Iraqi Journal of Veterinary Sciences

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Morphological and molecular diagnosis of microfilaria species in blood of naturally infected sheep in Mosul city, Iraq

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Article information

Article history:
Received 7 May 2025
Accepted 26 September 2025
Published 6 April 2026

Keywords:
Sheep
Microfilaria
PCR
Electron microscope

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Abstract

The present study aimed to use morphometric and genetic characteristics to identify the species of filarioid worms that infest sheep in Mosul city, Iraq. by using microscopic examination (ME) of the blood smears stained with MGG- Quick stain, Modified Knott technique and conventional polymerase chain reaction technique for the diagnosis of disease. From July 2024 to October 2024, three hundred of blood samples Blood samples were drawn randomly from sheep in various regions of Mosul city. The total percentage of microfilaria infection is 56% during investigation by the modified Knott technique. The results of morphological study showed different type of *Microfilaria* spp. diagnosis depended in shapes and measurement, such as *Setaria* spp., *Onchocerca* spp., *Dipetalonema* spp., *Elaeophora* spp. The length range (102-600) µm and width range (11-21) µm. also Ultrastructure of microfilaria (Mf) was imaged through scanning electron microscopies, in a high degree of accuracy that is not provided by traditional light microscopes that identity of the most common genera was confirmed through examination using a Scanning Electron Microscope (SEM), which contributed to enhancing the accuracy of diagnosis of *Setaria* spp. and *Onchocerca* spp., also Molecular analysis the gene sequencing and phylogenetic tree analysis of the microfilaria identified three isolates of *Setaria digitata* under accession number LC850259.1, PV523263.1 and PV523264.1 as well as two isolates of *Filaria latala* under accession number, LC850256.1 and LC850257.1, our DNA analysis revealed that the *Setaria digitata* isolates from Mosul, Iraq showed a close similarity to the Japanese isolates EF196090.1 and EF196088.1, demonstrating a high level of genetic similarity ranging from 54% to 85%. Likewise, the *Elaeophora schneideri* isolate from Mosul city exhibited strong similarity to the American isolates KT878990.1 and KT878976.1, with similarity values ranging from 85% to 99%. Furthermore, the *Filaria latala* isolates from Mosul city showed a high degree of global similarity to the French isolate KP760377.1, with a similarity value of 93%. Four different genera of microfilariae were diagnosed based on morphological and morphometric characteristics, while diagnosis of the most common microfilariae genera was confirmed using scanning electron microscopy, also were diagnosis of five species of microfilariae larvae was confirmed using molecular technique (PCR).

DOI: 10.33899/ijvs.2025.159935.4290,

Introduction

Ovine microfilariosis is a vector borne disease occurs in sheep and goat resulting from the infection with microfilaria of multiple filarial nematodes (1,2). Filarial worm infections affect a broad range of species. (Filarioidea, Onchocercidae), which are spread by flies, mosquitoes, and ticks, among other haematophagous arthropods (3). Adult nematodes typically lodge in the cephalic arteries to cause elaeophorosis, which restricts blood flow and causes

ischemic necrosis, blindness, and sublingual oral impactions (4). When sheep and goats infected with *Setaria* spp. may be suffer from fetal neurological disorder Cerebrospinal nematodiasis (CSN) which characterized by dysfunction of central nervous system lead to motor weakness, ataxia, lumber paralysis and death (2,5). Also, Microfilaria can be seen in arteries, peritoneal cavity, and subcutaneous tissue. This can result in skin filariasis, which is characterized by lesions that resemble scabies and can reach the eye and cause blindness (6,7). Microfilaria produce local inflammation and have been present for (8). The parasites produced numerous pathological symptoms, the most significant ones are intense itching, skin stiffness, crust formation, skin thickening, and development of numerous nodules on the skin's surface, and hair loss (9). Detection of microfilaria by using multiple methods such as Modified Knott's technique was very sensitive (10). Blood samples were examined using Modified Knott's approach to detect sheathed microfilaria with a rounded front end and a pointed posterior end, ranging in length from 200 to 625 μm (11). Tested 150 sheep blood sample by Knott`s concentration techniques and revealed that 64% of the population was infected, recorded that the percentage of the infection with microfilariae was 56% in sheep blood during investigation by modified Knott technique (12). Examination of blood sample from sheep and goats detecting microfilaria with total percentage of infection in sheep was 18.8% while in goats was 22% according to Knott's concentration test (13). Scanning Electron Microscopic (SEM) is important method for diagnosis of the fine structure of *Setaria* spp. and enabled us to affirm classification for each worm (14,15). Showed that, using scanning electron microscopy, there were distinct prominences around the mouth and a feature resembling a knob at the tail was successfully discovered the morphological differences between adult worm *Setaria digitata* and *Setaria labiatopapillosa* through SEM studies (16,17). Using SEM for description the anterior structure in anterior end of male adult *Setaria digitata* (18). The Polymerase Chain Reaction (PCR) assay characterized by increased sensitivity and specificity in diagnosis (19). It proved to be more effective than Giemsa staining and the Modified Knots technique (20). Used the IpSdS repetitive sequences that were cloned and sequenced from the *S. digitata* genome to create a probe (21). Genetic diversity of *Setaria* parasites sequences analysis of the 12S rDNA and the mitochondrial cytochrome C oxidase subunit I (COXI) genes (22). The aim of study was to use morphometric and genetic characteristics to identify the species of filarioid worms that infest sheep in Mosul, Iraq.

Materials and methods

Ethical approval

The study was approved by the institutional animal care and use committee of Veterinary Medicine College, University of Mosul (UM.VET.2024.034 decision number in 1/9/2024).

Collection of blood samples

Three hundred sheep blood jugular vein (5 ml each) sample were collected in EDTA tubes were chosen at randomly from Al-Saadon abattoir of Mosul city from July 2024 to October 2024 and on that same day, they were moved to the parasitological lab at the University of Mosul's College of Veterinary Medicine. The blood sample was divided into two parts, firstly, the blood samples was examined by using number of stains example MG quick stain (Giemsa staining), Methylene blue stain, Acridine orange stain blood smear, secondly, and the remainder of sample was kept in the refrigerator at -4ºC to undergo the PCR technique.

Microscopic examination of blood

A total of 300 blood smears were prepared, air dried, then stained with MGG Quick stain (Bio-Optic, Italy) Giemsa stain and examined under a light microscope at (X10, X4) with immersion oil (X100), (Leitz, Germany) (13). At the same time the blood smears were stained with methylene blue by using Modified knot technique One milliliter of blood was placed in a 15 ml test tube, and 9 ml of 2% formalin solution was added. The tube was then centrifuged at 1500 rpm for 5 minutes. After centrifugation, the supernatant was discarded, and the sediment was retained. One drop of 1% methylene blue stain was added to the sediment, and the tube was shaken well to ensure proper mixing. A drop of the stained sediment was then taken using a Pasteur pipette and placed on a glass slide. A cover slip was applied, and the preparation was examined under a light microscope using X4 and X10 objective lenses (23).

Scanning electron microscopy (SEM)

Scanning Electron Microscope (SEM), model EVO 110, supplied by Zeiss, a renowned German company specializing in the manufacturing of advanced microscopy equipment, using to study the microscopic structure of the parasite with a high degree of accuracy that is not provided by traditional light microscope Microfilariae samples were pre-cooled at 4 °C for 30 minutes, then immersed in a fixation solution containing 2.5% glutaraldehyde and 1.5% formaldehyde in phosphate-buffered saline (PBS) for two hours. After fixation, the samples were washed three times with PBS, each wash lasting 10 minutes, Subsequently, the samples were gradually dehydrated using a graded ethanol series with the following concentrations and durations: 30% (10 minutes), 50% (10 minutes), 70% (10 minutes), 80% (10 minutes), 90% (10 minutes), and 100% (three times for 30 minutes each), After dehydration, the samples were coated with a thin layer (4 nm) of gold-palladium using a Leica EM ACE200 sputter coater (Leica Microsystems). The specimens were then mounted on aluminum stubs for

examination using a scanning electron microscope (SEM) (24).

DNA Extraction

The genomic DNA (gDNA) of Microfilariae was extracted from a 500 µl blood sample using Geneaid DNAn kit from (Qiagen, Germany). To rehydrate the DNA pellet, add 100 µl of hydrate solution. Stored at -20ºC until a genomic DNA estimation.

Polymerase chain reaction

Table 1 shows the sequences of primers used in PCR to diagnose 18SDNA region Microfilaria where specific primers are used to amplify the DNA. The second table illustrates the PCR program used to amplify the DNA of Microfilarial nematode using the primers (Table 1). This program contains the steps of denaturation, primer annealing, and extension, facilitating accurate and rapid diagnosis. PCR reactive mixing was equipped with 1 µl of primers, 10 µl of the master mix, four µl of template DNA and four µl of PCR grade water in a 20 µl vessel containing, Following the completion of the PCR with a thermocycler (Optimum 96 G Germany), the multiplication reaction was completed by using the bespoke software mentioned in table 2.

Sequencing and phylogenetic analysis

PCR amplicons were sent to Psomagena Company (USA) for purification and sequencing after they tested positive for Microfilariae spp. using the PCR technique. The, ITS1 partial sequences were subjected to multiple sequence alignment using the GenomeNet online tool. Following this, the NCBI BLAST from NCBI was used to compare the sequences with other sequences available in GenBank. With MEGA11 software, the Likelihood method on the Tamura Nei model and bootstrap analysis with 1000 resampling (25). In addition, the constructed phylogenetic tree used the 18S rRNA gene sequence of LC804379.1 *Dirofilaria ursi* as an outgroup.

Statistical analysis

The results were analyzed using the IBM SPSS version 22 statistical program, using the two-sided Chi-square test and the Fischer test to determine the significant differences between the factors and the infection rate ($P < 0.05$) (25).

Table 1: Types of the primers and their sequence.

Primers	Sequence	bp	Reference
Para F	GCAGCAGCAGTAGCACTTTC	370	(26)
Para R	CAGCGGGTAATCTCGACTGA		
COI intF	TGATTGGTGGTTTTGGTAA		(27)
COI intR	ATAAGTACGAGTATCAATATC		

Table 2: Steps of the conventional PCR scheme

Stage	Temperature	Time	Cycle number
Initial denaturation	95	6 min.	1
denaturation	95	45 sec.	
Annealing	55	1.0 min.	35
Extension	72	1.0 min.	
Final extension	72	5 min.	1

Results

Microscopic results

Microscopic examination of infected blood sample findings demonstrated that, throughout the inquiry using the modified Knott technique. The percentage of infection in sheep males (54%) and females (58%) did not significantly differ from one another were 168 sheep samples that tested positive for microfilariae under a microscope, yielding a total percentage of 56%. in Table 3.

Table 3. Total rate of infection with microfilariae in sheep by using Modified Knott technique

Gender of animals	No. of the examined animals	No. of the positive animals	Percentage %
Males	150	81	54a
Females	150	87	58a
Total	300	168	56

The identical letters indicate that there is no significant difference at the probability level ($P<0.05$).

Morphological and Measurement study

Through microscopic analysis of sheep blood smears stained with MG Quick Stain Giemsa, four taxa of microfilaria were identified based on the larvae's morphometric characteristics, and using Omaxtoup view program to measure of microfilaria length and width, *Setaria* species are among the recognized genera. They found that the microfilaria had a sheath with a slender, with a sharply pointed anterior end and a gradually tapered posterior end,

with lengths varying between 250-400 µm (Figure 1). The microfilariae of *Onchocerca* spp. are unsheathed with a broad anterior end and a gradually tapered posterior end and measure 350-600 µm (Figure 2). The microfilariae of *Elaeophora* spp. are unsheathed microfilariae, with a rounded anterior end and a tapered, blunt posterior end and measure 239-279 µm (Figure 3). The microfilariae of *Dipetalonema* spp. unsheathed microfilariae, with a sharply pointed anterior end and a curved posterior end terminating in a hook-like tail and measure 250-300 µm (Figure 4).

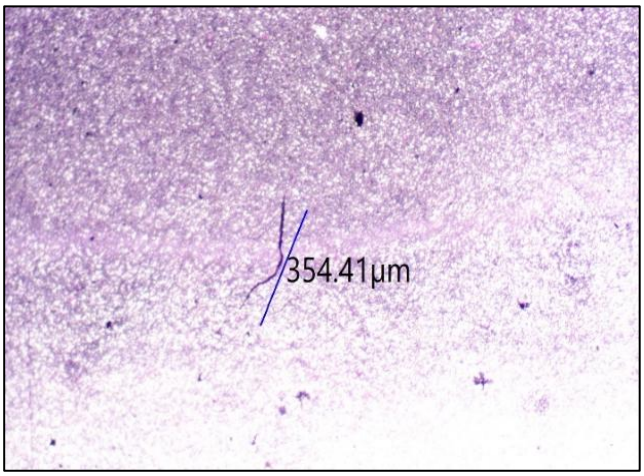

Figure 1: Blood smear stained with Giemsa stain which appear microfilaria of *Setaria* spp. (X4) which appear sheathed, slender, with a sharply pointed anterior end and a gradually tapered posterior end.

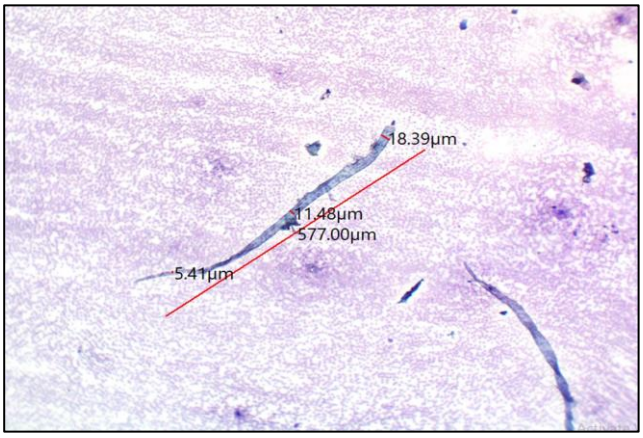

Figure 2: Blood smear stained with methylene blue stain microfilaria of *Onchocerca* spp. (X10) which appear unsheathed microfilariae, with a broad anterior end and a gradually tapered posterior end.

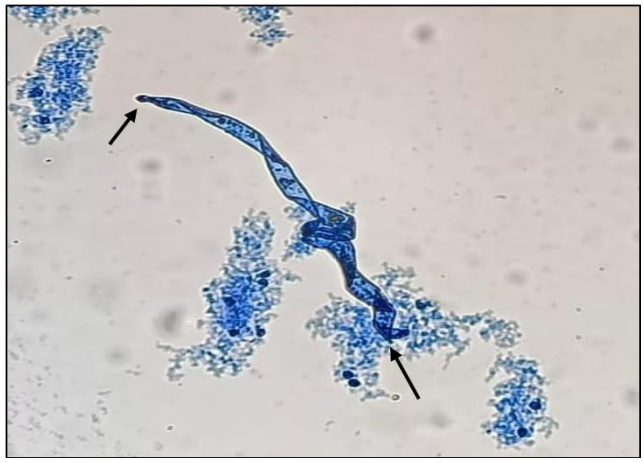

Figure 3: Blood smear stained with methylene blue stain which appear microfilaria of *Elaeophora* spp. (X10) unsheathed microfilariae, with a rounded anterior end and a tapered, blunt posterior end.

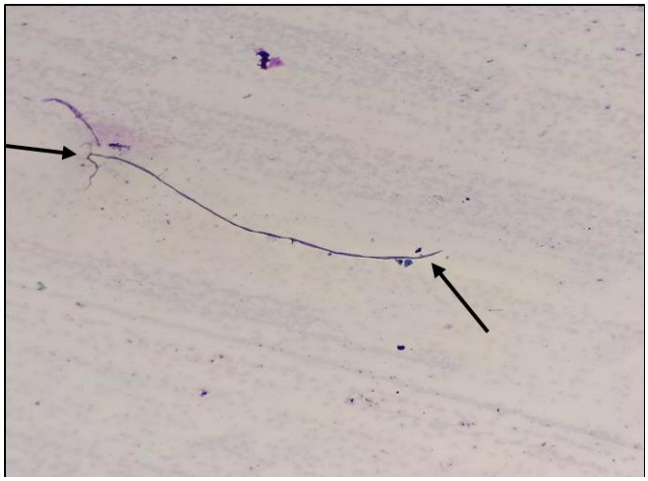

Figure 4: Blood smear stained with Giemsa stain which appear microfilaria of Dipetalonema spp. (X10) unsheathed microfilariae, with a sharply pointed anterior end and a curved posterior end terminating in a hook-like tail.

Scanning electron microscopy study

This study shows the importance of the electron microscope in describing the morphological features of microfilariae. The examination of microfilaria by using (SEM) revealed sheathed microfilaria characterized by a rounded anterior end and a pointed posterior end, belonging to *Setaria* spp (Figure 5). Additionally, an unsheathed microfilaria was observed featuring, a hook on the cephalic cap, which is attributable to *Onchocerca* spp (Figure 6).

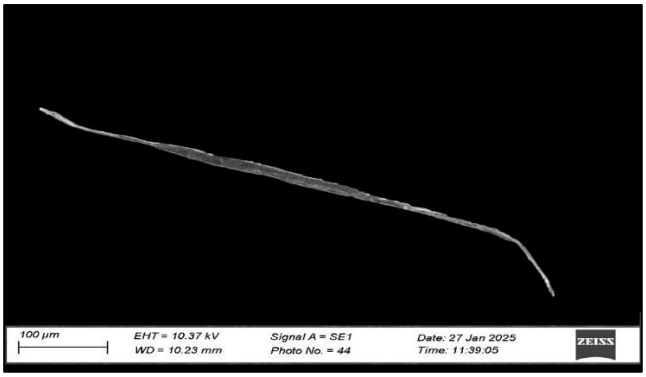

Figure 5: Microfilaria of *Setaria* spp. under Scanning electron microscope.

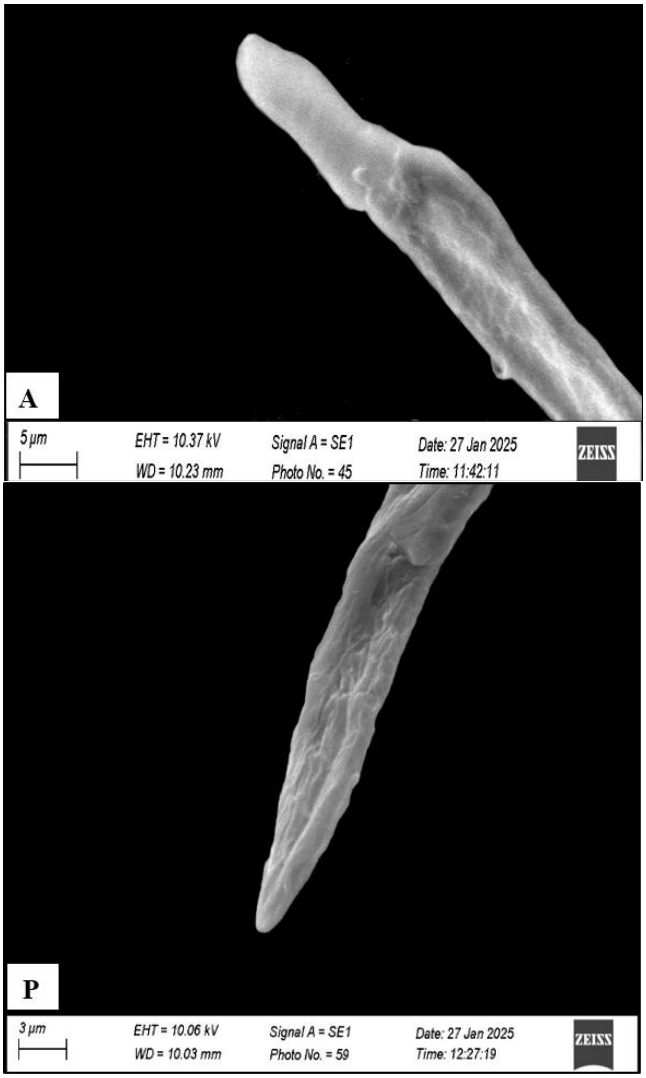

Figure 6: Microfilaria under Scanning electron microscope which appeared Anterior (A) and Posterior (P) ends of *Onchocerca* spp.

Molecular analysis study

For detecting ovine microfilariosis infection, the outcomes of the PCR assay and blood smear examination were contrasted *Filaria latala, Onchocerca volvulus, Setaria digitata, Elaeophora schneideri, Dipetalonema yatesi* Amplified DNA fragments are represented by the bright bands in the picture. One amplified DNA sample from a fifteen sheep blood sample is represented by each band. Fifteen blood samples have fifteen distinct bands that show the presence of microfilaria DNA. For the first time, a single microfilaria sequence was found in Mosul city from fifteen sheep blood samples using six sequence analysis. GenBank received one of these sequences (n=1), which was entered under the entry number LC850256, LC850257, LC850258, LC850259, LC850260, LC850261, PV523263, PV523264.

The bright bands in the figure represent amplified DNA fragments. Each band represents one amplified DNA sample from a single sheep blood sample. There are fifteen clear bands indicating the presence of *Microfilariae* spp. (Figure 7).

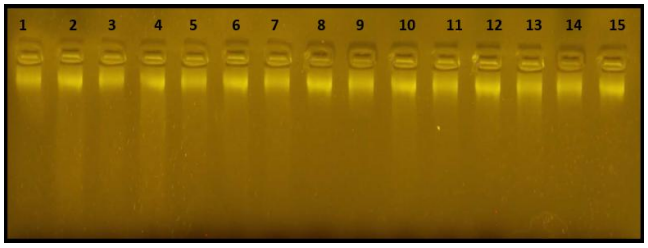

Figure 7: The genome extraction and separated by 1% agarose gel electrophoresis.

The results indicate that some of the tested samples contain DNA from the *Microfilariae* spp., based on the presence of bands at the expected 370 bp size. This demonstrates the effectiveness of using PCR to diagnose the presence of Microfilariae in the tested samples (Figure 8 and 9).

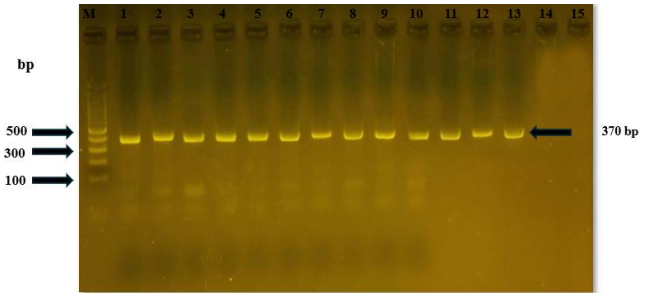

Figure 8: PCR reaction of Microfilariae spp. based on the 18sDNA region and amplification product of 370 bp. (Lanes 1-13: Positive Microfilaria samples; Lanes 14, 15: Negative controls).

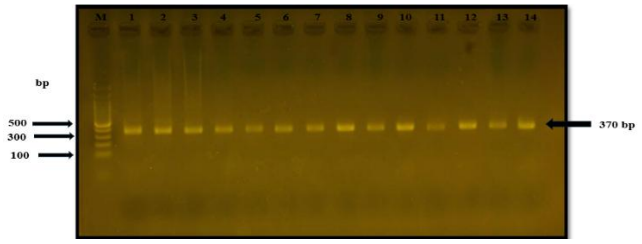

Figure 9: Results of PCR amplification by electrophoresis lanes shows a band size of 370 bp (Lanes 1-14: Positive Microfilaria samples).

These sequences were 100% identical to those found in the NCBI GenBank, including (EF196088.1) in Japan, (*Setaria digitata*) (KP760377.1) in France *Filaria latala* voucher ;(KT878990.1, KT020850.1) in USA, with *Elaeophora schneideri* (Table 4). Furthermore, the MEGA12 program's neighbor-joining phylogenetic tree analysis proved that *Microfilaria* Spp. native sequences were 100% similar to the GenBank sequences of the same species. As an outgroup, *Thelazia callipaeda* (GenBank: AM042549.1) was used to root the tree (Figure 10).

Table 4: Utilize NCBI to compare the genomes of local *Microfilaria* spp. strains with those in GenBank BLASTn associated with the same disease

No.	Name of isolate	Accession no.	Name of gene	Country	Percent Identity %
1	*Setaria digitata isolate SL/Sd/7*	EF196088.1	*5.8S r RNA gene*	Japan	100%
2	*Filaria latala voucher 62YT MNHN*	KP760377.1	*28S r RNA gene*	France	100%
3	*Elaeophora schneideri isolate ES-WY7*	KT878990.1	18S r RNA gene	USA	100%
4	*Elaeophora schneideri isolate ES-CA1*	KT020850.1	18S rRNA gene	USA	100%
5	*Setaria digitata* isolate SL/Sd/9,	EF196090.1	5.8S r RNA gene	Japan	99.7%
6	*Elaeophora schneideri isolate*	KT878976.1	ES-WY2 18SrRNA gene,	USA	99.7%

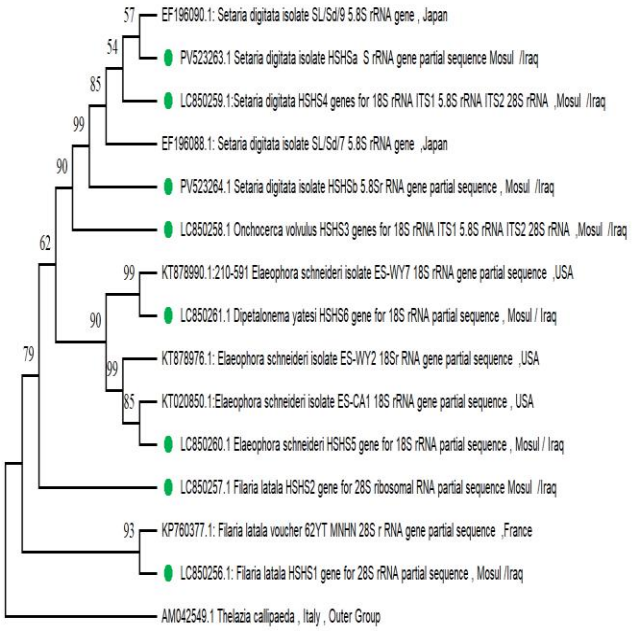

Figure 10: Represents the phylogenetic tree of the isolated *microfilaria species* for this study, constructed using the Neighbor-Joining method and the 28S rRNA gene. Bootstrap support levels are indicated by numerical values at branching (1000 replicates). The outgroup was *Thelazia callipaeda* (GenBank accession number: AM042549.1). Circle (●) represent the *Setaria digitata*, *Filaria latala*, *Onchocerca volvulus*, *Elaeophora schneideri*, *Dipetalonema yatesi* Local sequences.

The phylogenetic analysis based on ribosomal RNA genes (5.8S, 18S, ITS1, ITS2, and 28S) revealed that the *Setaria digitata* isolates from Mosul, Iraq (HSHSa, HSHSb, HSHS4), clustered closely with Japanese isolates (EF196090.1 and EF196088.1), showing high similarity with bootstrap values ranging from 54% to 85%, indicating moderate to strong evolutionary relationships. The *Onchocerca volvulus* isolate from Mosul (HSHS3) formed a distinct branch but remained within the filarial clade. Iraqi *Elaeophora schneideri* (HSHS5) grouped strongly with U.S. isolates (KT878990.1, KT878976.1, KT020850.1) with bootstrap support of 85–99%, reflecting a very close genetic relationship. Similarly, *Diptolena yatesi* (HSHS6) clustered nearby, suggesting related ancestry. The *Filaria latala* isolates from Mosul (HSHS1, HSHS2) shared high similarity with the French isolate (KP760377.1) with 93% bootstrap support, indicating minimal genetic divergence. Overall, the tree demonstrates that the Iraqi isolates are genetically close to international strains, suggesting conserved evolutionary lineages across geographical regions (Table 5) (Figure 10).

Also, we studied the different infection patterns of microfilariae in sheep, three infection patterns were observed: The single infection pattern, which recorded the highest infection rate at 50%; followed by the double infection pattern, with a total infection rate of 30%; and finally, the mixed infection pattern, which recorded a rate of 20%. Significant differences were observed among the three infection patterns at a probability level of ($P < 0.05$) (Figure 11).

Table 5. Genomic DNA for microfilaria spp. isolates that were entered into the gene bank, including the 28S rRNA and 18S RNA, ITS1, 5.8S rRNA, and ITS2 sequence

Accession No. of 16S rRNA gene	Pathogen	Local Strain
LC850256.1	*Filaria latala*	*Filaria latala* HSHS1 gene
LC850257.1		*Filaria latala* HSHS2 gene
LC850258.1	*Onchocerca volvulus*	*Onchocerca volvulus* HSHS3 genes
LC850259.1	*Setaria digitata*	*Setaria digitata* HSHS4 genes
LC850260.1	*Elaeophora schneideri*	*Elaeophora schneideri* HSHS5 gene
LC850261.1	*Dipetalonema yatesi*	*Dipetalonema yatesi* HSHS6
PV523263.1	*Setaria digitata*	*Setaria digitata* isolate HSHSa
PV523264.1	*Setaria digitata*	*Setaria digitata* isolate HSHSb

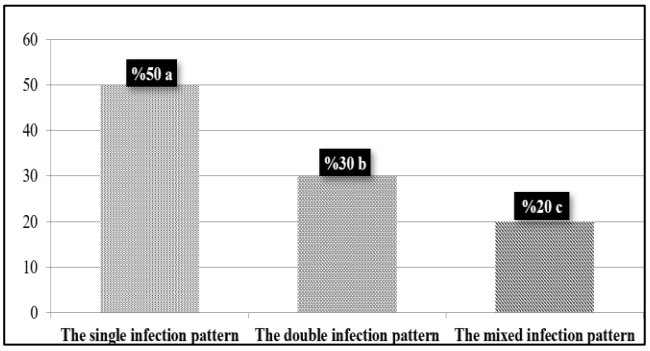

Figure 11: The relationship between infection patterns and the percentage of infection with microfilariae in sheep.

Discussion

The results we obtained may be attributed to the increase in the incidence of the disease. The elevated infection rate may be due to the presence of intermediate hosts, like mosquitoes and flies, that facilitate the transmission of microfilaria (11). Our findings align with those of (24) and the research conducted by which documented a high infection rate of microfilariae in sheep. Additionally, (25-27) observed elevated percentages of microfilariae in farm animals.

When utilizing an electron microscope for the diagnosis of microfilariae, it was identified two genera of microfilariae belonging to *Setaria* spp. and *Onchocerca* spp. (28) detected microfilaria of *Setaria labiatopapillosa* by using (SEM) technique (14). SEM was used to categorize the amount of distinct *Setaria* species in the peritoneal cavities of Korean cattle by examining distinctive characteristics at the mature worms' anterior and posterior ends (29). Using a scanning electron microscope, *Onchocerca volvulus* Microfilariae from Guatemala were analyzed. The species' anterior end is distinguished by the presence of a circular cephalic cap with a V-shaped hook.

Molecular analysis was conducted utilizing the polymerase chain reaction (PCR) technique to amplify the 18S rRNA gene, yielding a product of 370 bp. The positive isolate identified five types of microfilariae such as *Filaria latala*,*Onchocerca volvulus*, *Setaria digitata*, *Elaeophora schneideri*, *Dipetalonema yatesi*. Prior studies have discovered *S. digitata*, *S. marshalli*, and *S. labiatopapillosa* in cattle and buffaloes from different parts of Iran. Nevertheless (30) revealed that *S. labiatopapillosa* was the sole *Setaria* spp. isolated from cattle in southwestern Iran (31). Differences in temperature, the availability of suitable intermediate hosts, and the various livestock management techniques used in the various regions could all be reasons for the disparities in the results (32). The phylogenetic analysis of *Microfilaria spp.* isolates from Mosul, Iraq, based on ribosomal RNA gene sequences (18S, 5.8S, ITS1, ITS2, and 28S), reveals significant insights into their genetic relationships with globally reported strains. The local *Setaria digitata* isolates (HSHSa, HSHSb, and HSHS4) clustered within the same clade as Japanese reference isolates (EF196090.1 and EF196088.1), with moderate to high bootstrap support values (54–85%). This close genetic affinity suggests a conserved lineage that may be attributed to either a shared evolutionary origin or limited genomic divergence across geographic boundaries. Such findings support the notion that *S. digitata* is a genetically stable species with a potentially broad distribution.

The Iraqi *Onchocerca volvulus* isolate (HSHS3) formed a separate but related branch within the filarial group, reflecting its distinct taxonomy while maintaining evolutionary proximity to other Onchocercidae members. Notably, the *Elaeophora schneideri* isolate from Iraq (HSHS5) grouped with three U.S. isolates in a highly supported monophyletic clade (85–99% bootstrap), indicating strong genetic conservation and suggesting that *E. schneideri* populations from different continents may share a recent common ancestor or be part of an underrecognized global transmission cycle.

In a similar context, *Diptolena yatesi* (HSHS6) from Iraq showed phylogenetic relatedness to the *Elaeophora* group, confirming their close evolutionary linkage within the family Onchocercidae. Furthermore, the *Filaria latala* isolates (HSHS1 and HSHS2) clustered tightly with a French isolate (KP760377.1), with 93% bootstrap support, reflecting minimal sequence variation. This suggests that *F. latala* exhibits high genetic stability across diverse geographical

locations, which may be indicative of conserved structural and functional regions within the rRNA genes.

Overall, the phylogenetic tree supports the hypothesis that the Iraqi filarial nematodes belong to globally conserved lineages, with relatively low divergence from their international counterparts. The high bootstrap values across most clades enhance the credibility of these relationships and affirm the suitability of ribosomal RNA regions as molecular markers for taxonomic resolution and epidemiological tracing of filarial nematodes. These findings highlight the importance of molecular surveillance in understanding the genetic landscape and potential transmission dynamics of filarial parasites in endemic regions.

Conclusion

The most common genera were confirmed through Scanning Electron Microscopy such as *Onchocerca* spp. and *Setaria* spp., while five species of microfilariae were identified by using the molecular technique (PCR) such as *Filaria latala*, *Onchocerca volvulus*, *Setaria digitata*, *Elaeophora schneideri*, *Dipetalonema yatesi*, Three infection patterns were observed: the single infection pattern, which recorded the highest infection rate at 50%; followed by the double infection pattern, with a total infection rate of 30%; and finally, the mixed infection pattern, which recorded a rate of 20%.

Acknowledgment

We thank the Veterinary Medicine College, University of Mosul, for support.

Conflict of interest

None.

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التشخيص الشكلي والجزيئي لأنواع الميكروفيلاريا في دم الأغنام المصابة طبيعيًا في مدينة الموصل، العراق

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الخلاصة

هدفت هذه الدراسة إلى استخدام الخصائص الشكلية والقياسية (المورفومترية) والجينية لتحديد أنواع اليرقات الخيطية الدقيقة (الديدان الفيلارية) التي تصيب الأغنام في مدينة الموصل، العراق، وذلك باستخدام الفحص المجهري لمسحات الدم المصبوغة بصبغة جيمسا السريعة وتقانة التركيز المحورة والتفاعل المتسلسل لإنزيم البلمرة التقليدي لتشخيص المرض، حيث في الفترة الممتدة من أب ٢٠٢٤ إلى تشرين الأول ٢٠٢٤، تم جمع ٣٠٠ عينة دم بشكل عشوائي من أغنام في مناطق مختلفة من مدينة الموصل. بلغت نسبة الإصابة الإجمالية باليرقات الخيطية الدقيقة (الميكروفيلاريا) ٥٦% باستخدام تقانة التركيز المحورة، كما أظهرت نتائج الدراسة الشكلية (المورفولوجية) وجود أنواع مختلفة من اليرقات الخيطية الدقيقة (الميكروفيلاريا)، وتم الاعتماد في التشخيص على الشكل والقياسات، مثل الأنواع التالية الستيريا، الأنكوسيركا، الديبيتالونيما والإليوفورا حيث تراوح طولها بين ١٠٢ إلى ٦٠٠ ميكرومتر، وعرضها بين ١١ إلى ٢١ ميكرومترًا. كذلك تم تصوير البنية الدقيقة للميكروفيلاريا باستخدام المجهر الإلكتروني الماسح، والذي أتاح درجة عالية من الدقة لا توفرها المجاهر الضوئية التقليدية، وقد ساهم هذا الفحص في تأكيد هوية الأجناس الأكثر شيوعًا، وبشكل خاص يرقات الستيريا والأنكوسيركا. كشف التحليل الحمض النووي أن عزلات سيتيريا ديجتاتا من مدينة الموصل، العراق تشابهت بشكل وثيق مع العزلات اليابانية EF196090.1 وEF196088.1، مما يُظهر درجة عالية من التشابه الجيني بلغ ٥٤% ٥٨%، كذلك أظهرت عزلة اليوفورا شيندريني من مدينة الموصل تشابه قوي مع العزلات الأميركية KT878990.1، KT878976.1 بلغت ٨٥% ٩٩%، كما أظهرت عزلات فيلاريا لاتالا من مدينة الموصل تشابهًا عالميًا مع العزلة الفرنسية KP760377.1 بنسبة بلغت ٩٣%. تم تشخيص اربعة اجناس مختلفة من اليرقات الخيطية الدقيقة بالاعتماد على المواصفات الشكلية والقياسية، كما تم تأكيد تشخيص الاجناس الأكثر شيوعا لليرقات الخيطية الدقيقة باستخدام المجهر الالكتروني الماسح، كذلك تم تأكيد التشخيص لخمسة أنواع من اليرقات الخيطية الدقيقة بالاعتماد على التقنية تفاعل البلمرة المتسلسل.

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