZ012358, PZ012359, PZ012360, PZ012361, and PZ012362) with NCBI-BLAST deposited isolates is presented in Table (6). This analysis provides insights into the genetic similarity between the local and deposited isolates, helping to better understand their evolutionary relationships. The five local isolates showed 97.54–99.66% sequence identity with internationally deposited S. vulgaris strains from Turkey (MF489225, MF489226), China (KP693439), Iran (OP317639), and Australia (AJ228251), confirming species-level identification.

Figure (3) shows the evolutionary phylogenetic analysis of *Strongylus vulgaris* conducted by applying the Maximum Likelihood technique. This was analyzed using the partial region of the ITS1 gene, estimated through the use of the Tamura-Nei model. The MEGA11 software was used to perform evolutionary analyses, which enables detailed visualization of the evolutionary links of the S. vulgaris isolates. This analysis involved 10 nucleotide sequences with a total of 296 positions in the final dataset.

Figure (4) illustrates the results of the multiple sequence alignment of the identified *Strongylus vulgaris* sequences targeting the ITS1 gene. This was done to reveal similarities and dissimilarities between the sequences, providing a better perspective of the genetic variation among the analyzed isolates. The analysis was performed with MEGA software, which gives a detailed perception of the sequence variations.

Table 6: The NCBI-BLAST Homology Sequence identity (%) between local *Strongylus vulgaris* (PZ012358, PZ012359, PZ012360, PZ012361, and PZ012362) and NCBI-BLAST deposited isolates

Samples	Obtained Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical to	GenBank Accession number	Country	Identity (%)
1	PZ012358	*Strongylus vulgaris*	MF489225	Turkey	99.66
2	PZ012359	*Strongylus vulgaris*	KP693439	China	99.32
3	PZ012360	*Strongylus vulgaris*	OP317639	Iran	99.32
4	PZ012361	*Strongylus vulgaris*	MF489226	Turkey	98.64
5	PZ012362	*Strongylus vulgaris*	AJ228251	Australia	97.54

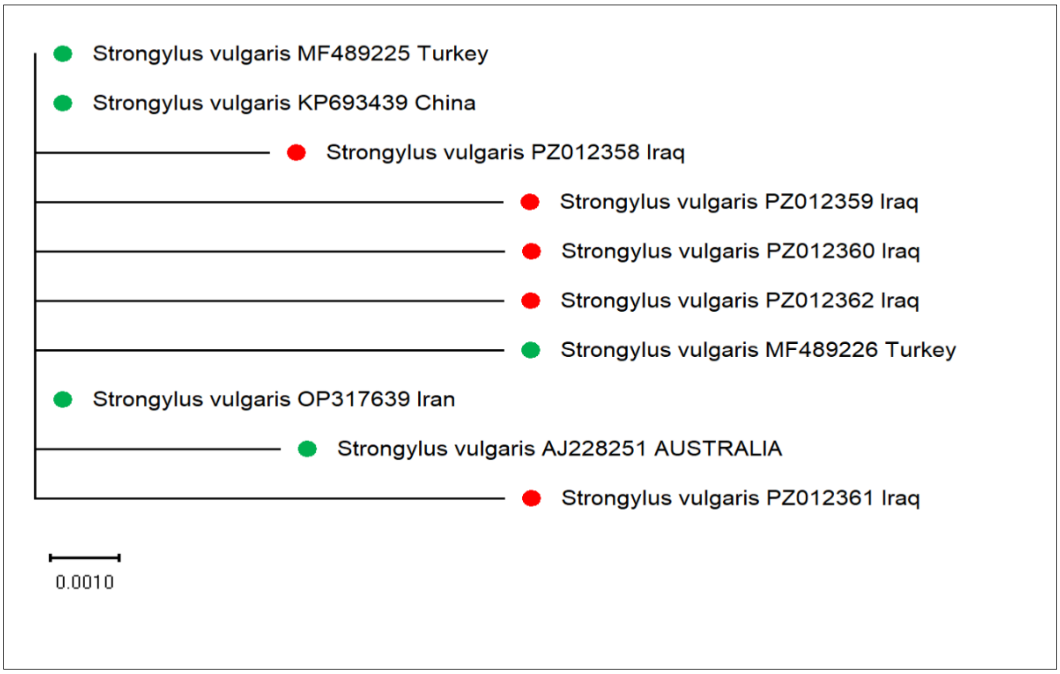

Figure 3: Evolutionary tree analysis of Strongylus vulgaris by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 10 nucleotide sequences. There were total of 296 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

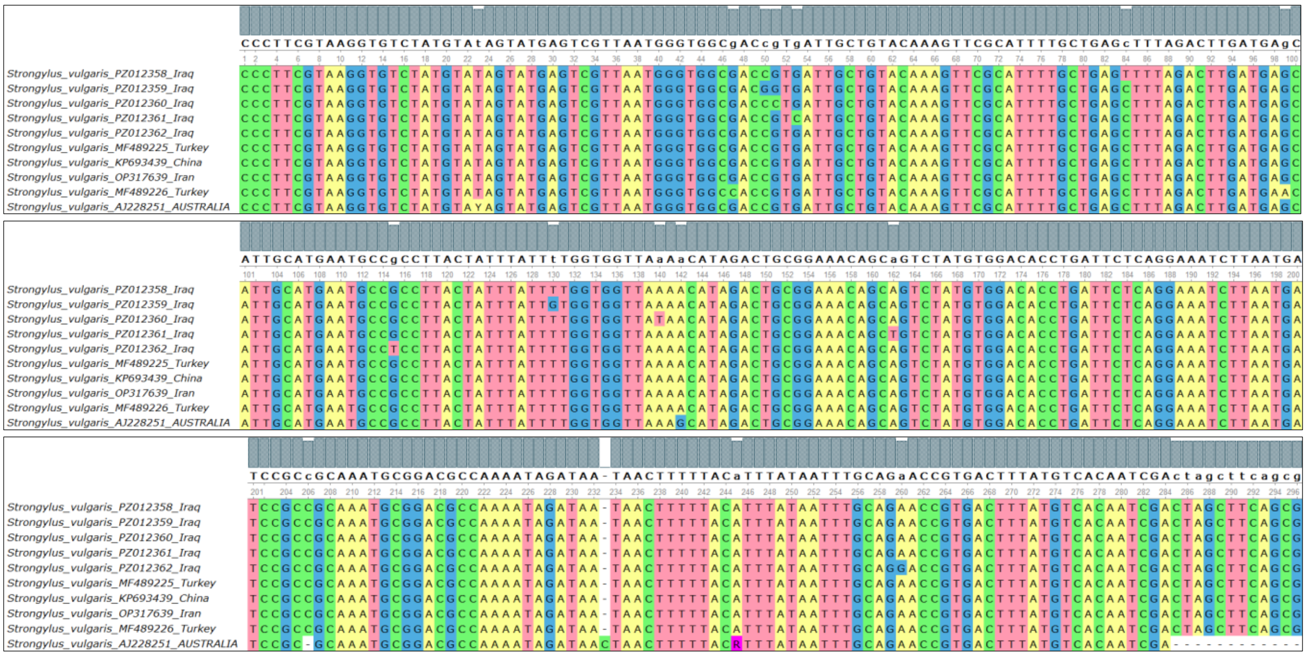

Figure 4: Multiple alignment of the identified *Strongylus vulgaris* sequences targeted the ITS1 gene to show the similarity and differences within the sequences. Analysed by Mega software.

DISCUSSION

Strongylus vulgaris is widely recognized as the most pathogenic large strongyle nematode infecting horses, with its migrating larvae causing verminous arteritis, thrombus formation in the cranial mesenteric artery, and potentially fatal non-strangulating intestinal infarctions [1, 2]. The present study reports, for the first time, the molecular identification and phylogenetic characterization of *S. vulgaris* in horses from Al-Najaf Province, central Iraq. The overall infection rate by microscopic examination was 34.5% (69/200), while PCR-based molecular analysis confirmed infection in 30.0% (30/100) of examined samples. These rates indicate a moderate but significant endemicity of the parasite within the equine population of the region.

In the context of neighboring countries, this prevalence is consistent with data from Iran, where *S. vulgaris* has been reported across multiple provinces [5], in a study evaluating larval culture and conventional PCR methods for the detection of *S. vulgaris* in equines from different provinces of Iran, demonstrated that PCR consistently outperformed larval culture in sensitivity, a finding consistent with the current study where the PCR rate (30.0%) was slightly lower than the microscopic prevalence (34.5%). The comprehensive review by [4], covering parasitic diseases of equids in Iran from 1931 to 2020, reported that infection rates with large strongyles including *S. vulgaris* varied widely between 4.4% and 69.2% depending on the region, diagnostic method, and management conditions. More recently [9], characterized the diversity of strongyle communities in horses in northern Iran using morphological and nemabiome metabarcoding approaches, confirming the presence of *S. vulgaris* and demonstrating that parasite community composition varied significantly among locations and management systems. These Iranian studies collectively confirm that *S. vulgaris* is endemic across the Middle East region, and the current findings from Al-Najaf Province are consistent with this regional pattern.

Regarding North Africa [3], reported an infection rate of 15.85% (39/246) for *S. vulgaris* in donkeys from Egypt using microscopic examination and larval culture, with ITS2 sequencing revealing approximately 95% identity with sequences from Germany, China, and Turkey. A subsequent Egyptian study by [6], provided morphomolecular characterization of *S. vulgaris* using ITS2 gene sequencing, reporting 97–99% sequence homology with global isolates from Turkey, China, and Australia. These data suggest that *S. vulgaris* populations across the Middle East and North Africa are genetically conserved, which is supported by the current phylogenetic analysis where the five local Iraqi isolates (PZ012358–PZ012362) clustered closely with internationally deposited *S. vulgaris* sequences, confirming high ITS1 gene conservation across geographically diverse populations. In Turkey [7], recorded *S. vulgaris* as one of the most prevalent equine parasites at a rate of 40.8% in horses from Kirikkale Province, higher than the current study and potentially reflecting differences in management practices, climate, and anthelmintic use.

Regarding gender, male horses exhibited a higher, though statistically non-significant, infection rate (38.75%) compared to female horses (31.67%) ($P > 0.05$). This absence of a significant gender effect is consistent with [3], who similarly reported no statistically significant difference in *S. vulgaris* infection rates between male and female donkeys in

Egypt ($P = 0.69$). In Iran [10], also found no significant gender-based difference in large strongyle infection rates in horses from Hamedan Province. The comparable infection rates between sexes likely reflect similar exposure levels to infective larvae in the pasture environment. However, hormonal modulation of immune responses, particularly during gestation and lactation in mares, may alter susceptibility under different management conditions [4].

With respect to age, adult horses showed a significantly higher infection rate (42.0%) compared to foals (27.0%) ($P \leq 0.05$). This finding is consistent with data from Egypt, where [3], reported higher *S. vulgaris* infection rates in animals older than three years. In Iran, multiple studies reviewed by [4], similarly documented greater strongyle burdens in adult horses. Furthermore [11], in a Swedish prevalence study following ten years of prescription-based anthelmintic use, confirmed that patent *S. vulgaris* infection occurred across all age groups, though the long prepatent period of approximately six months [1], means that infections acquired earlier in life become detectable as patent adult burdens only later. The higher prevalence in adult horses in the current study may therefore be attributed to their longer cumulative exposure to infective L3 larvae during grazing [5].

Seasonal variation revealed the highest prevalence in December (45.0%) and January (42.5%), while the lowest was observed in November (20.0%). Although these differences did not reach statistical significance ($P > 0.05$), the trend toward higher infection rates during cooler winter months is biologically consistent with the known epidemiology of equine strongyle parasites. Cooler and more humid conditions in Al-Najaf Province during winter favor the development and survival of free-living larval stages of *S. vulgaris* on pasture, increasing the availability of infective L3 larvae [4]. In Turkey [7], similarly noted seasonal fluctuations in strongyle infection rates in association with environmental conditions.

The PCR-based confirmation of *S. vulgaris* in 30.0% of tested samples ($P < 0.001$) validates the use of molecular tools for species-specific identification of this parasite. The discrepancy between microscopic (34.5%) and PCR (30.0%) prevalence reflects the higher specificity of PCR, which exclusively detects *S. vulgaris* DNA, whereas microscopy detects the morphologically identical eggs of all strongylid species [3-5]. This limitation of morphological egg identification at the species level is well established [4-12], further underlined the importance of species-specific molecular confirmation alongside coproscopic methods in a study on German horse farms that recorded high seroprevalence of *S. vulgaris* infection. Phylogenetic analysis using the Maximum Likelihood method with the Tamura-Nei model in MEGA11 [13], revealed that the five local isolates clustered with globally deposited *S. vulgaris* sequences, consistent with findings of [3-6], for Egyptian isolates and [9], for Iranian isolates. To the best of our knowledge, this represents the first molecular identification and phylogenetic characterization of *S. vulgaris* in horses from Al-Najaf Province, Iraq.

CONCLUSION

The findings of this study confirm that *Strongylus vulgaris* is prevalent in equine populations in Al-Najaf Province, Iraq, with an overall microscopic prevalence of 34.5% and a PCR-confirmed prevalence of 30.0%. This represents the first molecular identification and phylogenetic characterization of *S. vulgaris* in this region. Adult horses were significantly more susceptible to infection compared to foals, and higher infection rates were observed during cooler winter months. Phylogenetic analysis demonstrated high ITS1 gene conservation between local isolates and global *S. vulgaris* strains. These results underscore the importance of implementing regular parasite surveillance programs and strategic anthelmintic treatment protocols in equine management in the region. Providing horses with clean water and uncontaminated feed, combined with routine fecal egg count monitoring and targeted anthelmintic treatment, would substantially reduce the burden and transmission of *S. vulgaris* in Al-Najaf Province.

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Data Availability

All data generated or analyzed during this study are included in this manuscript. The five sequences are deposited in NCBI GenBank under accession numbers PZ012358–PZ012362.

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