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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

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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 *et al*., 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 (Amarante *et al*., 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, and Trichuris spp. In a prior study by El-Khabaz *et al*. (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).

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

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 40 grams allocated for both examination methods.

Concentration method:

The sample is ground by adding distilled water, then filtered through medical gauze and transferred to 7 cm^3 tubes. Next, 3 cm^3 of formalin solution and 3 cm^3 of acetic acid are added. The tubes are centrifuged at 5000 rpm for 10 minutes, 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 & 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 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 ($P > 0.05$).

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Table 1: Prevalence of Gastrointestinal Parasites in Sheep Feces

Sign	Culture method	Concentration method	
P>0.05	35	35	No. examined feces sheep
	23 (65.7%)	11 (31.4%)	Infection rate

Note: 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 µm, with lengths ranging from 20 µm to 1033 µm. The average length of the anterior part of the larvae was 33 µm, with variations between 6 µm and 275 µm. Meanwhile, the average length of the posterior (tail) part was 31 µm, ranging from 3.2 µm to 4 µm. Based on these measurements, the larvae were classified into three categories: long, medium, and short. Significant differences (P ≤ 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 Tables 2, 3, and 4.

Table 2: Mean Total Length of Isolated Larvae Groups

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

** High significant differences

Table 3: Mean Length of the Anterior Part of Isolated Larvae Groups

Small larvae	Medium larvae	Long larvae	
9.62±0.97	18.2±.81	51.9±7.11	Mean±SE
** **			Sign

** High significant differences

Table (4) Average length 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	Mean±SE
** **			Sign

** 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 µm, 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 µm, 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 µm and a sharp, long tail, while 75% were identified as *Trichostrongylus* spp., measuring 100 µm 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 µm. These larvae were noted for their thick, dark brown appearance (Fig 5).

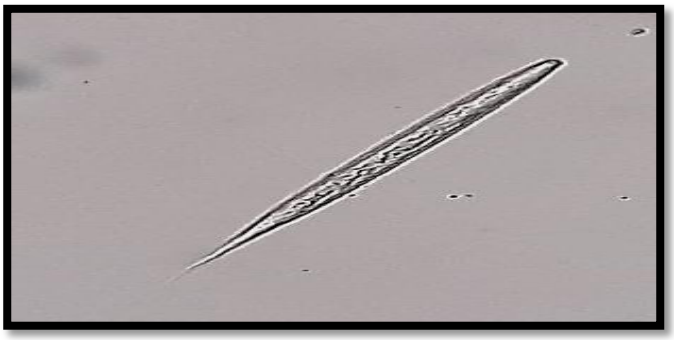

Fig 1: First-Level Larva (L1) of Haemonchus contortus
The arrow indicates the pointed short tail.

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.

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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.

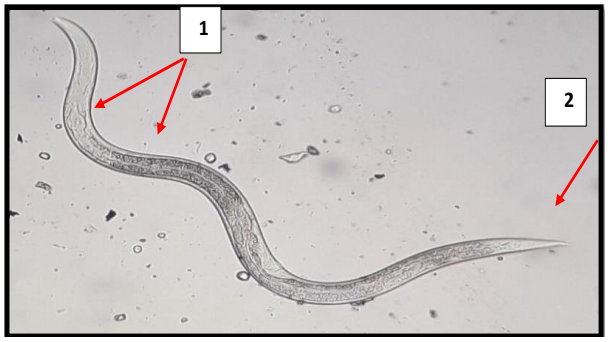

Fig 5: Larva of Dictyocaulus spp.

Arrow (1) indicates the shape of the esophagus and intestinal cells containing food residue, while arrow (2) points to the short tail.

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.

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

Normal saline	Distilled water	
1.33±0.49	9.2±1.5	Mean± SE
P≤0.05		Sign

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). 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 a 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 Hailegebriel *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 Blatt 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 Habtemchael *et al*. (2018) in Ethiopia, 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 Wyk (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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References

1. Abd El-Aal, S.A and Nosseur M. El-Sayed (2006) Prevalence of Gastrointestinal Nematode Parasites in Sheep and Parasite-Control Practices in Wady EL-Hayat-Libya Dept. Parasitology, Fac. Vet. Med. Zagazig University
2. Abebe, R., Meberahtu, G., Solomon, M., Fufa, A. and Alemayehu, R. (2010) Gastrointestinal Nematode Infection in Small Ruminants under the Traditional Husbandry Systems during the Dry season in Southern Ethiopia. Pp. 1112-1117.
3. AS El-Khabaz, K., & I Arafa, M. (2014) Study on the prevalence of internal parasitic infection in diarrheic and apparently healthy sheep at Assiut governorate, Egypt. Zagazig Veterinary Journal, 42(3), 157-168.

DOI:

4. Cabaret, J. (1983) The nematode parasites of large intestine of sheep in the Middle-Atlas (Morocco). Folia Parasit, 30, 117-121.
5. Coop, R. L., Holmes, P. H. (1996) Nutrition and parasite interaction. Int. J. Parasitol. 26: 951-62.
6. Dikmans, G., & Andrews, J. S. (1933) A Comparative Morphological Study of the Infective Larvae of the Common Nematodes Parasitic in the Alimentary Tract of Sheep. Transactions of the American Microscopical Society, 52(1), 1–25. https://doi.org/10.2307/3222221
7. do AMARANTE, A. F. T., & Amarante, M. R. V. (2016) Advances in the diagnosis of the gastrointestinal nematode infections in ruminants. Brazilian Journal of Veterinary Research and Animal Science, 53(2), 127-137.
8. Elmajdoub Layla O, Shimaa Sobhy Sorour, Mosaab A Omar and Alsaghir Om Assaed. (2022) Investigation and Prevalence of Gastrointestinal Parasites of Equestrian Clubs Horses in Misurata, Libya. Archives of Veterinary and Animal Sciences 4(1).
9. Gatehouse, G. (2012) Misrata votes for brighter Libyan future. BBC News. 2012-02-20. Retrieved on 2012-02-22).
10. Gill, J.H.; Redwin, J.M. (1995) Cryopreservation of the first stage larvae of trichostrongylid nematode parasites. International Journal for Parasitology, v.25, n.12, p.1421-1426.
11. Gray, S. J., & Kennedy, J. P. (1981) Gastro-intestinal parasites in sheep in an arid environment. Australian Journal of Experimental Agriculture, 21(109), 179-182.
12. Habtemichael1, Y. G., Meron Dejene1 and Solomon Mosu Eniyew. (2018) Prevalence of gastrointestinal helminth parasites and identification of major nematodes of cattle in and around Bishoftu, Oromia Region, Ethiopia. Journal of Veterinary Medicine and Animal Health, 10(7), pp. 165-172.
13. Hailegebriel, T., B. Petros, T. Endeshaw. (2017) Evaluation of parasitological methods for the detection of Strongyloides Stercoralis among individuals in selected health institutions in Addis Ababa, Ethiopia. Ethiop. J. Health Sci., 27, pp. 515-522, 10.4314/ejhs.v27i5.10
14. Hayat, C. S., Hussain, S. M., Iqbal, Z., Hayat, B. and Akhtar, M. (1996) Effect of parasitic nematodes on haematology and productivity of sheep. Pak. Vet. J. 16: 81-3. Heise, M., Epe, C. and Schnieder, T.
15. Jensen, J.R., Merlo, A. & Vieira-bressan, M.C.R. (2000) cryopreservation of first and infective stage larvae and the infectivity test by anigical inoculation of the nematodes *Haemonchus placei* and *Cooperia punctata*. Rev. Bras. Parasitol. Vet., 9, 1, 175-177.
16. Knoll, S., Dessì, G., Tamponi, C., Meloni, L., Cavallo, L., Mehmood, N. & Varcasia, A. (2021) Practical guide for microscopic identification of infectious gastrointestinal nematode larvae in sheep from Sardinia, Italy, backed by molecular analysis. Parasites & vectors, 14(1), 1-14.
17. Pugh, D. G. and Baird, N. (2012) Sheep & goat medicine. USA: Elsevier Health Sciences. pp. 216-238.
18. SOULSBY, E.J.L. (1982) Helminths, Arthropods and Protozoa of domesticated animals. 7th edn., Bailiere Tindall and Cassel, London 809 pp.
19. Suarez, V. H., Cristel, S. L. and Busetti, M. R. (2009) Epidemiology and effects of gastrointestinal nematode infection on milk productions of dairy ewes. Parasite. 16: 141-7.
20. Taylor, M.A., Coop, R.L. & Wall, R.L. (2007) Veterinary Parasitology. Third edition. Oxford: Blackwell Publishing Ltd.
21. Urquhart, G. M., Aremour, J., Dunchan, J. L., Dunn, A. M. and Jeninis, F. W. (1996) Veterinary parasitology 2thedition, The University of Glasgow, Blackwell sciences, Scotland, 3-137.
22. Van Wyk, J. A., Cabaret, J., & Michael, L. M. (2004) Morphological identification of nematode larvae of small ruminants and cattle simplified. Veterinary parasitology, 119(4), 277-306.
23. Veena, M., Puttalakshmamm, G. C., Ananda, K. J., & Sengupta, P. P. (2020) Morphological and molecular characterization of gastrointestinal nematodes in sheep. Int. J. Curr. Microbiol. App. Sci, 9(4), 2564-2575.
24. Marchi Blatt J, Cantos GA. (2003) Evaluation of techniques for the diagnosis of Strongyloides stercoralis in human immunodeficiency virus (HIV) positive and HIV negative individuals in the city of Itajaí, Brazil. Braz J Infect Dis. Dec;7(6):402-8. doi: 10.1590/s1413-86702003000600008. PMID: 14636480.

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