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Original Article

Gastrointestinal nematodes infections in ruminants: Prevalence and Anthelmintic efficacy

Nahla. H Sallam[1], Asmaa, Z. Assasa[1], Amal, K. EL-Gayer[1] and Ahamed. A. Abdel Aal[1]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Suez Canal University

Abstract:

This study investigates the prevalence of gastrointestinal nematodes in ruminants in Ismailia, Port-said and Damietta cities, during a period from October 2016 to September 2017. A total of 130 fecal samples were collected from sporadic cases and 60 abomasal samples and 36 small intestinal samples were collected from slaughtered animals in abattoirs to detect the adult nematode parasites. The results indicated that sheep was infected with 5 species of nematodes 3 in abomasum and 2 types in small intestine. The prevalence of the infection were 26.15%, 30% and 11.11% in faecal , abomasal. and small intestine samples respectively. The highest prevalence of the nematodes was in young animals (71.42% in ovine sp., 14.28% in bovine sp. and 0.0% in caprine sp). Higher prevalence was in male sheep 60% than ewe 30%. Damietta was the highest in prevalence (47.6%) then in Ismailia (29.16%) and 0.0% in Port-said. The most prevalent species of nematodes in abomasum was *Haemonchus contortus* (26.67%), *Ostertagia* sp. (10%) and *Trichostrongylus axei* (3.33%). In small intestine, were *Cooperia* sp. (11.11%) and *Nematodirus* sp. (5.56%). Faecal egg count reduction test was carried out on Ivomec super & Albendazole to demonstrate the efficacy of anthelmintics, and proved the Ivomec was more potent than Albendazole in treatment of GINs.

Key words Ruminants-Nematodes-*H. contortus*- Histopathological changes

Introduction:

Ruminants are considered a major source of meat and milk productions for human consumption in Egypt. Egypt possesses large population of livestock which is managed by smallholder farmers.Gastro-intestinal nematode infection was one of the most problems on health all over the world, it was affected the health of several people and animals causing huge economic loss in ruminants farming **(Muluneh *et al.*, 2014)**.

Haemonchus was regarded as one of the most prevalent and highly pathogenic, possessed the highest biotic potential and had a prominent ability to develop resistance against most widely used anthelmintics and a unique survival strategy due to great biological and ecological plasticity. Hence compared to other gastrointestinal nematodes, *Haemonchus* was the most important parasite of domestic ruminants especially in sheep and goats **(Kumsa *et al.*, 2008).**

Anthelmintic treatment considered the most common method to control of GINs in ruminants although the cost of medication and anthelmintic resistance was a good documented phenomenon **(El-Gayar, 2002).** Currently, the primary control strategy for nematode infections was the use of chemical treatments **(Ahmed, 2010).**

Material and methods:

1. The Study area & animals:
This study was carried out on ruminants animals (cattle, buffalo, sheep and goats) which are collected from Ismailia, Port-said and Damietta. 130 faecal samples (64 from sheep, 18 from goat, 34 from cattle, 14 from buffalo) were collected from lived animals, sixty abomasal (54 from sheep, 4 from cattle and 2 from buffalo) & 36 small intestinal (32 from sheep, 2 from cattle and 2 from goat) samples were collected from abattoirs. The sex and age of studied animals were recorded. Parasitological examination were done in Faculty of Veterinary Medicine, Suez Canal University.

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2. Laboratory procedures:

2.1. Faecal examination:

(A) Macroscopic examination**:**
Each faecal sample was inspected by the naked eyes and with the aid of a magnifying hand lens for the presence of any gross parasites. Blood or mucous, colour, odour and consistency of the faeces were recorded.

(B) Microscopic examination**:**
Concentration flotation technique**:**
i. Saturated Salt Centrifugal fecal floatation technique according to **(Zajac and Conboy, 2012):**

2.2 Abattoir samples examinations:

(A) Worm counts:
Following slaughter, the abomasum and small intestine of each animal was removed, the abomasum opened along its greater curvature and the content collected in a container. Recovery and counting of worms from the abomasum and small intestine were carried out using similar technique to that described by **Bissat *et al*., 1996.** The nematode worms were collected, preserved, identified and counted **(Demissie *et al.* 2013** and **Vadlejch *et al.* 2014).** Complete identification of the nematodes was carried out after permanent mount acc. to **Glycerol Jelly Method: (Fleck & Moody, 1993)**

2.3 Histopathological examination:
(A) Tissue samples: For histopathologicl examination, abomasal tissues collected from slaughtered animals were sectioned. Sections of adjacent grossly uninvolved tissue were also collected. The sections were fixed in 10% neutral buffered formalin.

(B) Histopathological studies**:** Abomasum specimens were fixed in 10% neutral buffered formalin for 72 hrs. Tissue samples of 1 to 2 mm thickness were dehydrated in graded alcohol and cleared in xylene and embedded in paraffin blocks. The 4-5µ thick serial sections were taken with rotator microtome on clean grease free slides processed and stained with Haematoxyline & Eosin stain **(Luna, 1968)**.

3. Evaluation of the efficacy of some anthelmintics by using faecal egg count reduction test (FECRT):

- **(A) Animals used for the evaluation of the efficacy of the selected anthelmintics:**
- Ten of identified infected animals were detected by concentration flotation technique as they were positive for *Strongyle*-type eggs were selected for evaluation of the efficacy of Ivomec super (Ivermectin, Clorsulan) and Albendazole (Albendazole 2.5%).
- All of the studied infected ruminants were divided into two similar groups, each of 5 animals which used for treatment trial.
- All animals (10) in both group were examined pretreatment for *Strongyle*-type eggs which were counted using McMaster egg-count technique (pretreatment count).
- The first group (5animals) was treated by Ivomec super (Ivermectin, Clorsulan) while the second group was treated by Albendole (Albendazole 2.5%).

(B) The selected anthelmintics:

Table (1): Anthelmintics generic name, route of administration and recommended dose

Trade name	**Generic name**	**Route of administration**	**Recommended dose**
Ivomec Super	Ivermectin, Clorsulan	subcutaneous injection	1 ml / 50 kg bodyweight
Albend-azole	Albendazole 2.5%	oral drench	10ml /50 kg. bodyweight

(C) Egg counting technique:

Egg count was determined for positive samples only using the McMaster technique according to **Soulsby (1982)**.

$$\text{No. of eggs/gram} = \frac{\text{Total No.of eggs counted}}{\text{No.of counting chamber}} \text{ X } 200$$

(E) Efficacy %: (Knox, 2002):

Efficacy Reduction of FEC % =

$$\frac{\text{pre}-\text{post}}{\text{pre}} \times 100$$

Results:

In the current study on the gastrointestinal parasites in ruminants in Ismailia, Port-Said and Damietta governorates, out of 130 faecal samples of ruminants animals examined (64 from sheep, 18 from goat, 34 from cattle, 14 from buffalo) 34 samples (26.15%) were found to be positive for the gastrointestinal nematodes egg and out of 60 abomasal samples (54 from sheep, 4 from cattle and 2 sample from buffalo) 18 samples (30%) were found to be positive for the abomasal samples andout of 36 small intestine samples (32 from sheep, 2 sample from cattle and 2 sample from goat) 4 samples (11.11%) were found to be positive for the small intestine samples of different age, sex, species and origin of animals were examined. The detected nematodes were (*Haemonchs*, *Ostertagia*, *Trichostrongylus*, *Cooperia* and *Nimatodirus*) were recovered.

1. The prevalence:

Table (2): Prevalence of (GINs) in ruminants in relation to age of the animals

	Age	No. of infested/ No of examined			Prevalence (%)			P-value		
Sample / Spp.		Faecal sample	Abomasal sample	S.I. sample	Fae.	Ab.	S.I.	Fae.	Ab.	S.I.
Bovine	Young Adult	4/28 0/20	0/4 0/2	0/2 0.0	14,28 0.0	0.0 0.0	0.0 0.0			
Ovine	Young Adult	30/42 0/22	18/36 0/18	4/24 0/8	71,42 0.0	5 0.0	16.67 0.0	0,008	٠.008	0,54
Caprine	Young Adult	0/10 0/8	_ _	0.0 0/2	0.0 0.0	_ _	0.0 0.0			

Table (3): Prevalence of GINs in ruminants in relation to sex of the animals

	Sex	No. of infested/ No of examined			Prevalence (%)			P-value		
Samples / Sp.		Faecal sample	Abomasal sample	S.I. sample	Fae.	Ab.	S.I.	Fae.	Ab.	S.I.
Bovine	Male Female	2/22 2/30	0/6 0.0	0/2 0.0	9.1 6.67	0.0	0.0	0.83		
Ovine	Male Female	24/40 6/20	14/46 4/8	4/30 0/2	60 30	30.43 50	13.33 0.0	0.055	0.498	0.058
Caprine	Male Female	0/6 0/12	_ _	0.0 0/2	0.0 0.0	_ _	0.0 0.0			

Fig. (1): Monthly prevalence of GINs

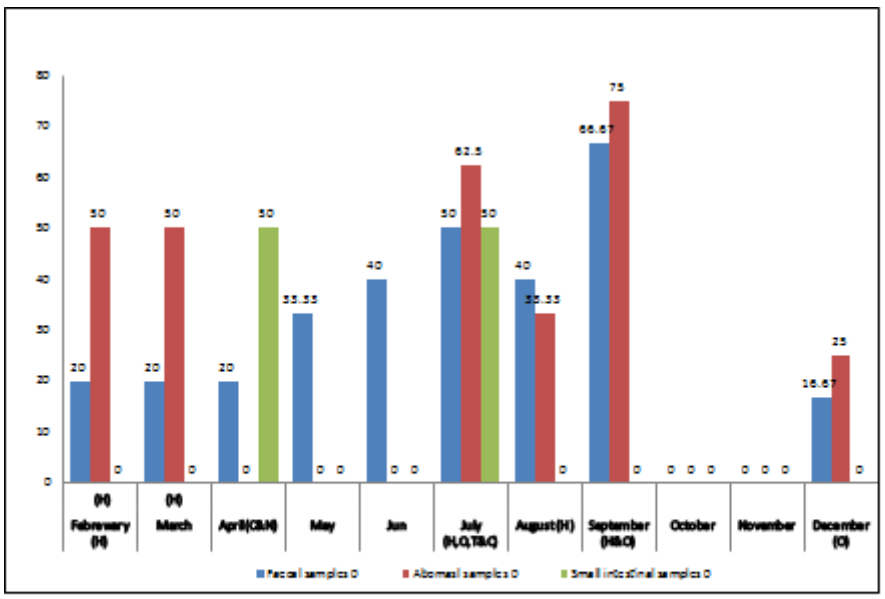

Table (4): The prevalence of GINs in relation to location of the animals

2. Morphological features:

	No. of infested/ No of examined			Prevalence (%)			P-value		
Sample / Location	Faecal sample	Abomasal sample	S.I. sample	Fae.	Ab.	S.I.	Fae.	Ab.	S.I.
Ismailia	14/48	4/20	2/12	29.16	20	16.67	0.0004	0.0033	0.413
Port-said	0/40	0/12	0/10	0.0	0.0	0.0			
Damietta	20/42	14/28	2/14	47.6	50	14.28			

Fig. (2): *Strongyle*-type egg

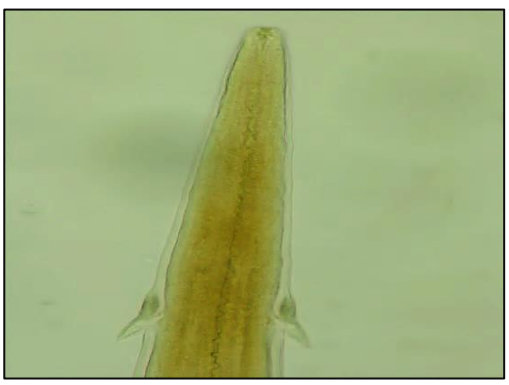

Fig. (3): *Haemonchus contortus* anterior en X10

Fig. (4): *Haemonchus contortus* male spicules and Y shaped dorsal ray X10

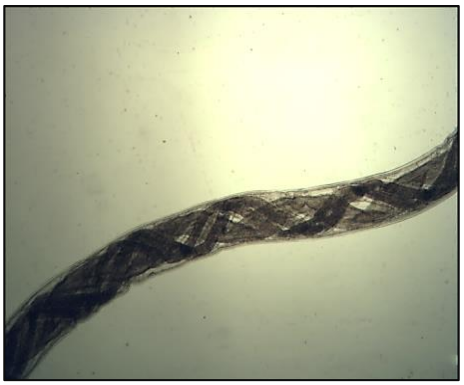

Fig. (5): *Haemonchus contortus* female white uterus winding around the red blood-filled intestine giving a twisted or barber`s pole appearance X4

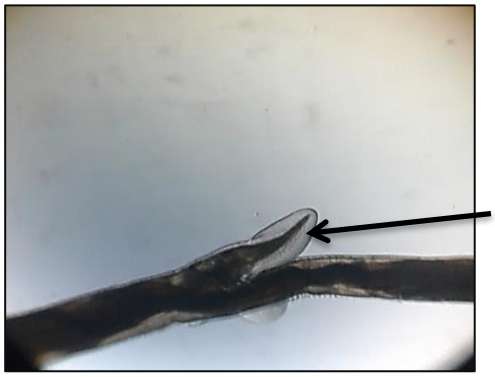

Fig. (6): *H. contortus* female vulvar flap, linguiform type (black arrow) X4

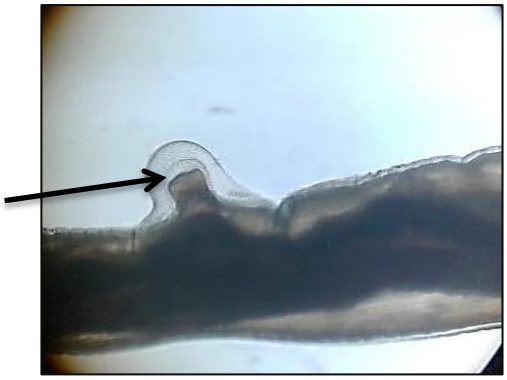

Fig. (7): *Haemonchus contortus* female vulvar flap, knob type (black arrow) X4

Fig. (8): *Nematodirus* sp. anterior end with cephalic swelling (black arrow) X10

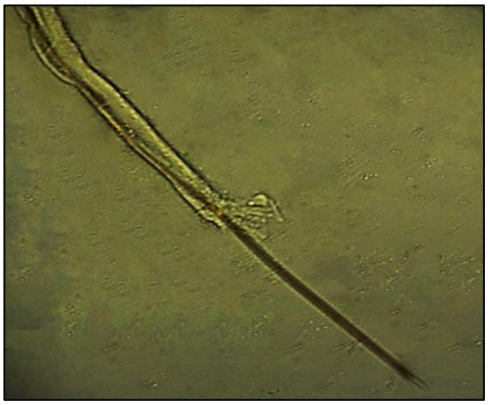

Fig. (9): *Nematodirus* sp. male bursa (a) and spicules (b) X4

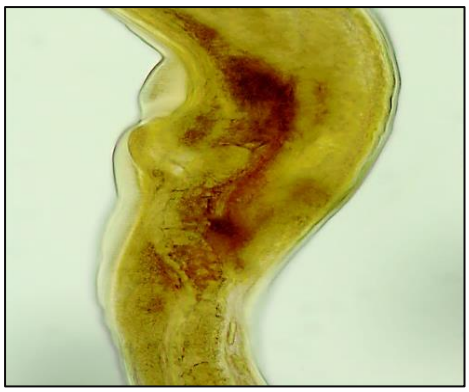

Fig. (10): *Nematodirus* sp. female vulvar region (black arrow) X20

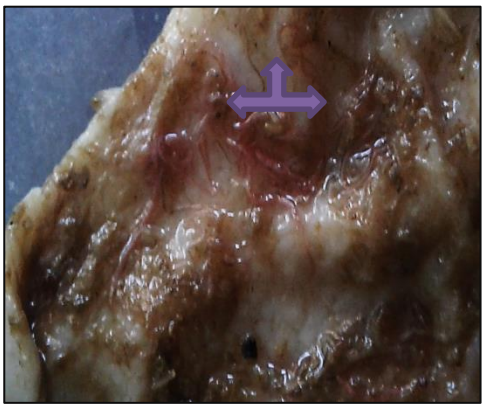

Fig. (11): Portion of abomasum with adult *Haemonchus* worms (arrows)

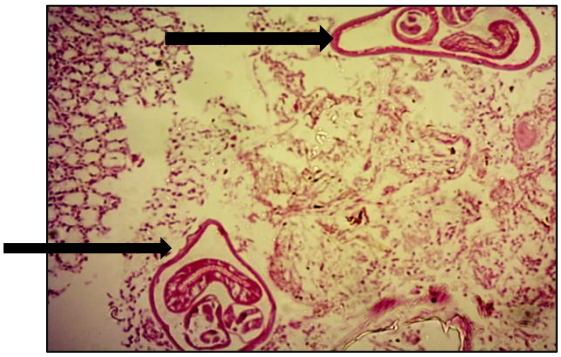

Fig. (12): Sheep, abomasum showing two cross sections of *Haemonchus contorus* (black arrow) with massive destruction and desquamation of the superficial layer of mucosa in the lumen. H&E, X 200.

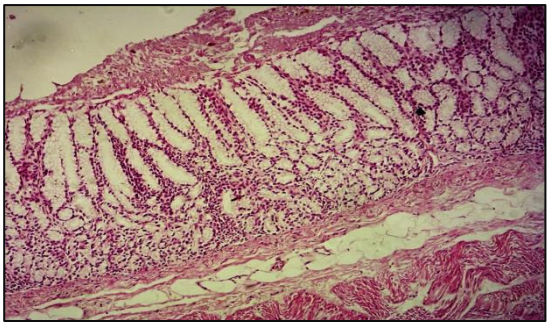

Fig. (13): Sheep, abomasum showing necrosis of the superficial layer of mucosa, diffuse mucinous degeneration of submucosal glands with leukocytic infiltrations. H&E, X 200.

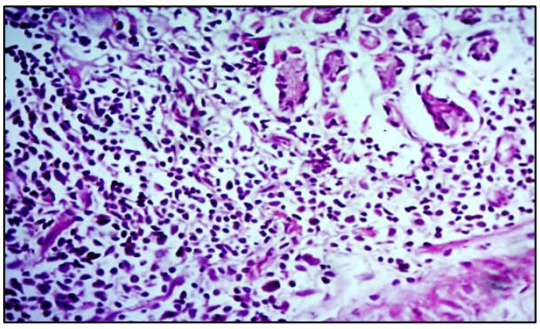

Fig. (14): Sheep, abomasum higher magnification of previous figure showing massive necrosis of the glands which was replaced by leucocytic infiltrations mainly lymphocytes, macrophages and few plasma cells. H&E, X 400.

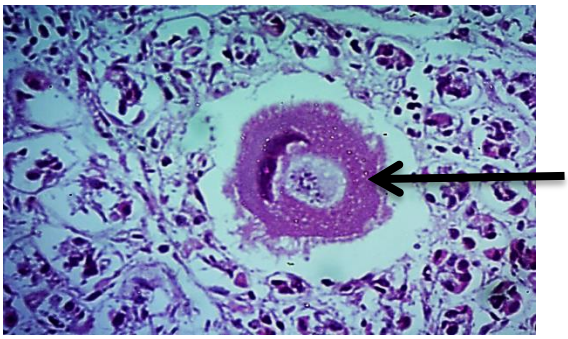

Fig. (40): Sheep, abomasum higher magnification of previous figure showing larva of *ostertagia* in gastric gland (black arrow) gland (black arrow) X 400.

4. Evaluation the efficacy of some anthelmintics by using faecal egg count reduction test (FECRT):

Efficacy of used anthelmintics on GINs infesting the selected group of sheep:

Table (5): Changes in mean egg per gram in Ivomec super (Ivermectin, Clorsulan) treated sheep group.

		Changes in mean egg/gram in examined sheep				
	Animal number	Before treatment	1st wk after ttt	2nd wk after ttt	3rd wk after ttt	4th wk after ttt
Treated group	1	1500	200	100	100	100
	2	1000	200	100	0	0
	3	500	0	0	0	0
	4	700	100	0	0	0
	5	1200	200	100	0	100
Mean egg /gm		**980**	**140**	**60**	**20**	**40**
Efficacy %			**85.71**	**93.87**	**97.95**	**95.9**

Table (6): Changes in mean egg per gram in Albendazole (Albendazole 2.5%) treated sheep group.

		Changes in mean egg/gram in examined sheep				
	Animal number	Before treatment	1st wk after ttt	2nd wk after ttt	3rd wk after ttt	4th wk after ttt
Treated group	1	500	0	0	0	0
	2	400	0	0	0	0
	3	1700	200	200	100	200
	4	900	100	0	0	100
	5	1300	200	100	100	200
Mean egg /gm		**960**	**100**	**60**	**40**	**100**
Efficacy %			**89.58**	**93.75**	**95.83**	**89.58**

Discussion:

1. Prevalence status:

The prevalence of gastrointestinal nematodes infection were 26.15%, 30% and 11.11% in faecal , abomasal. and small intestine samples respectively The current results were higher than those reported by **Eisa & Esmail (2003)** (28.9%), **Abouzeid *et al*. (2010)** (27.5%)**, Khalafalla *et al.* (2010)** (10.4%), **Awraris *et al*. (2012)** (27.57%)**, Kabaka *et al.* (2013)** (13.8%), **Mir *et al*. (2013)** (21.93%), **Swapna and Nithinya (2015)** (19.36%) and **Sultan, *et al*., (2016)** (26.79%)**.** The fluctuation in prevalence of GINs might be due to extensive use of anthelmintics by the farmers, difference in agro-climatic conditions that could support prolonged survival and development of infective larval stages of most nematodes **(Muluneh *et al.*, 2014)**.

In the present study, the age susceptibility of the examined animal infected with GINs was observed all over the year with the highest percentage of infection and significantly high in young animal (2-6 months) but the infection was nil in adult animals (more than one year old). The high prevalence of GINs in young animal than adults may be due to the lack of immunity which acquired by continuous infection due to bad management especially space insufficiency and continuous indiscriminate treatments which only minimizes the intensity of infection without efficient integrated control measures **(Lemma and Abera, 2013)**. This result was similar with that recorded by **Regassa *et al.* (2006)**, **Pfukenyi *et al.* (2007), Abouzeid *et al.* (2010), Awraris *et al.* (2012), Kakar *et al.* (2013), Lemma and Abera (2013), Lashari *et al.* (2015), Swapna and Nithinya (2015).**

The present study revealed that sex of the animals shows significance variation at ($p \leq 0.05$) with GINs infection in case of faecal samples of sheep as the prevalence of infection in males was 60% and in females was 30%, this may be due to stimulatory effects of estrogens and inhibitory effect of androgens on immune responses (**Lashari *et al.*, 2015)** and **(Desta, 2015).**

The study further revealed that the highest prevalence of GINs egg was during September (66.67%) followed by July (50%) then in months (June and August) (40%) then in May (33.33%) and then in months (February, March and April) (20%), the lowest were found in December (16.67%) and with (0.0%) in the other months. Also adult worms in abomasal samples were higher in September (75%) then in July (62.5%) then in February and March (50%) then in August month (33.33%), and the lowest prevalence was recorded in December (25%) and (0.0%) in other months. These results were agreed with **Nwosu *et al.* (2007)** and **Mir *et al.* (2013)** as their results recorded that *Haemonchus* and *Trichostrongylus* species

attained peak counts together in both goats (June) and sheep (August).

Concerning adult worms in abomasum, 30% of the examined abomasal samples were infested with at least one species of nematodes worms. The most prevalent species was *Haemonchus contortus* (26.67%) then *Ostertagia* sp. (10%) and *Trichostrongylus axei* (3.33%) from the examined abomasal samples, and *Cooperia* sp. (11.11%) and *Nematodirus* sp. (5.56%) from examined small intestine samples. These results were in line with results of **Raza *et al*. (2009)** as the prevalence of *H. contortus* in sheep was 37.18% in all examined animals. **Sultan *et al.* (2010)** in Gharbia Governorate, Egypt, **Owhoeli *et al.* (2014)** and **Varadharajan and Vijayalakshmi (2015)** recorded that the most prevalent abomasal nematode was *H. contortus*.

The present study revealed that location of the animals showed high significance variation so in case of faecal and abattoir samples. The highest prevalence of nematodes were recorded in Damietta (47.6% - 50%) then Ismailia (29.16% - 20%) and with (0.0%) in Port-said city. In our opinion the absence of infection in Port-said city may be due to the good managemental activities of the farmer such as housing of the animals, good feeding and periodical using of anthilmintics, similar findings were observed by **Kabaka *et al.* (2013)**.

2.Morphological status:

The morphological status including that of *Haemonchus contortus* recovered from 16 abomasal samples of the sheep. The male worms were reddish in color while the females were with white uterus which spirally wound around the red intestine, giving the appearance of a barber`s pole.The male length was 12.6-19.4mm, the dorsal lobe of the bursa was supported by Y shaped dorsal ray and the spicules were barbed. The female was 14.4-25 mm in length, in most of the specimens, the vulva was covered with a conspicuous large flap, the flap was linguiform, in a few specimens it was reduced to small knob, this feature were agreed with finding of **Lichtenfels *et al.* (1986** & **1994), El-Gayar (2002), Rauf Tak *et al.* (2014)** and **Saminathan *et al.* (2015)**.

In case of description of *Ostertagia* sp. which was collected from the 6 abomasal samples of sheep. The male measured 6.2-7.5 mm long, the spicules were pigmented brown, equal, short and trifurcated distally. The female length of *Ostertagia* was 8.1-9.2 mm and the female tail was slender and curved in a C-shape manner. These morphological characters were similar to those described by **Lichtenfels and Pilitt (1991)** and **El-Gayar (2002)**.

Trichostrongylus axei were collected from 2 abomasal samples of sheep. The worms were small, Cervical papillae were lacking. The male was 2.5-6 mm long and the female was 3.5-8mm long. The

spicules of male were dark brown in color, and unequal. This description was similar with those obtained by **Boomker (1986), Knight (1962), Soulsby (1982)** and **Ghasemikhak *et al.* (2011).**

In the present study, *Nematodirus* sp. were collected from 2 specimens small intestine of sheep. They have an inflated cuticle around the anterior end with 14-18 longitudinal ridges on the cuticle. This result was similar to that obtained by **Lichtenfels and Pilitt (1983)**, **Hoberg *et al*. (1986)** and **Hoberg and Rickard (1988)**.

Cooperia sp. were collected from 4 specimens of small intestine. The cuticle of the anterior end formed a cephalic swelling with transverse striations and the rest of the body cuticle beard 14-16 longitudinal ridges. The female was 5.7-7.5 mm long. This description was similar to that recorded by **Lichtenfel (1977)** and **Soulsby (1982).**

3.Histopathological findings:

In gross lesions, there were a watery content of the abomasum which partially covered with free blood. Numerous adult worms and petechial hemorrhage were found on the abomasal mucosa. This result was agreed with **Tehrani *et al.* (2012)** and **Saminathan *et al.* (2015).**

Current study referred to histopathological changes on the examined abomasum of sheep which showed cross section of *Haemonchus contorus*. The worms was seen embedded between mucosa and submucosa with massive destruction and desquamation of the superficial layer of mucosa that lead to focal necrosis of the superficial layer of mucosa. Submucosal layer was showed prominent leukocytic infiltrations. This result was agreed with that obtained by **El-Gayar (2002)** and **AL-Hasnawy (2014).**

4. Efficacy of anthelmintics:

In the present study, the efficacy of two anthelmintics Ivomec super (Ivermectin) and Albendazole in treatment of gastrointestinal nematodes naturally infecting animals was evaluated by using faecal egg count reduction test (FECRT). Results revealed that Ivomec super was more efficient as it induced marked reduction in mean egg /gm from which was 980 before treatment to become 40 in the 4^{th} week after treatment. The means epg of this group were (980) pre-treatment and (140, 60, 20 and 40) post-treatment with efficacy 85.71%, 93.8%, 97.95% and 95.9% in 1^{st}, 2^{nd}, 3^{rd} and 4^{th} week after treatment respectively. This finding was nearly similar to that obtained by **Mckellar *et al.* (1991),** Moreover **Goudie *et. al.* (1993), Walsh *et. al.* (1995)** and **Khaled *et al.* (2004).**

Conclusion:

From this study, it could be concluded that:

- Ovine gastrointestinal nematodes were the major helminthosis parasites in ruminants.
- The age, monthly variations and the location of the animals were very important factors associated with gastrointestinal nematode infection.
- The most prevalent type of gastrointestinal nematode was *Haemonchus* sp. which cause severe pathogenic effect in infected animals.
- Ivomec super as an anthelmintic was more effective than Albendazole in treatment of GINs, so it was recommended to treat infected ruminants with this anthelmintic in months with high prevalence (June, July, August and September).

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الملخص العربى

دراسات وصفية وبيولوجيةعلي الديدان الاسطوانية المعدمعوية فى المجترات

نهله حامد أحمد سلام [١]،أسماء زكريا عساسه [١]، أمل كمال عباس الجيار[١]، أحمد انور السيد عبد العال[١]

[١]قسم الباراسيتولوجيا - كليه الطب البيطري - جامعه قناه السويس

اجريت هذه الدراسة للبحث عن إصابه المجترات بالديدان الإسطوانية المعدمعوية فى ثلاثة مدن الاسماعيلية وبورسعيد ودمياط فى الفترة من اكتوبر ٢٠١٦ الى سبتمبر ٢٠١٧ وتم فحص ١٣٠ عينة براز من الحيوانات الحية بصورة عشوائية وكذلك فحص ٦٠عينة من المعده الرابعة و٣٦ عينة من الامعاء الدقيقة من الحيوانات المذبوحة فى المجازر ثم تم إجراء الصفة التشريحية لها للبحث عن الديدان الاسطوانية واظهرت النتائج إصابة الاغنام ب (ثلاثة أنواع من الديدان الإسطوانية فى المعدة الرابعة و نوعين من الديدان الإسطوانية فى الأمعاء الدقيقة) وكانت نسبة اصابة المجترات فى عينات البراز ٢٦.١٥% وفى عينات المجزر (٣٠% فى المعدة الرابعة و ١١.١١% فى الأمعاء الدقيقة) وقد سجل وجود فروق معنوية فى إنتشار الطفيليات بين أعمار الحيوانات حيث أعلى نسبة إصابة فى الحيوانات الصغيرة من عمر ٦-١٢ شهربنسبة إصابة تصل الى ٧١.٤٢% فى الأغنام و١٤.٢٨% فى الابقار وكذلك نسبة انتشار الإصابة فى ذكور الحيوانات اكثر من الإناث حيث فى ذكور الأغنام وصلت النسبة الى ٦٠% وفى الإناث ٣٠% كما وجدت فروق معنوية فى معدل الإصابة من حيث منشأ الحيوان حيث سجلت محافظة دمياط أعلى نسبة فى معدل الإصابة فكانت النسبة ٤٧.٦% وتليها محافظة الاسماعيلية وسجلت ٢٩.١٦% ولم تسجل النتائج ظهور إصابة فى محافظة بورسعيد وكذلك وجدت فروق معنوية فى معدل الإصابة باختلاف شهور السنة حيث كان شهر سبتمبر اكثر الشهور انتشارا للمرض بنسبة ٦٦.٦٧% وكان اكثر الانواع انتشارا فى المعدة الرابعة ديدان ***الهيمونكس*** بنسبة ٢٦.٦٧% ثم ديدان ***الاوسترتاجيا*** بنسبة ١٠% واخيرا ديدان ***التريكوسترونجيلوس*** بنسبة ٣.٣٣% اما فى الامعاء فسجلت النتائج ديدان ***الكوبيريا*** بنسبة ١١.١١% وكذلك ديدان ***النيماتوديرس*** بنسبة ٥.٥٦% كما اشارت النتائج إلى وجود تغيرات نسيجية واضحة بوجود تنخر في الطبقة المخاطية وتثخن بالطبقة العضلية وفرط التنسج للخلايا الجداريه مع الخزب بالاضافه إلى وجود ارتشاح للخلايا الالتهابية متعددة الأشكال وتليف كامل لبعض الخلايا الغديه للمعدة الرابعة. وقد تم عمل اختبار تناقص عدد البيض بإثنين من مضادات الديدان الإسطوانية وهما الأيفوماك سوبر و الألبندازول وتبين تفوق الأيفوماك سوبر على الألبندازول فى علاج الديدان الإسطوانية فى المجترات.