



## Identification of nematodes third stage larvae of ruminant animals

### Abstract

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The present study was carried out to identify the infective nematode larvae of cattle and sheep. Also, to differentiate some important nematodes infecting ruminants at the generic and species levels as a method helping for epidemiological studies. For this purpose, faecal samples were randomly collected from cattle and sheep, examined using standard floatation technique and positive samples with Strongyle type eggs were identified using coproculture. Identification of third stage larvae was based on the shape of the head, tail and sheath extending beyond it, size measurements of both larva and sheath (including; anus to tip of sheath and end of tail to tip of sheath). The morphometrical characters were studied for six 3<sup>rd</sup> stage larvae of gastrointestinal nematode genera infecting cattle and sheep. Namely, *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Strongyloides*. A key for the identification of these larvae is given and photographs are also provided to assist in their identification.

Key words: Larva identification, nematodes, cattle, sheep.

## Introduction

Ruminant animals are hosts to a great variety of nematode parasites, some of which can cause significant morbidity or mortality. The majority of them are members of the family Trichostrongylidae, which includes the most pathogenic and economically important nematode parasites of ruminants. Knowing which of these species and at what relative levels they are infecting animals is extremely important for preventing and managing parasitic disease (Jurasek et al., 2010). Due to differences in the pathogenicity of the common worm genera; identification of the genus of the nematodes is essential in evaluating the importance of worm infection and / or the efficacy of anthelmintic treatment (van Wyk et al., 2004). The similarities in size and shape of the eggs of different species of gastrointestinal nematodes makes their differentiation is extremely difficult, (Dikmans and Andrews., 1933; Ministry of Agriculture,

fisheries and food, 1977). The standard method for identifying eggs of Trichostrongyle nematodes in faeces is culturing the faeces for 10–14 days and isolating the third stage (L3) larvae, which can be identified to genus level. Identification of third stage infective larvae in cultured ruminant faeces is challenging but not formidable (Bowman, 2014), which need an experienced worker to distinguish between those of different genera, (Ministry of Agriculture, fisheries and food, 1977; van Wyk et al., 2004; Jurasek et al., 2010 and Taylor., 2010). The present study has been conducted to identify and clarify the morphological differences between genera of some nematodes infecting ruminants specially those of Trichostrongyle nematodes.

## Material and methods

In the present study, faecal samples were collected from cattle and sheep of various ages, sex and breeds reared in large organized

private farms, small private non organized farms and animals owned by small scale farmers in Dakahlia province, Egypt. All faecal samples were collected directly from rectum or immediately after defecation from individual animal in well-labeled sterile polyethylene sac and transferred in icebox to the laboratory of parasitology, Faculty of Veterinary Medicine, Mansoura University to be examined for the presence of gastrointestinal nematodes.

Examination of faecal samples was done by the standard floatation techniques using saturated salt solution. Positive samples with Strongyle type eggs were cultured for 7-14 days, then recovery of larvae using modified *Baerman's* technique following the guidelines illustrated by **Ministry of Agriculture, fisheries and food (1977)** and **Zajac and Conboy (2012)**. Then, drop of culture fluid mixed with drop of lugol's iodine for killing and staining of recovered larvae for morphological identification. Characteristic 3<sup>rd</sup> stage larvae of strongylid parasites were identified based on the shape of the

head, tail and sheath extending beyond it, and size measurements of both larva and sheath lengths (including; anus to tip of sheath and end of tail to tip of sheath), then compared with different keys illustrated by various investigators.

For size measurements, an ocular micrometer was used and microscopic calibration reveals that each division of ocular micrometer with 10x objective lens equals to 10  $\mu\text{m}$  and with 40x objective lens equals to 2.5  $\mu\text{m}$ . Total length of each larva was measured using 10x objective lens, while lengths from anus to tip of sheath and from end of tail to tip of sheath were measured using 40x objective lens.

## Results and Discussion

There has been significant progress in species differentiation of Strongylid nematodes specially those of Trichostrongylids in ruminant animals, starting from the differentiation of certain Strongylid parasite species/genera based on their egg morphology (**Shorb; 1939; Georgi and McCulloch; 1989**), to the development of molecular assays

for the specific identification of nematodes and staining methods, such as the lectin-binding assay which selectively stains the eggs of *H. contortus*, (**Jurasek et al., 2010**). Faecal culture and larval identification is the most widely used and best method currently available (**van Wyk et al., 2004** and **Jurasek et al., 2010**). Limitations of faecal culture and advanced techniques have been reported and well discussed in publication of **Roebera and Kahn; 2014** and finally they stated that the technology has not replaced microscopy but should be used together.

Strongylid type eggs found during routine floatation technique were oval, thin shelled, colorless and measured 70 -100  $\mu\text{m}$  in length by 35 - 50  $\mu\text{m}$  in width, (**Plate; I, 1 and 2**), while that of *Strongyloides* spp. were thin shelled with rather bluntly two ends, containing well developed larvae and measured 48 – 60  $\mu\text{m}$  in length by 25 - 33  $\mu\text{m}$  in width, (**Plate; I, 3**). The morphometrical characters were studied for six gastrointestinal nematode genera 3<sup>rd</sup> stage larvae of

cattle and sheep, including: *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Strongyloides*.

#### 1. *Strongyloides*:

The infective larva of this genus obtained from sheep is slender; short sized and measured 550-650  $\mu\text{m}$  in length. The most characteristic is the presence of very long esophagus which extending nearly for 40% of the body length. Absence of tail sheath and high magnification shows that the tail is bifid, (**Plate; I, 5 and 6**). However, being identified from the shape and size of their eggs, *Strongyloides* larvae may be present in faecal cultures that necessitate their differentiation from other nematodes larvae and the most characteristic is the esophagus as different authors described its length in proportion to its body; more than one-third (**Keith, 1952; Hansen and Shivnani; 1956; Bowman, 2014**) or nearly half length (**Ministry of Agriculture, fisheries and food; 1977**).

#### 2. *Oesophagostomum*:

This is a medium sized larva, measured around 800  $\mu\text{m}$  in length as the body has a rather stumpy appearance and killed larvae with iodine usually lie in a characteristic semicircular position (**Plate; I, 7**), which agreed with **Keith, 1952**. The tail of the sheath tapers rapidly to end in a characteristic long, fine filament where, length from anus to tip of sheath was 210–250  $\mu\text{m}$  and from end of tail to tip of sheath was 150-190  $\mu\text{m}$ . Rounded head and 16–24 triangular intestinal cells could be identified, (**Plate; II, 1 and 2**).

### 3. *Ostertagia* and *Trichostrongylus*:

Differentiation between those two genera is difficult based on morphology of L3 (**Zajac and Conboy, 2012; Roeber et al., 2013; Roebera and Kahn, 2014**) due to short tail sheath in both especially in ovine larvae. Demarcation between those two genera being dependent on the larval size as medium or large sized L3 of *ostertagia*, while short sized L3 of *Trichostrongylus*. Also, tail of *Trichostrongylus* is bearing one or two tuberosities, (**Ministry of Agriculture, fisheries and food;**

**1977**). Moreover, the head of ovine *Ostertagia* has a slight shoulder close to its anterior end giving it a square appearance, while absent in *Trichostrongylus* that makes its head rounded (**Lancaster and Hong; 1987**).

Our results showed that *Ostertagia* infective larvae measured 900–1000  $\mu\text{m}$  in cattle species and 800–950  $\mu\text{m}$  in sheep species. Lengths from anus to tip of sheath were 150–175 $\mu\text{m}$  in cattle and 95–130  $\mu\text{m}$  in sheep, from end of tail to tip of sheath were 60–75  $\mu\text{m}$  in cattle and 30–50  $\mu\text{m}$  in sheep. Head squared and measurements illustrated that the tail sheath being shorter in sheep species, (**Plate; II, 3 and 4**).

The measurements of *Trichostrongylus* 3<sup>rd</sup> stage larvae showed that the total lengths were 700–750  $\mu\text{m}$  in cattle species and 700–800  $\mu\text{m}$  in sheep species. Lengths from anus to tip of sheath were 90–100  $\mu\text{m}$  in cattle and 95–110  $\mu\text{m}$  in sheep; from end of tail to tip of sheath were 30–40  $\mu\text{m}$  in both cattle and sheep, which in turn reflects the similarity between ovine *Ostertagia*

and *Trichostrongylus* 3<sup>rd</sup> stage larvae. Characteristically, tail of *Trichostrongylus* bearing one or two tuberosities while, tail of *Ostertagia* being rounded at its end, (**Plate; II, 5 and 6**)

#### 4. *Haemonchus*:

Medium sized larva measured 750–850  $\mu\text{m}$  in cattle species and 680–800  $\mu\text{m}$  in sheep species with rounded head. Lengths from anus to tip of sheath were 150–190 $\mu\text{m}$  in cattle and 135–150 $\mu\text{m}$  in sheep; from end of tail to tip of sheath were 85–100  $\mu\text{m}$  in cattle and 68–80  $\mu\text{m}$  in sheep. The tail of the sheath tapers to end in a whip-like, medium sized filament and usually kinked, (**Plate; II, 7 and 8**)

#### 5. *Cooperia*:

Large sized larva with squared head, measured 850–1000  $\mu\text{m}$  in cattle species and around 900  $\mu\text{m}$  in sheep species. Lengths from anus to tip of sheath were 145–190 $\mu\text{m}$  in cattle and 140–155 $\mu\text{m}$  in sheep; from end of tail to tip of sheath were 80–100 $\mu\text{m}$  in cattle and 70–85 $\mu\text{m}$  in sheep. Medium sized sheath tapering to fine point (*C. oncophora*).

Characteristically, Head bearing two refractile oval bodies at anterior end of the esophagus, (**Plate; II, 9 and 10**). Morphometrical parameters of tail sheath indicated that the longer tail sheathed larvae of *Cooperia oncophora* was the isolated species, which agreed with **Dikmans and Andrews, 1933; Keith, 1952; Hansen and Shivnani; 1956; Bowman, 2014.**

Previous studies on infective larvae of gastrointestinal nematodes infecting cattle and sheep illustrated several valuable keys which is useful to get an approximation of genera present (**Roebera and Kahn; 2014**), but definite diagnosis of species involved is very difficult, however some authors described specifically the morphological characters of some species based on culturing of eggs obtained from nematodes of slaughtered sheep (**Dikmans and Andrews; 1933**) and cattle (**Hansen and Shivnani; 1956**) where, *C. oncophora* is the only species that can be identified easily from other *Cooperia* species. No attempt was made to make a detailed study of

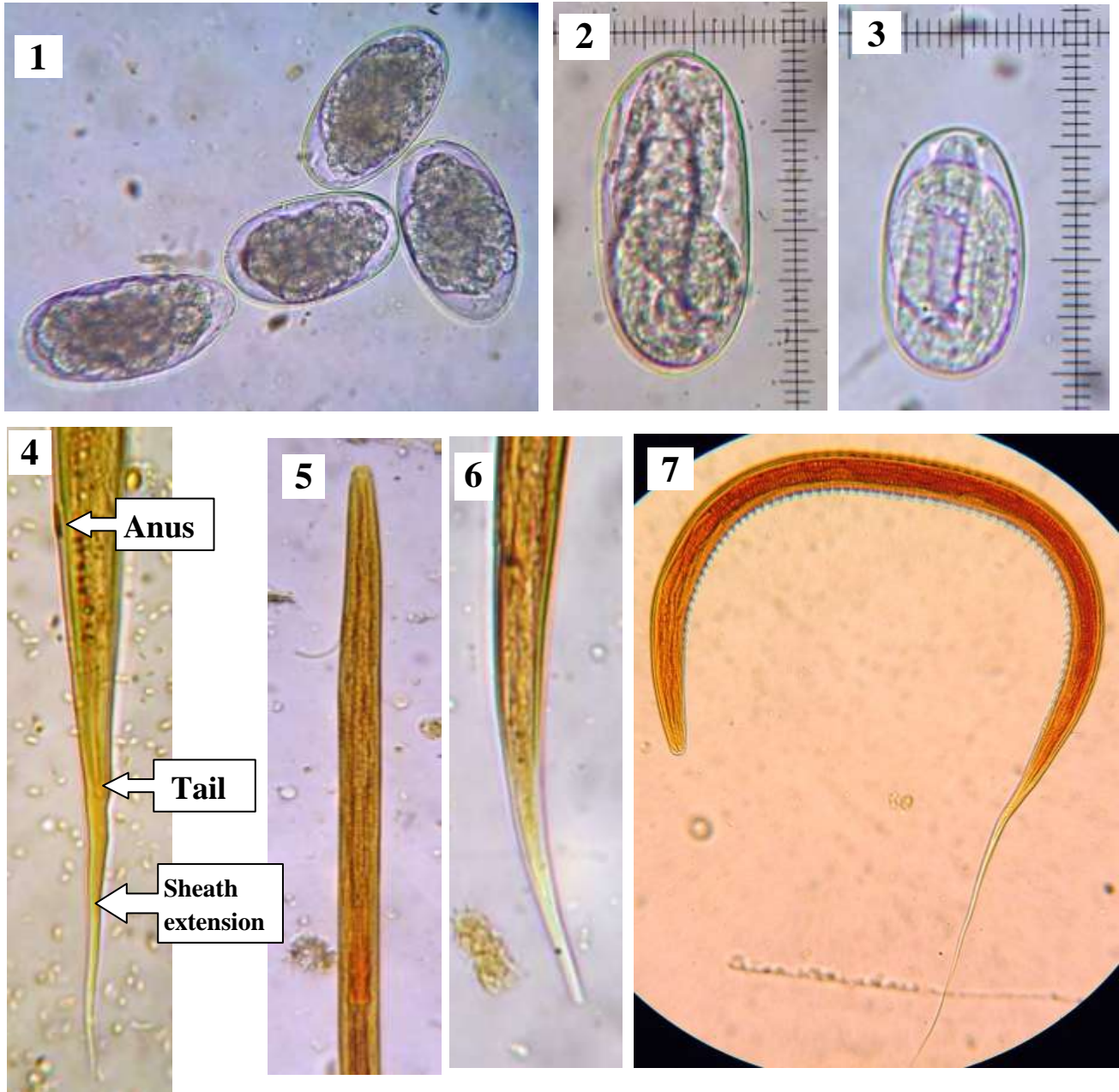
larval morphology and study aiming to clarify characters useful for the quick diagnosis. However, some authors gave detailed larval morphology including intestinal cells and esophagus which complicate the identification. In some genera as *Oesophagostomum* and *Chabertia*, the shape and number of intestinal cells is of great importance in differentiation between them as *Oesophagostomum* has 16–24 triangular intestinal cells, while *Chabertia* has 24–32 rectangular intestinal cells and both are similar in having long thin filamentous sheath tail (**Dikmans and Andrews; 1933**).

Size measurements cannot be considered alone as there is a substantial overlap in length measurements, which increases the difficulty of accurate identification (**Roebera and Kahn; 2014**) and other characteristics would have to be taken in consideration before a definite generic diagnosis (**Dikmans and Andrews., 1933**). Therefore, **van Wyket al. (2004)** developed a simplified approach for identification

of larvae which is based on the mean length of the sheath extension.

It isn't necessary to illustrate the different keys obtained by various authors but they represented useful information to our studies and helping in identification of different genera (**Dikmans and Andrews, 1933; Keith, 1952; Hansen and Shivnani, 1956; Ministry of Agriculture, fisheries and food, 1977; van Wyk et al., 2004; Zajac and Conboy, 2012; Bowman, 2014**).

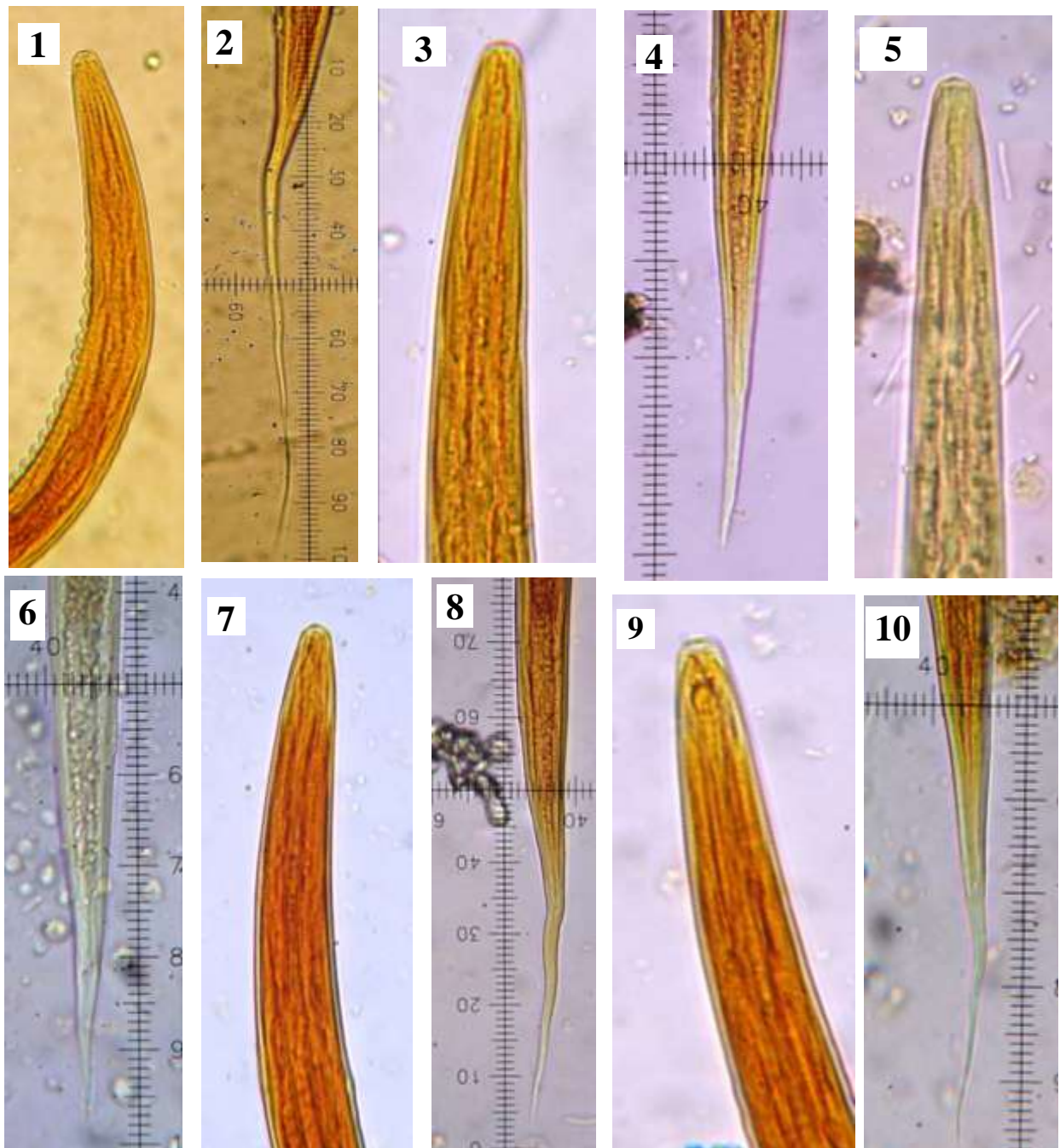
Simplified key of obtained morphometrical parameters of six nematodes genera illustrated in table (1). Photographs also provided to assist in their identification, which includes photos of the whole larva, as well as anterior and posterior extremity.



**Plate (I):** Different diagnostic stages of gastrointestinal nematodes recovered from examined cattle and sheep.

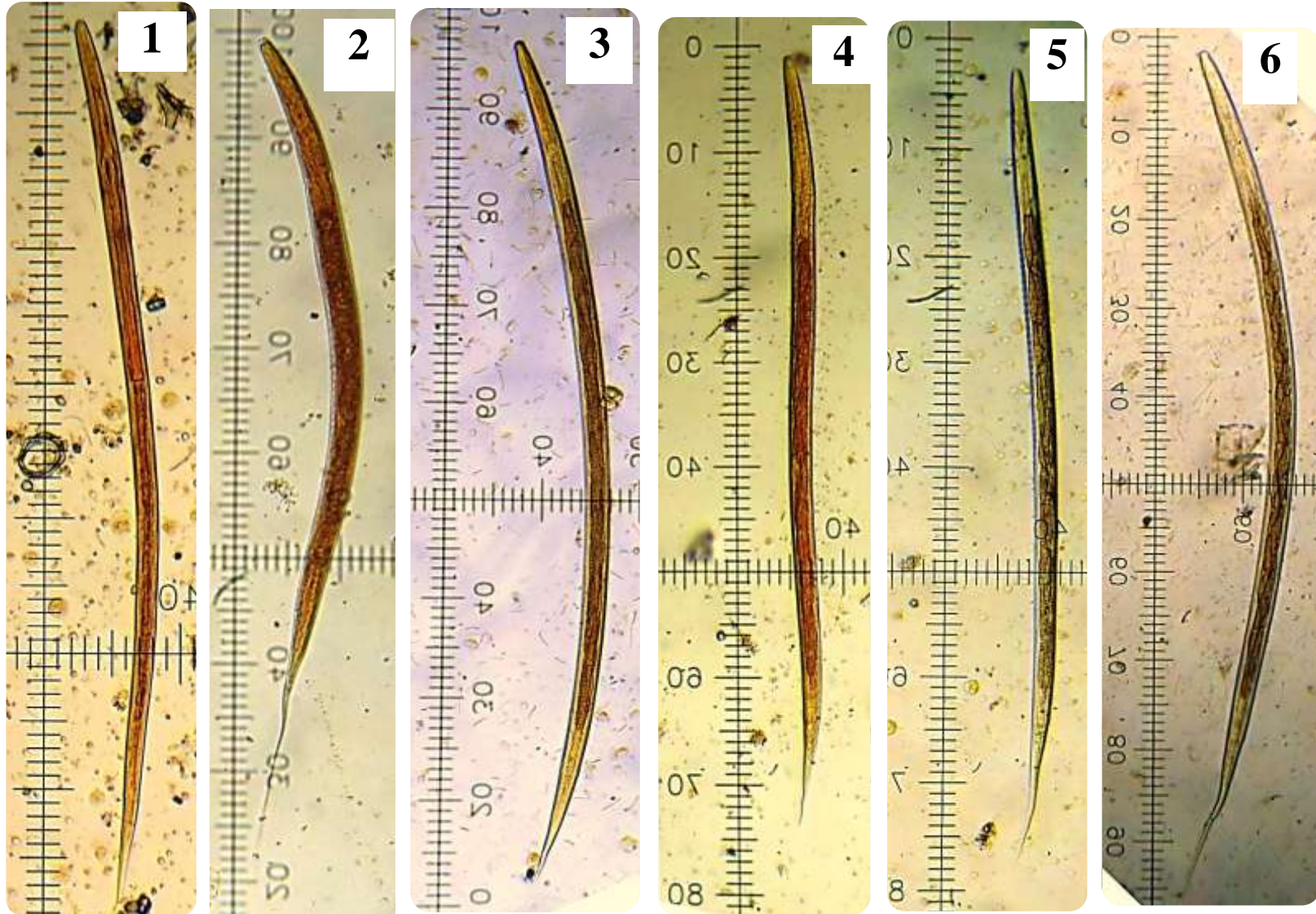
- 1) *Strongyle* type egg (X10).
- 2) Larvated *Strongyle* type egg (X40).
- 3) *Strongyloides* spp. egg (X40).
- 4) Trichostrongylid 3<sup>rd</sup> stage larva posterior end (X40).
- 5) *Strongyloides* spp. 3<sup>rd</sup> stage larva, anterior end (X40).
- 6) *Strongyloides* spp. 3<sup>rd</sup> stage larva, posterior end (X40).
- 7) *Oesphagostomum* spp. 3<sup>rd</sup> stage larva (X40).





**Plate (II):** Gastrointestinal nematodes 3<sup>rd</sup> stage larvae (anterior and posterior end).

- 1) *Oesophagostomum* 3<sup>rd</sup> larval stage anterior end (X40).
- 2) *Oesophagostomum* 3<sup>rd</sup> larval stage posterior end (X40).
- 3) *Ostertagia* spp. 3<sup>rd</sup> stage larva, anterior end (X40).
- 4) *Ostertagia* spp. 3<sup>rd</sup> stage larva, posterior end (X40).
- 5) *Trichostrongylus* spp. 3<sup>rd</sup> stage larva, anterior end (X40).
- 6) *Trichostrongylus* spp. 3<sup>rd</sup> stage larva, posterior end (X40).
- 7) *Haemonchus* spp. 3<sup>rd</sup> stage larva, anterior end (X40).
- 8) *Haemonchus* spp. 3<sup>rd</sup> stage larva, posterior end (X40).
- 9) *Cooperia* spp. 3<sup>rd</sup> stage larva, anterior end (X40).
- 10) *Cooperia* spp. 3<sup>rd</sup> stage larva, posterior end (X40)



**Plate (III):** Third stage larvae (L3) revealed from coproculture of cattle and sheep (X10).

1. *Strongyloides* spp. 2. *Oesophagostomum* spp. 3. *Ostertagia* spp. 4. *Trichostrongylus* spp. 5. *Haemonchus* spp. 6. *Cooperia oncophora*

**Table (1): Proposed key for identification of six gastrointestinal nematode genera 3<sup>rd</sup> larval stage (length in µm):**

Genus	Overall length	Anus to tip of sheath	End of tail to tip of sheath	Special features
<i>Trichostrongylus</i>				
Cattle	700–750	90–100	30–40	Head rounded, short sized larva, tail of sheath short and bearing one or two tuberosities.
Sheep	700–800	95–110	30–40	
<i>Ostertagia</i>				
Cattle	900–1000	150–175	60–75	Head squared, large sized larva and tail of sheath shorter in sheep.
Sheep	800–950	95–130	30–50	
<i>Haemonchus</i>				
Cattle	750–850	150–190	85–100	Head rounded, medium sized larva with medium length and kinked sheath tail.
Sheep	680–800	135–150	68–80	
<i>Cooperia</i>				
Cattle	850–1000	145–190	80–100	Head squared with two refractile oval bodies at anterior end of the esophagus, large sized larva, medium-length sheath that tapering to fine point.
Sheep	Around 900	140–155	70–85	
<i>Oesophagostomum</i>				
Cattle	Around 800	210–250	150-190	Rounded head, medium sized larva, long thin sheath tail and 16–24 triangular intestinal cells.
<i>Strongyloides</i>				
Sheep	550-650	-	Absent.	Short sized larva with very long esophagus and absence of tail sheath.

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## الملخص العربي

### تحديد هوية الديدان الاسطوانية التي تصيب المجترات من خلال المرحلة اليرقية الثالثة

صلاح احمد ابو الوفا      يارا محسن القباني      السيد نبيل الالفي

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أجريت هذه الدراسة لتحديد نوع اليرقات المعدية للديدان الاسطوانية التي تصيب الابقار والاعنام في مصر ، وايضا للتمييز بين بعض الديدان الاسطوانية الهامة التي تصيب المجترات عموما كوسيلة مساعدة تستخدم في تحديد هوية هذه الطفيليات، وكذلك الدراسات الوبائية المتعلقة بها. ولهذا الغرض تم تجميع عينات البراز عشوائيا من الابقار والاعنام حيث فحصت باستخدام تقنية التعويم القياسية، وبالنسبة للعينات المصابة ببيض عائلة الاسترونجيل فقد تم التمييز بينهم بواسطة الاستزراع اليرقي لاستخراج المرحلة اليرقية الثالثة حيث تتباين فيما بينها من خلال شكل الراس والذيل والغمد) بما في ذلك قياس الاطوال من فتحة الشرج الى نهاية الغمد و ايضا المسافة بين نهاية الذيل الى نهاية الغمد ، وكذلك قياسات وحجم كل يرقة بشكل عام). وقد تم دراسة الوصف الظاهري للمرحلة اليرقية الثالثة لستة ديدان من الديدان الاسطوانية التي تصيب المجترات وهي هيمونكس ، اوستارتاجيا، تريكوسترونجيلس، كوبيريا، اوسيفاجوستومام واسترونجيلويدز. وقد تم وضع مفاتيح التفريق بين هذه اليرقات مدعما ذلك بالصور الايضاحية.