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FIRST DOCUMENTATION OF *NEMATODIRUS FILICOLLIS* FROM SHEEP IN IRAQ

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ABSTRACT : The present study was conducted to determine the parasitic infection with intestinal nematode species *Nematodiruis* spp. of sheep slaughtered in different areas from the slaughterhouses of Basrah, Iraq. Three hundred sixty sheep were examined during 2019- 2020. The nematodes were isolated from small intestine and kept into alcohol / glycerin for further diagnosis. It is worth commending that the diagnosis of *Nematodiruis* species was confirmed through the nomenclatural acts and registered in the Iraqi Natural History Research Center when a deposit number for Nematodes (sheep) *Nematodiruis filicollis* (Rudolphi, 1805) F: Anoplocephalidae: (INHM. 2020 Nem. 1.2). When the samples of nematodes were deposited in the helminth collection at the Museum of the University of Baghdad, this repository is considered as a confirmative for diagnosis and morphological analysis. Mixed infection with other parasites were reported with *Moniezia* spp., and significant increases were reported with *Cystericus tenuicollis* that considered importantly associated with *Nematodirus* infestation among the examined sheep. For our knowledge, this study is the first Iraqi report associated with confirmation of adult *Nematodirus filicollis* parasite in sheep.

Key words : *Cystericus tenuicollis*, *N. spathiger*, Lucida camera, Basrah province.

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INTRODUCTION

Nematodirus spp. (Nematoda: *Anoplocephalidae*) considers an important pathogenic parasite in small intestines of domesticated and ruminants animals throughout the world taxonomy (Taylor, 2016). *Nematodirus* nematodes are composed of more than 45 species, many of which are distributed in ruminants, and their distribution are generally global throughout the world (Nadler *et al*, 2000 and Oliver *et al*, 2014). *Nematodirus filicollis* was recognized as a major parasites of lambs (Pomroy, 1997). The clinical signs of disease in lambs with *Nematodirus* infections include the loss of appetite, sometimes failure to gain weight or weight loss, profuse diarrhea, and dehydration (Vlassoff and McKenna, 1994). The outbreak of disease commonly occurs suddenly. Lamb with subclinical parasitism usually results in a significant reduction in carcass value and a persistent decrease in milk production and wool growth (Miller *et al*, 2012). In previous studies, parasites are considered to be a major problem in ruminants by causing disease, death and production loss (Islam *et al*, 2017).

Nematodirus species having a direct life cycle and comprises a stage within the host and a stage outside the host. Each worm in the host has been ingested from larvae contaminated pasture. The females reside in the small intestines and lay eggs which are passed out in the feces of the host (Familton and McAnulty, 1997). Especially in the case of *Nematodirus* spp., which may expend many months as an egg on pasture (Vlassoff *et al*, 2001). Taxonomic identification of *Trichostrongyloid* nematode is usually the species being identified by the characteristics of each males and females which usually not identifiable. The six species of *Nematodirus* have been previously identified by characteristics of the limb spicule and group bursa (Becklund and Walker, 1967). Although, the morphological similarity of eggs, there was no absolute correlation between worm burden and egg output (Demeler *et al*, 2010). Infections by adult worms can be

easily diagnosed and controlled, but infection by migrating and inhibited larvae are difficult to diagnose (Auda, 2020). The parasite eggs were observed in the feces of infected sheep as a part of stool examination methods during previous studies conducted inside Iraq (Al-Robaiee *et al*, 2019; Gatie and Nejiban, 2019). Therefore, *Nematodirus filicollis* have not been reported as being a parasitic worm for sheep in Iraq, and this study represent the first report in lambs infected with *Nematodirus*.

MATERIALS AND METHODS

Study area and Samples collection

Basrah was selected for this study in different random areas by organizing weekly visits to the central slaughterhouses for sheep in Basrah Governorate (Garmat Ali, Al-Jumhuriya, Al-Mawfakia, Al-Jumhuriya, Al-Jubaylah and Al-Hakimiah). Basrah is one of the Southern governorates. On one day every week, regular visits were made to various areas of Basrah Governorate, where the central slaughterhouses are for the random slaughter of sheep,during 2019- 2020. Totally, 360 small intestine samples of sheep was examined and used to diagnose and identify parasitic worms. Samples were made regardless of age and sex of a host. However, the complete data concerned the age and sex of infected animals were reported. The samples were transferred to Parasite Research Laboratory at the College of Veterinary Medicine, University Basrah, Basrah, Iraq.

Laboratory study

Procedures for examination and collection of parasites

The small intestine was sectioned surgically using special surgical tools, and a longitudinal incision was made to detect the presence of parasitic worms in its contents or their attachment to epithelium of small intestine. *Nematodirus* spp. were obtained through using the modified techniques (Hansen and Perry, 1994). Nematodes were collected, contents of small intestine were emptied into a bucket, intestinal mucosa was washed separately in clean tap water and the mucous layer was examined with a magnifying glass with a high power light to detect it due to their very small size and accuracy. The washers were combined with the contents of small intestine, mixed well and passed through an aperture of a mesh sieve and all retained material was kept along with the parasites. The isolated worms were washed several times with distilled water, transferred to sterile plastic containers, and preserved into 70% alcohol, and then in glycerol alcohol (10 parts glycerol and 90 parts 70% alcohol). The preserved nematodes were smeared on glass slides in anhydrous glycerol,and examined under an optical lens of light microscope at ×4, ×10 and × 40.

Fixation and preservation clearing

Nematodirus filicollis were fixed immediately after collection in 70 percent alcohol. The gums in this vial were partially coated until all ethyl alcohol evaporated for at least 24-72 hours before the microscopic inspection to allow morphological studies. Females and males were described, and nematodes were microscopically recognized by the standard taxonomic keys (Taylor, 2016). The morphology of spicules and bursas were the key characteristics used for identification of male *Nematodirus filicollis* and photographed where appropriate. After identification, the following measurements were made for many worms: body length, spicule length, anterior part length. These features were measured at 10× eyepiece and 40× objectives, calibrated initially using a stage micrometer and presented as the range (measurements micrometers).

Taxonomic identification (Morphology)

The phenotypic characteristics (color and length of *Nematodirus* of adult nematode part) of collected worms were recorded and measured with a microscope standard ruler.*Nematodirus* males were randomly selected from all examined small bowels. For each worm, three morphometrical spicule parameters, total length (TL) and distance from hook to tip of the right spicule (THr) were measured using the ocular micron. The discrimination feature (DF), together with these three male spicule measurements was used to classify each male worm's species (Jacquiet *et al*, 1997). These worms were sent to the Registration System of the Iraqi Center for Natural History Research and the Museum, University of Baghdad, while, the samples of nematodes were deposited at the museum to confirm diagnosis and morphological analysis.

Percentage infection of slaughtered sheep

The number of infected animals and uninfected animals was recorded as personal data, and tables of collected information and the percentage of parasitic worms infection were used for the total number of animals that were collected during the study period.

RESULTS AND DISCUSSION

The findings for morphology of study samples and identification of worms found in small intestine of slaughtered sheep was showed an identity for the genera of Nematodes and named as *Nematodirus*. Based on the identification keys, gastrointestinal nematode species were highlighted as *Nematodirus filicollis. Nematodirus*

spp. was identified in small intestine of 360 sheep examined, 26 were positive to *Nematodirus* helminths infestation were found at different age, sex, species, and origin were examined. Detected nematodes were *Nimatodirus filicollis* showing a prevalence at 7.22% (Table 1).

Tabel 1 : Total resultsto detect *Nematodirus* spp. in slaughtered sheep.

Species	Total No.	Infected	
		No.	(%)
Nematodirus filicolis	360	26	7.22%

The characteristics of the adult *Nematodirus* spp. as a filiform worm with the cuticle of head being inflated, while on the body, there are between 14 and 24 synlophe. The mouth is oval, and buccal cavity is very short. In male, bursa had two large lateral lobes, and spicules were slender, tubular, filiform and greater than 0.5 mm long, copulatory bursa, lateral view, showing distribution of bosses and position of bursal rays. Female was larger than males with vulva, a transverse slot that opens in the middle third of the body. The posterior was truncate with a short slender spin. The body characteristics of parasites are white. These nematodes were harvested from the small intestinal mucosa and bottom trays after repeated washings. They appeared as whitish filiform, and can distinguish between male and female.

Adult *Nematodirus* worm was slender, white in colour, and moderately long with a coiled appearance. The anterior part is thinner than posterior. Length of males was apprximately 10-15 mm, and for females, it was approximately 15-24 mm. Adult had a small, but distinct, cephalic vesicle (blister like inflation over head) and the cuticle (outer covering) hold about 14-18 longitudinal ridges. The male bursa (an external male copulatory appendage, used to grip the female during mating) had elongate lateral lobes and the spicules (needle-like mating structures) are long and slender, and the tips of the spicules are joined except terminate in small expansion (Figs. 1-8).

The spicules was vary in shape and this a useful feature for species differentiation. The worm also had ventral rays that were parallel and found close together. The female worm had a short tail with a slender terminal appendage (Taylor, 2016). The infective larvae was noticeably longer than other trichostrongyloids and having eight large elongated intestinal cells with a prominently longer sheath tail and evident terminal appendages (Van Wyk *et al*, 2004). The mean length was approximately 912-1018µm (Thomas, 1957).

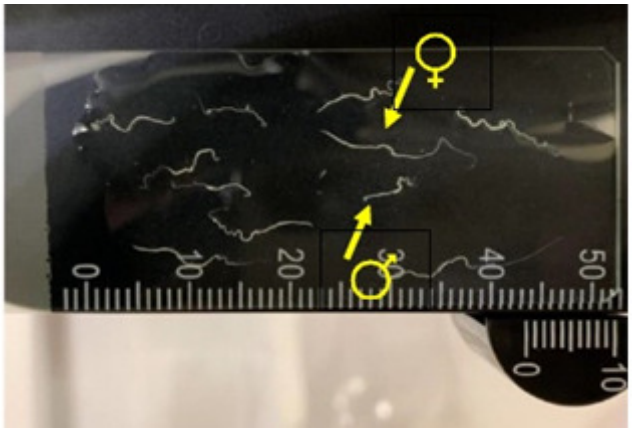

Fig. 1 : Adult *Nematodirus* spp. on slide with normal saline and ruler for microscopically stage.

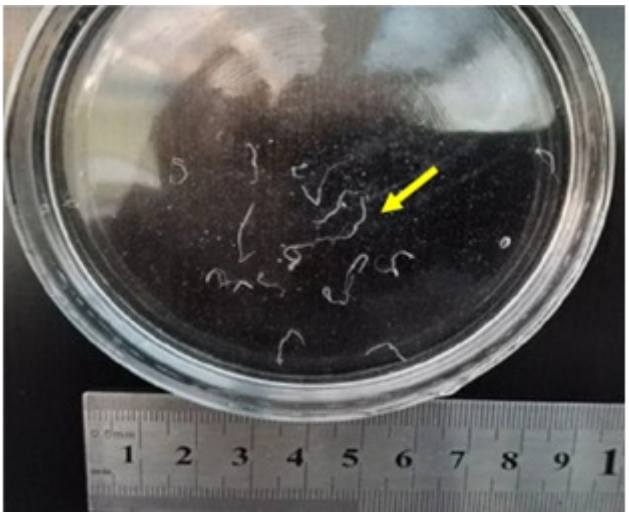

Fig. 2 : Adult of *Nematodirus* spp. in petrydish with normal saline and ruler.

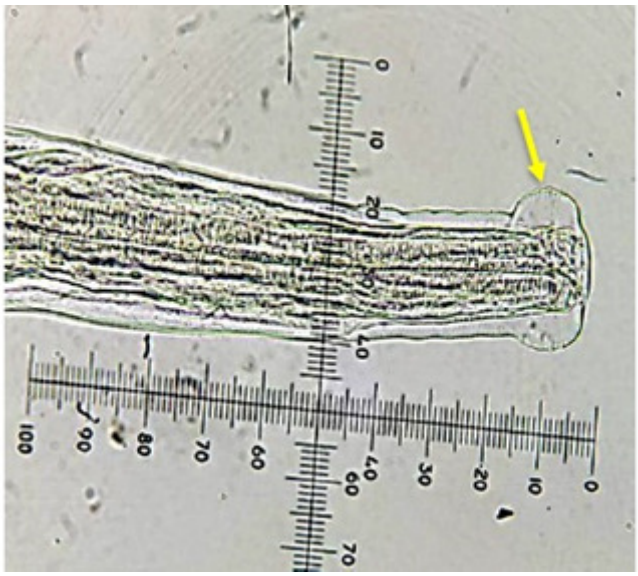

Fig. 3 : Oval mouth of *Nematodirus* spp. without cervical papillae × 10.

The *Nematodirus* eggs were distinctive and roughly twice the size of typical trichostrongyloid egg, measuring

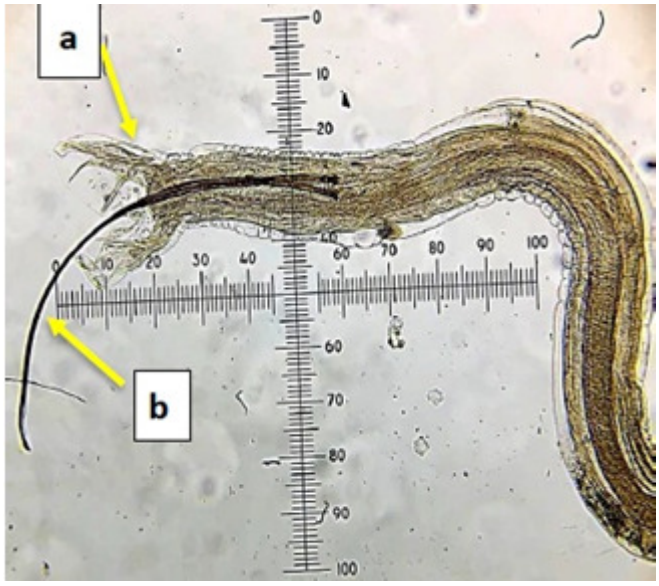

Fig. 4 : Posterior end of male *N. filicollus* with bursa and spicules ×4.

Fig. 6 : Caudal end of male *N. filicollus* × 4.

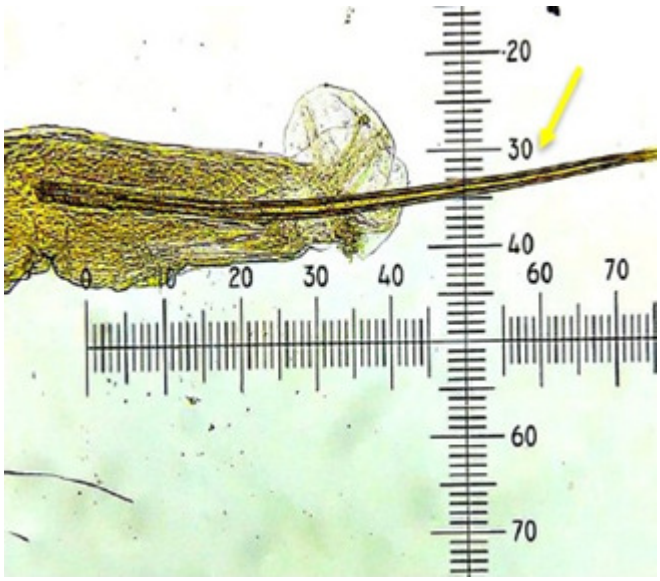

Fig. 5 : Ventral view of male spicule *N. filicollus* with bursa ×10.

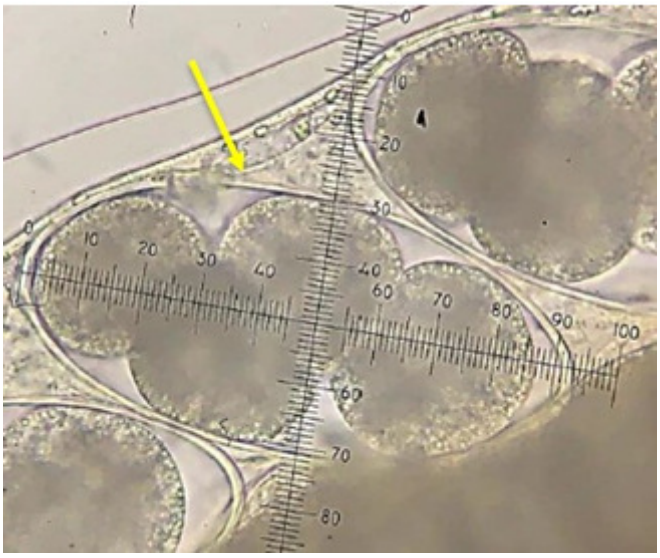

Fig. 7 : Egg of *Nematodirus* spp. shows single profile in genital tract near digestive system ×40.

approximately 210µm × 100µm (L×W) and were easily distinguishable from other trichostrongyloid eggs. Additionally, they were ovoid, colourless and thin-shelled (Taylor, 2016). When the eggs passed in feces, they contain two to eight large granular cells, which are taking up nearly two thirds off egg. The development of larvae takes place exclusively within the egg membranes and the egg will not hatch before the larva has reached the infective stage, providing that the environmental requirements have been met. The morphological characteristics are microscopic and are used for identification (Kates and Turner, 1955).

The egg size of *Nematodirus* spp. was 200 ±3µ long and 95±2µ wide. Presented thin sheath, without color and conical ends. The fact that the eggs are colorless, their morphology with conical ends (Figs. 9-11). Copulatory bursa (lateral view) showed distribution of bosses and position of bursal rays (Fig. 12).

This study showed that sheep in Basrah is a host for a number of nematode species. The animals were both adult males, female and it may be that other infections are associated with younger animals of various ages and sexes. The findings were compared with previous studies on helminth infections in related species in different countries. Iran and turkey (Naem and Gorgani, 2011; Gönenç *et al*, 2018). The eggs are large, usually, over 150µm long. Interestingly, Oliver (2015) mentioned that *Nematodirus* spp. for males as having the same length, with the adult male 10-15 mm long, while the female was larger, measuring 15-23mm in length. Lichtenfels and Pilitt

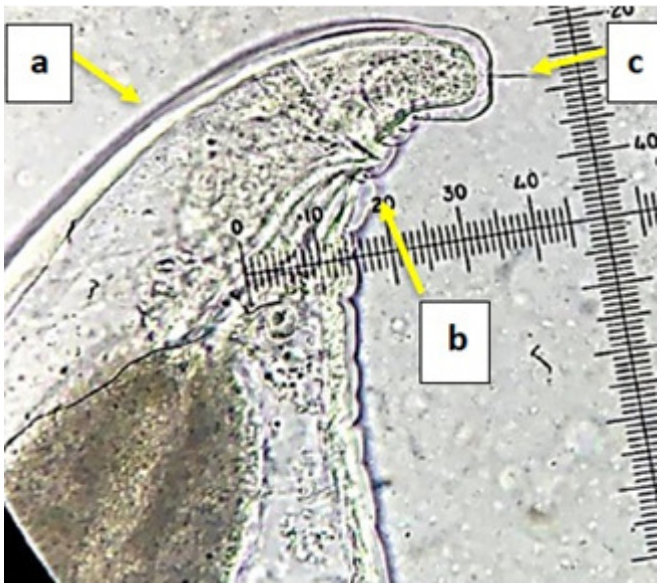

Fig. 8 : Posterior end of female *Nematodirus* spp. with a spin ×40.

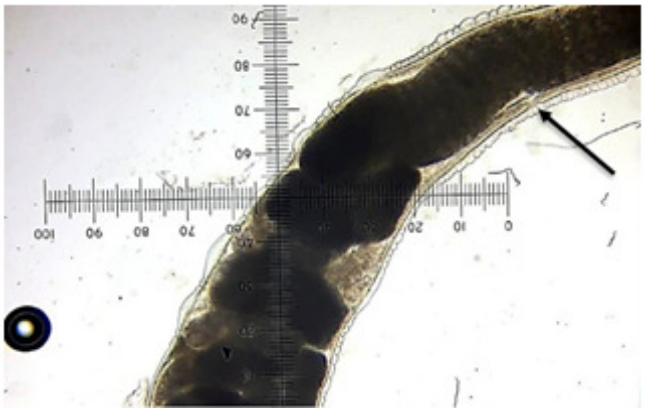

Fig. 9 : Oesophageo-intestinal junction of *Nematodirus* spp. ×10.

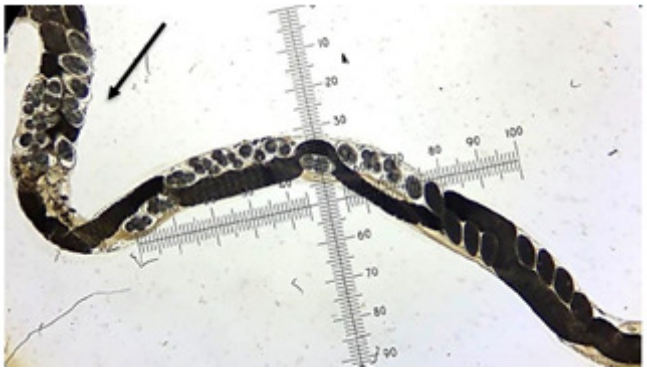

Fig. 10 : Female *Nematodirus* spp. shows accumulation of eggs in a uterus with genital tract ×100. Female vulvar region (black arrow) × 20.

(1983), one of the primary diagnosis between the adults of the two species is the male spicules. In *N. spathiger,* they are 0.7-1.10 mm long and end in a spoon-shaped enlargement, whereas the spicules of *N. filicollis* are 0.68-0.95 mm long and end in a narrow, pointed piece. Helminth parasites of the digestive tract are also widespread among domestic sheep and goats in Iraq. The

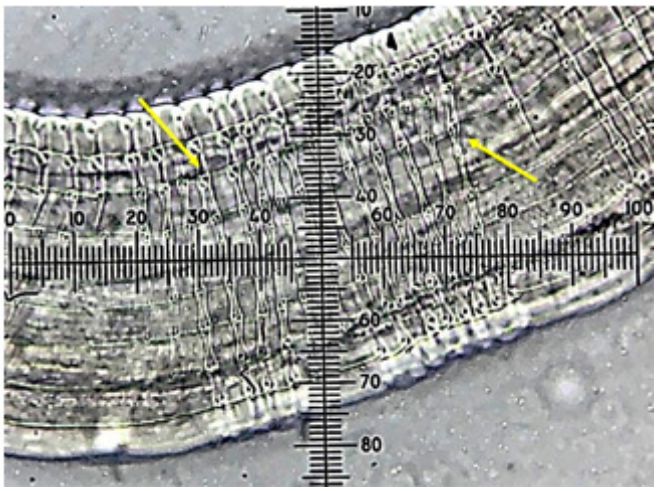

Fig. 11 : Cephalic extremity of *Nematodirus filicollus*, shows detail of synlophe Longitudinal ridges on cuticle 14-18 as transversally striated with wide × 40.

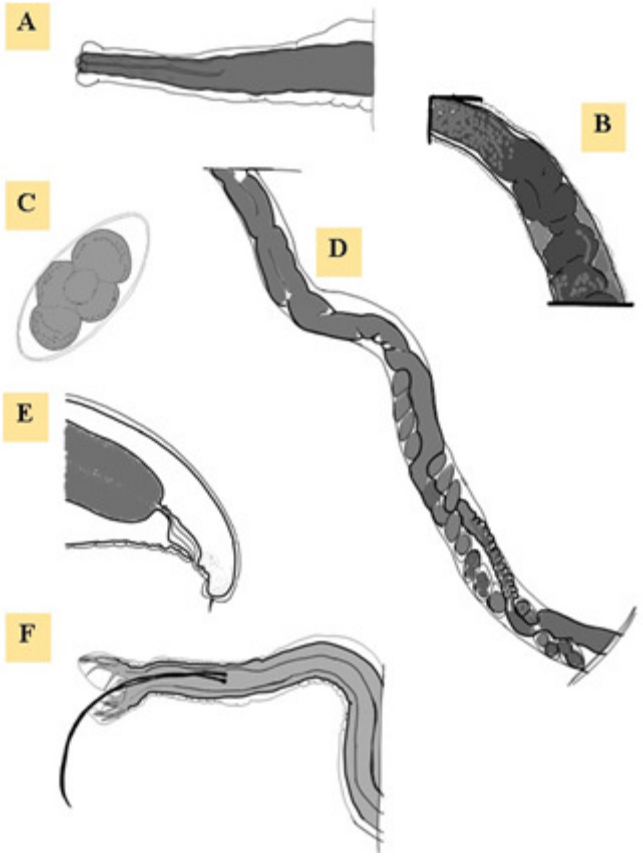

Fig. 12 : *Nematodirus filicollus* in sheep. **(A):** Mouth opening with cephalic extremity and esophagus filiforms of anterior part, **(B):** Tubular structure of Oesophageo-intestinal junction *Nematodirus* spp., **(C):** Eggs in examined feces show the six to eight large granular cells, **(D):** Uterine tube (lateral view) shows the vestibula and egg in oviduct irregulae polar thickenings in the shells, appear uterine and intestinal twisting, **(E):** Copulatory bursa of male (ventral view) shows distribution of bursal rays with terminal portion of spicules, **(F):** Female tail (lateral view) shows the anus and terminal spin.

distribution of *Nematodirus*spp. in domestic sheep were studied in only egg inspectionas it reported in Duhok and Thi-Qare, identified by eggs isolated from sheep faeces (Mohammed Ameen, 2017; Gatie and Nejiban, 2019). This is the first record of intestinal nematodes from 360 sheep in Basrah, Iraq. The study emphasizes the need for more studies that may help design appropriate strategies to control parasites of helminths in sheep. Importing sheep from neighbouring countries could be a source of gastrointestinal nematode infection for domestic livestock in their living area. In addition, there is a need for further investigation into the environment, seasonality, and the effect of parasites on local sheep populations in Iraq. We recommend a study supplement including the age, monthly variations, and the location of the animals were very important factors associated with gastrointestinal nematode infection. Types of gastrointestinal nematodes identified in sheep slaughtered in Basrah, corresponded largely with the same species identified by authors, others in other countries (Indre *et al*, 2011; Salam *et al*, 2017; Gönenç *et al*, 2018). Observed assorted infection with *Moniezia* spp. were reported and *Cystericus tenuicollis* was considered as important as connected with *Nematodirus* infestation the examined sheep.

REFERENCES

Al-Robaiee I, Sabah Z, Ahmed K and Salih S A (2019) Diagnostic study of ovine gastrointestinal parasites in Kirkuk city, Iraq. *Adv. Anim. Vet. Sci*. **7**(9), 727-731.

Auda H M, Khalifa M M, Amin M M and El Bahy M M (2020) Value of pooled gastro-intestinal nematode coproantigens in the diagnosis of inhibited larvae infection in Egyptian Sheep. *J. Egypt. Soc. Parasitol.* **50** (1), 98-104.

Becklund W W and Walker M L (1967) Nematodirus of domestic sheep, *Ovis aries*, in the United States with a key to the species. *The J. Parasitol.* 777-781.

Demeler J, Küttler U, El-Abdellati A, Stafford K, Rydzik A, Varady M and Vercruysse J (2010) Standardization of the larval migration inhibition test for the detection of resistance to ivermectin in gastrointestinal nematodes of ruminants. *Vet. Parasitol.* **174** (1-2), 58-64.

Familton A S and McAnulty R W (1997) Life cycles and development of nematode parasites of ruminants. *Sustainable control of internal parasites in ruminants: Animal industries workshop* 67-79.

Gatie J A and Nejiban Z S (2019) Prevalence of gastrointestinal parasites infestation in sheep of Thi-Qar Province, Iraq. *Al-Qadisiyah J. Vet. Med. Sci.* **18**(1), 123-128.

Gönenç B, Hasan E M Ý R and andIacob O (2018) Digestive tract helminths of Turkish ibex (*Capra aegagrus aegagrus* Erxleben, 1877). *Ankara Üniversitesi Veteriner Fakültesi Dergisi* **65**(3), 247-251.

Hansen J and Perry B (1994) *The epidemiology, diagnosis and control of helminth parasites of ruminants*. International laboratory for Research on Animal Diseases, Nairobi, Kenya.

Indre D, Balint A, Hotea I, Sorescu D, Indre A and Darabus G (2011) Trichostrongyles species and other gastrointestinal nematodes identified in sheep from Timis county. *Bull. Univ. Agricult. Sci. Vet. Medicine Cluj-Napoca. Veterinary Medicine* **68**(2), 171-178.

Islam M S, Hossain M, Dey A, Abdul Alim M and Akter S (2017) Epidemiology of gastrointestinal parasites of small ruminants in Mymensingh, Bangladesh. *J. Adv., Vet. Anim. Res*. **4**, 356-362.

Jacquiet P, Cabaret J, Cheikh D and Thiam E (1997) Identification of *Haemonchus* species in domestic ruminant based on morphometrics of spicules. *Parasitol*. **83**, 82-86.

Kates K C and Turner J H (2011) An experiment 011 the combined Pathogenic effects of *Haemonchus contortus* and *Nematodirus spathiger* on lambs. *J. Helm*. **28**, 127-134.

Lichtenfels J R and Pilitt P A (1983) Cuticular ridge patterns of Nematodirus (Nematoda: Trichostrongyloidea) parasitic in domestic ruminants of North America, with a key to species. *Proc. Helminthol. Soc. Washington* **50**(2), 261-274

Miller C M, Waghorn T S, Leathwick D M, Candy P M, Oliver A M B and Watson T G (2012) The production cost of anthelmintic resistance in lambs. *Vet. Parasitol.* **186** (3-4), 376381.

Mohammed Ameen M G (2017) Prevalence of gastrointestinal helminthes in sheep from Bardarash district, Duhok Province. *ZANCO J. Pure Appl. Sci.* **28** (6), 166-173.

Nadler S A, Hoberg E P, Hudspeth D S and Rickard L G (2000) Relationships of *Nematodirus* species and *Nematodirus battus* isolates (Nematoda: Trichostrongyloidea) based on nuclear ribosomal DNA sequences. *J. Parasitol.* **86**(3), 588-601.

Naem S and Gorgani T (2011) Gastrointestinal parasitic infection of slaughtered sheep (Zel breed) in Fereidoonkenar city, Iran. *Vet. Res. Forum* **2**, 238-241.

Oliver A M B, Leathwick D M and Pomroy W E (2014) A survey of the prevalence of *Nematodirusspathiger* and *N. filicollis* on farms in the North and South Islands of New Zealand. *N. Zeal. Vet. J.* **62**(5), 286-289.

Oliver A M B (2015) Studies on the epidemiology of *Nematodirus spathiger* and *Nematodirus filicollis* in New Zealand: A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science at Massey University, Manawatu, New Zealand (*Doctoral dissertation*, Massey University).

Pomroy W E (1997) Internal helminth parasites of ruminants in New Zealand. In : *Sustainable control of internal parasites in ruminants*. (ed. Barrell G K), Animal Industries Workshop (pp. 11-23).

Sallam N H, Assasa A Z, Elgayar A K and Abd-ElAal A A (2017) Gastrointestinal nematodes infections in ruminants: Prevalence and Anthelmintic efficacy. *Egypt. Vet. Medical Soc. Parasitol. J.* **13**(1), 38-54.

Taylor M A and Coop R L (2016) *Veterinary parasitology*. 4th. ed. Chichester: Wiley Blackwell. pp. 22-24.

Thomas R J (1957) A comparative study of the infective larvae of *Nematodirus* species parasitic in sheep. *Parasitology* **47** (1-2), 60-65.

Van Wyk J A, Cabaret J and Michael L M (2004) Morphological identification of nematode larvae of small ruminants and cattle simplified. *Vet. Parasitol.* **119** (4), 277-306.

Vlassoff A and McKenna P B (1994) Nematode parasites of economic importance in sheep in New Zealand. *N Zeal. J. Zool.* **21**(1), 1-8.

Vlassoff A, Leathwick D M and Heath A C G (2001) The epidemiology of nematode infections of sheep. *N Zeal. Vet. J.* **49** (6), 213-221.