



Prevalence and Diagnosis of Gastro-intestinal Parasites from Libyan Local Sheep (*Ovis aries*)

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ABSTRACT

Parasitic diseases, particularly nematodes, are among the most significant pathogens affecting economically important animals such as sheep, leading to their deterioration, reduced productivity, and poor growth. Nematode infections are considered one of the most severe parasitic diseases in sheep. Therefore, this study aimed to examine fresh sheep manure samples collected from Misurata. A total of 35 samples were analyzed using two concentration methods and laboratory culture techniques. Additionally, the morphological and structural characteristics of nematode larvae were identified. Larvae were cryopreserved in liquid nitrogen with distilled water and saline solution to assess survival rates. The results indicated that the laboratory culture method yielded higher detection rates (66%) than the concentration method (31.4%). Based on morphological and structural traits, four genera of nematodes were identified. Among these, *Haemonchus contortus* was the most prevalent, followed by *Trichostrongylus* spp. and *Oesophagostomum* spp., with *Dictyocaulus* spp. (lungworms) being the least common. Cryopreservation of larvae in liquid nitrogen demonstrated a higher survival rate when distilled water was used (68%) compared to saline solution (38%), with statistically significant differences. This study highlights the importance of differentiating between the types of nematode larvae that infect sheep and small ruminants. Accurate and rapid diagnosis, as demonstrated here, is essential for epidemiological research and improving disease management strategies.

Key words: Gastrointestinal nematodes, Concentration method, Larvae, Larval culture, Morphology, Morphometry, Cryopreservation, Sheep.

INTRODUCTION

Sheep are regarded as one of the most important livestock species, reared primarily for their meat, wool, and, to a lesser extent, milk production. However, poor management practices and grazing conditions expose them to a wide range of parasitic infections (El-Khabaz and Arafa 2014). Parasitic diseases remain a global challenge, representing significant constraints to livestock health and productivity (Abebe et al. 2010). Among these, gastrointestinal parasitism is particularly critical for sheep farmers worldwide, as it acts as a major limiting factor for sheep productivity (Veena et al. 2020). Nematodes are the predominant parasites, with multiple species infecting cattle and small ruminants (Do Amarante and Amarante 2016).

Sheep are frequently infected by several species of nematodes. However, differentiating these species based solely on their eggs proves challenging due to the eggs' morphological similarities and overlapping sizes. Therefore, it is critical to determine whether the larvae exhibit distinguishing characteristics that allow them to be identified in mixed cultures (Dikmans and Andrews 1933).

In Libya, there are limited studies on gastrointestinal nematodes (GIN) in sheep. However, some research has been conducted to assess the prevalence of these parasites in ruminants. For instance, one study reported an overall GIN prevalence rate of 98% in sheep (Elmajdoub et al. 2022). Similarly, Abd El-Aal and Nosseur (2006) found a 100% prevalence rate of GIN in sheep, identifying genera such as *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*

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and *Trichuris* spp. In a prior study by El-Khabaz and Arafa (2014), the total helminth prevalence rate was 47.13%, with infections predominantly caused by *Nematodirus* spp., *Trichostrongylus* spp. and *Haemonchus contortus*. Studies from other regions have also highlighted the prevalence of gastrointestinal nematodes in sheep. For example, research conducted at Flowers Gap Research Station, Broken Hill, New South Wales, reported infections with *Oesophagostomum venulosum* (42.9%), *Chabertia ovina* (27.6%) and *Trichuris* spp. (59%) in sheep from Morocco (Gray and Kennedy 1981; Cabaret 1983; Nebyou et al. 2021; Jadidoleslami et al. 2022). In China, the infection rate of various parasite species in sheep was 55.7% (Cai et al. 2023). On the other hand, the infection rate of sheep in Pakistan was relatively high, reaching approximately 94% (Ruhollah et al. 2021). In Southern Ethiopia, the overall prevalence of nematode was (51.3%), (Ibsa et al. 2022). Moreover, in Norway the gastrointestinal parasites were high in sheep, (Gravdal et al. 2024).

Larvae identification often relies on morphometric and morphological keys, such as those developed by Dikmans and Andrews (1933) and Van Wyk et al. (2004). A study by Knoll et al. (2021) demonstrated that 73.5% of nematode larvae could be correctly identified microscopically and molecularly. Furthermore, classification based on sheathed tail length correctly grouped 91.8% of larvae into their respective preliminary categories.

Cryopreservation has emerged as one of the most effective techniques for preserving nematode species for research purposes. This method helps overcome challenges associated with serial passage in donor animals, such as changes in anthelmintic resistance or pathogenicity of field strains, which may affect comparability across studies (Gill and Redwin 1995). Jensen et al. (2000) further emphasized that the use of distilled water and physiological solutions is a practical and effective approach to cryopreservation, as it extends larval survival periods.

This study aimed to diagnose nematode larval infections in sheep and investigate the morphological and standard characteristics of larvae cultivated under controlled laboratory conditions.

MATERIALS AND METHODS

Ethical approval

This study received approval from the animal ethics committee of the Faculty of Science, Misurata University, Misurata, Libya.

This research was conducted in Misurata, located in northwestern Libya, from February to April 2021. Misurata sits on the Mediterranean coast at a latitude of 32°22'39.12"N and a longitude of 15°05'31.26"E. According to the 2012 census, the population of Misurata is approximately 500,000. The region typically experiences a local steppe climate (Gatehouse 2012).

A total of 35 sheep manure samples were randomly collected in the early morning from various sheep farms in Misurata.

The samples were collected in sterile plastic bags and subsequently transported to the laboratory. Each sample was weighed using a digital scale, with a total weight of

40g allocated for both examination methods.

Concentration method

The sample was ground by adding distilled water, then filtered through medical gauze and transferred to 7cm³ tubes. Next, 3cm³ of formalin solution and 3cm³ of acetic acid were added. The tubes were centrifuged at 5000rpm for 10min, resulting in the formation of several layers: the acetic acid layer on top, followed by impurities, then formalin, with the precipitate containing the parasite at the bottom (Soulsby 1982).

Culture method

The fecal samples collected in this study were processed following methods used in previous research (Knoll et al. 2021). A total of 100 larvae obtained from the culture were selected in varying sizes, and measurements were taken of their total length, the length of the anterior section, and the length of the posterior section, specifically the tail sheath, to identify the nematode species (Dikmans and Andrews 1933). Photographs were captured using a Canon digital camera with a resolution of 16 megapixels, utilizing magnification levels of 10x and 40x with a Leica optical microscope. Measurements were conducted using the ImageJ software, along with a graphic scale to correspond to the magnification level for each image.

Experiment of cryopreservation of nematode larvae in liquid nitrogen

Live larvae were collected and thoroughly washed multiple times with distilled water, then divided into two groups. The first group consisted of distilled water, with six replicates containing an average of 10 larvae each, totaling 60 larvae. The second group was prepared using a 0.9% saline solution mixed with drops of glycerin, also comprising six replicates with the same number of larvae as the first group. Both groups were placed in specialized freezing tubes and stored in liquid nitrogen for two weeks. Afterward, the tubes were removed and allowed to equilibrate at room temperature. The larvae were then examined under a microscope to assess the survival rate, which was determined by observing their movement or by adding iodine dye (Jensen et al. 2000).

Statistical analysis

Statistical analyses were conducted using the T-test and one-way ANOVA within the SPSS software to compare the average infection rates between the concentration method and the egg culture method. Additionally, these analyses aimed to identify the most effective freezing method for larvae and to evaluate significant differences in the lengths of the larvae for species identification. Differences were considered significant at a P-value of less than 0.05.

RESULTS

In this study, 35 samples of sheep manure were examined for gastrointestinal parasites using two methods: concentration and egg culture. The egg culture method demonstrated a higher diagnostic efficacy, with an

infection rate of 65.7%, compared to 31.4% for the concentration method. As shown in Table 1, there was no significant difference in the average infection rates between the concentration and culture methods ($P>0.05$).

Table 1: Prevalence of Gastrointestinal Parasites in Sheep Feces

Parameter	Number of Samples	Infection Rate (%)
Culture Method	35	23 (65.7)
Concentration method	35	11 (31.4)

There was no significant difference between the average infection rates of the two methods ($P>0.05$).

Longitudinal measurements of isolated larvae

The results from the egg development indicated that the average total length of the larvae was $133.4\mu\text{m}$, with lengths ranging from 20 to $1033\mu\text{m}$. The average length of the anterior part of the larvae was $33\mu\text{m}$, with variations between 6 and $275\mu\text{m}$. Meanwhile, the average length of the posterior (tail) part was $31\mu\text{m}$, ranging from 3.2 to $4\mu\text{m}$. Based on these measurements, the larvae were classified into three categories: long, medium and short. Significant differences ($P\leq 0.05$) were observed in the total lengths of the larvae, as well as in the lengths of the anterior and posterior sections, as detailed in Table 2, 3 and 4.

Table 2: Mean Total Length of Isolated Larvae Groups

Small larvae	Medium larvae	Long larvae	Mean SE±
3.08 ± 32.5	2.36 ± 72.4	27.6 ± 213.1	
			Sign

** High significant differences.

Table 3: Mean Length (mean±SE) of the Anterior Part of Isolated Larvae Groups

Small larvae	Medium larvae	Long larvae
9.62 ± 0.97	$18.2\pm .81$	51.9 ± 7.11

** High significant differences.

Table 4: Average length (mean±SE) of the Posterior part of the isolated larvae groups

Small larvae	Medium larvae	Long larvae
8.19 ± 1.18	14.6 ± 1.12	51.14 ± 8.14

** High significant differences.

Determination of morphological characteristics of isolated larvae

The results of this study revealed a diversity of nematode types among the isolated larvae. Notably, the presence of a sheath was observed. Four genera of nematode larvae were identified in sheep: *Trichostrongylus*, *Haemonchus*, *Oesophagostomum*, and *Dictyocaulus*. The highest infection rate was found in *Haemonchus contortus* larvae, accounting for 38% of the isolates. Among these, 34% were first-stage larvae (L1), which are small, not exceeding a total length of $50\mu\text{m}$, characterized by a slender, pointed anterior and a short tail (Fig. 1). The third-stage larvae (L3), which represent the infectious phase for sheep, comprised 66% of the isolates. These larvae have a total length of approximately $1032\mu\text{m}$, featuring a bullet-shaped anterior, a sultagani's esophagus,

and a long, twisted, thin tail (Fig. 2). Larvae from the genera *Trichostrongylus* and *Oesophagostomum* were present at a rate of 36%, exhibiting medium sizes. The anterior sections are covered with structures resembling scales or fine folds, along with a pale esophagus and a rounded mouth. Among these, 25% belonged to *Oesophagostomum* spp., with a total length of $90\mu\text{m}$ and a sharp, long tail, while 75% were identified as *Trichostrongylus* spp., measuring $100\mu\text{m}$ in total length, with a comparatively shorter tail than *Oesophagostomum* spp. (Fig. 3 and 4). Lastly, *Dictyocaulus* spp. constituted 19% of the isolates, characterized by a longer size, with a total length of $259\mu\text{m}$. These larvae were noted for their thick, dark brown appearance (Fig. 5).

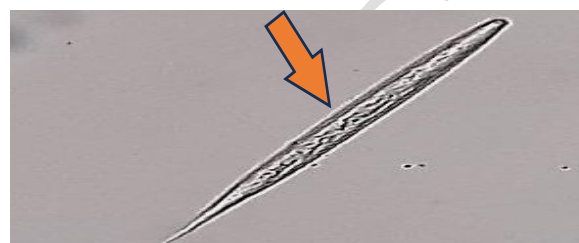


Fig. 1: First-Level Larva (L1) of *Haemonchus contortus*; The arrow indicates the pointed short tail.



Fig. 2: Third-Level Larva (L3) of *Haemonchus contortus*; Arrow (1) indicates the anterior part of the larva, arrow (2) shows the shape of the esophagus and arrow (3) points to the long twisted tail area.

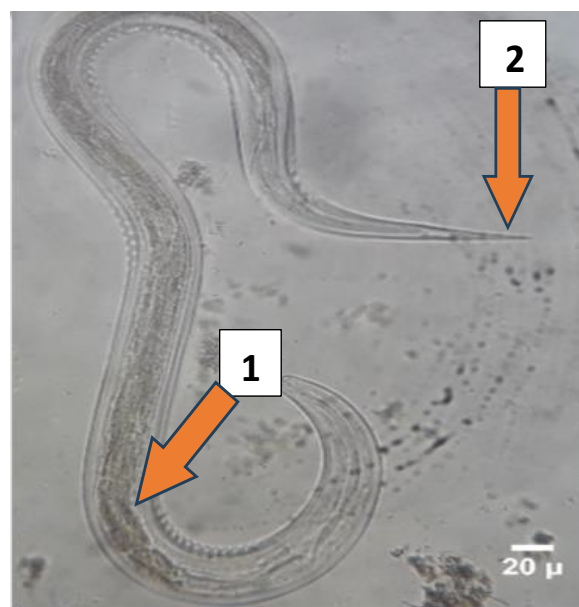


Fig. 3: Larva of *Oesophagostomum* spp.; Arrow (1) indicates the folds surrounding the outer shell, while arrow (2) points to the sharp tail area.

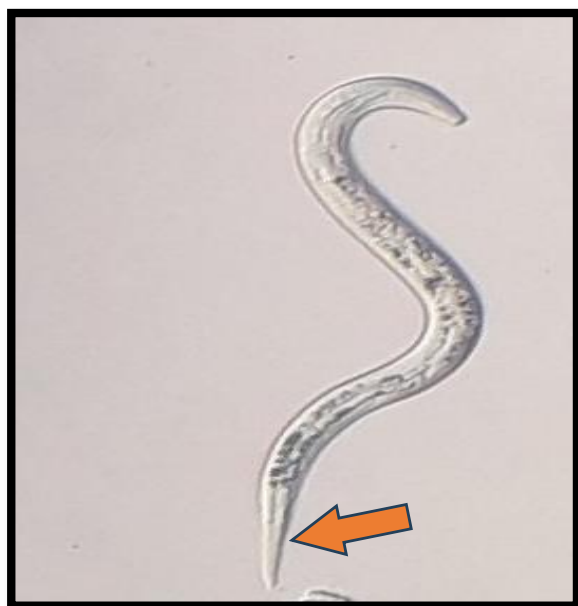


Fig. 4: Larva of *Trichostrongylus* spp.; the arrow indicates the short pointed tail.



Fig. 5: Larva of *Dictyocaulus* spp.; Arrow (1) indicates the shape of the esophagus and intestinal cells containing food residue, while arrow (2) points to the short tail.

Table 5: Average Survival of Larvae after Cooling from Liquid Nitrogen

Groups	Mean±SE
Normal saline	0.49±1.33a
Distilled water	1.5±9.2b

Values bearing different alphabets in a column differ significantly ($P < 0.05$).

Cryopreservation of nematode larvae in liquid nitrogen

The results of this study indicated that after the cooling process, the larvae frozen in liquid nitrogen and subsequently thawed at room temperature remained alive and exhibited movement and vitality. The survival rate of larvae in distilled water was 68%, whereas in saline solution, the survival rate was 38%. These differences were statistically significant.

DISCUSSION

Gastrointestinal parasitism is a significant concern for sheep farmers globally, as it limits sheep productivity and adversely affects the sheep industry (Coop and Holmes 1996). The economic losses incurred from gastrointestinal nematodes (GIN) impact meat and wool production, as well as sheep reproduction (Urquhart et al. 1996; Hayat et al. 1996; Suarez et al. 2009; Salehi et al. 2022). The clinical signs and sequelae associated with these infections vary

based on the parasite species present and the intensity of the infection. In sheep, symptoms can range from subclinical weight loss to severe conditions such as anemia, diarrhea and significant protein loss (Pugh and Baird 2012).

In this study, the culture method revealed the presence of nematode larvae at a rate of 65.7%, with no significant differences between the average infection rates of the concentration and development methods ($P > 0.05$). Although the egg development method requires more time, it proved to be more effective for diagnosing infections. This finding aligns with a study conducted in Ethiopia by Hailegebrie et al. (2017), which reported that the development method was more effective than other diagnostic methods, achieving a 97% accuracy rate. Similarly, a study in Brazil by Marchi and Cantos (2003) indicated that the larval development method yielded a positive identification rate of approximately 69.7% compared to other diagnostic approaches.

Morphometric measurements indicated a convergence between the lengths of the anterior and posterior sections of the larvae. This observation corroborates findings from Veena et al. (2020) in India, who noted that the lengths of the anterior and posterior parts of larvae of the same species and developmental stage tend to be similar.

The study also identified *Haemonchus contortus* larvae as the most prevalent, consistent with findings from Habtemichael et al. (2018) and Desalegn and Berhanu (2023) in Ethiopia, also with Elisa et al. (2021) in Colombia, which reported this species as the most common in sheep. Furthermore, larvae belonging to the genera *Trichostrongylus* and *Oesophagostomum* were noted to be of medium size, aligning with Van Wyk et al. (2004). In the long larvae group, a species from the genus *Dictyocaulus* spp., known as the filamentous lungworm, was identified, echoing findings by Taylor et al. (2007).

Regarding cryopreservation, the results indicated better survival rates for larvae stored in liquid nitrogen when placed in distilled water, as opposed to saline solution. Examination of larvae preserved in saline revealed instances of rupture, likely due to osmotic differences between the inside of the larvae and the saline environment. This outcome may also result from inadequate acclimatization time before cryopreservation; it is recommended that larvae be left in saline for at least 10 minutes prior to gradual exposure to liquid nitrogen, as confirmed by Jensen et al. (2000).

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Conflict of Interest: None.

Author's Contribution: Layla and Khadija devised the research concepts. Kholoud, Rowida, and Mabrooka conducted field and laboratory practices. Sara and Fatma composed the manuscript. All authors have reviewed and approved the final manuscript.

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