# STUDIES ON GASTROINTESTINAL NEMATODES INFECTION IN SHEEP WITH SPECIAL REFERENCE TO *HAEMONCHUS CONTORTUS*

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## ABSTRACT

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This study was carried out to investigate clinical, epidemiological and histopathological findings associated with gastrointestinal nematodes infection in sheep with special reference to *Haemonchus contortus* infection during the period from January, 2008 to June, 2010 at Gharbia Governorate, Egypt. The prevalence of gastrointestinal nematode among examined sheep was 62.38%. *Trichostrongylus* spp. was the most detected nematode. The highest prevalence was reported in females, during spring and in the age group over 2 years. The clinical findings observed in infected sheep were emaciation and pale mucous membrane. Infected sheep showed anemia and decrease in serum total proteins, albumin and globulins levels.

***Keywords:*** *Sheep, Gastrointestinal nematode, Prevalence, Egypt.*

## دراسات على إصابة الأغنام بالديدان الأسطوانية المعد-معوية مع التركيز على الهيمونكس كونتورتس

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أجريت هذه الدراسة على اصابة الاغنام بالديدان الاسطوانية بالجهاز الهضمى خلال الفترة من يناير ٢٠٠٨ إلى يونية ٢٠١٠ في محافظة الغربية على عدد ٣١٩ حيوان وقد ثبت اصابة عدد ١٩٩ منهم بمعدل انتشار ٦٢,٣٨ ٪. وكانت أهم أنواع النيماتودا التي تم الكشف عنها التريكوسترونجيلس بأعلى نسبة انتشار كانت في الاناث في فصل الربيع وفي الفئة العمرية الأكثر من عامين. وكانت أهم الاعراض الاكلينيكية الهزال وابيضاض الغشاء المخاطي والحيوانات المصابة كانت تعاني من الانيميا ونقص في بروتينات الدم.

## INTRODUCTION

Gastrointestinal nematode (GIN) parasitism is arguably the most serious constraint affecting sheep production worldwide. Economic losses are attributed to decreased production, costs of prophylaxis and treatment, and deaths of the infected animals. (Miller and Horohov, 2006).

Nematode species especially those fed on blood such as *Haemonchus contortus* and *Bunostomum* were calculated. Blood serum total

*trigonocephalum* are responsible for specific clinical symptoms and great economic losses to small ruminant industry. Although Haemonchus species infect all ruminants, the severity of *Haemonchus contortus* infection is more pronounced in sheep where it is linked to severe anemia, diarrhea, loss of body weight and death (Agarwal and Banerjee, 2007). The objective of this study is to investigate the clinical, epidemiological, and histopathological findings associated with gastrointestinal nematodes in sheep at Gharbia Governorates, Epypt.

## MATERIALS and METHODS

### 1. Animals:-

Three hundred and nineteen sheep belonging to 8 flocks of different age and sex (Table 1) were used in this study during the period from January, 2008 to June, 2010. All flocks were depending on grazing.

### 2. Clinical examination

All sheep used in this study were subjected to clinical examination according to. Kelly (1984).

### 3. Samples:-

### 3.1. Fecal samples

Fecal samples were examined macroscopically and microscopically using concentration floatation technique and the positive samples were subjected to fecal egg count, fecal culture, and larval identification according to Soulsby (1982).

### 3.2. Blood samples

Two blood samples were collected from each infected animal; one with anti coagulant to be used for hematological examination and the other without to be used for serum separation. These samples were collected via jugular vein puncture. Erythrocytic cell counts (RBCs), hemoglobin concentration (Hb) and Packed Cell Volume (PCV) were estimated according to Coles (1989). Moreover, MCV, MCH and MCHC also proteins, albumin and globulins were determined using commercial kits according to Doumas (1971) and Henry (1974).

### 4. Epidemiological investigations

Some epidemiological parameters associated with gastrointestinal nematodes infection were estimated according to Martin *et al.* (1987).

### 5. Post mortem examination:-

Five *Haemonchus contortus* infected sheep were slaughtered and their abomasa were examined grossly and histopathologically. Adult worms were collected for identification. Suitable portion of the infected abomasum, showing gross lesions was collected and fixed in 10% neutral buffered formalin solution to be used for histopathological examination.

### 5.1. Identification of adult worms from infected abomasa:-

Adult worms were collected from infected abomasa, washed in physiological saline, fixed in glycerin-alcohol then embedded in Canada-blasm on glass slides and identified according to Soulsby (1982).

### 5.2. Histopathological examination:-

The fixed infected abomasa were embedded in paraffin wax. Five microns thick paraffin sections were prepared and stained with Haematoxylene and Eosin (H&E) and examined microscopically according to Drurag and Wallington (1980).

### 6. Statistical analysis:-

Statistical analysis was carried out by using statistical soft ware program (GMP for windows version 5.1, SAS Institute, Cary, NC, USA). Differences between means at $P < 0.05$ were considered significant.

The prevalence of gastrointestinal

## RESULTS

**Prevalence and percentage of different gastrointestinal nematodes**

Out of 319 examined sheep, 199 were found to be infected with gastrointestinal nematodes via parasitological examination representing 62.38%. (Figure 1). The highest infection was recorded by *Trichostrongylus* species was 59.25%, (Haemonchms 30%), followed by *Strongyloides* (13.79%) then *Trichuris* (0.94%) and *Nematodirus* (0.63%). Concerning the seasonal distribution of parasitic infestation, higher prevalence was recorded in spring (71.59%) followed by autumn (70.31%) then winter (68.91%) and finally summer (43.01%).

The prevalence of the recovered 3rd stage larvae from fecal culture revealed that the predominant species in winter were *Haemonchus* (30%) followed by *Bunstomum* (20%) and *Trichostrongylus* species (20%). While, in spring were *Haemonchus* (30%) and *Trichostrongylus* species (30%) and in summer *Bunstomum* was the most predominant species (30%) followed by *Haemonchus* (20%) and *Strongyloides* (20%) then in autumn *Haemonchus* was the most predominant species (30%) followed by *Trichostrongylus axei* (20%).

nematodes in relation to animal age and sex was illustrated in Table (2 & 3).

**Clinical findings:-**

Clinical findings were illustrated in Table (4).

**Hematological findings:-**

The hematological and serum biochemical changes that associated with gastrointestinal nematodes infestation in the examined sheep were illustrated in Table (5 and 6).

**Post mortem findings:-**

**Gross lesions**

Thickening, hyperemia and small ulceration in addition to petechial hemorrhage at the site of worm attachment were observed in infested abomasa. Abomasal contents were dark in color and the adult worms were present grossly.

**Identification of the recovered worms**

The detected worms were identified as *Haemonchus* spp.

**Histopathological findings**

Histopathological changes in abomasa infected with *Haemonchus*, were illustrated in Figures (2 - 6)

**Table 1:** Locality, number, sex and ages of the examined animals.

| Locality | No. of animals | Sex and age | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Male | | | | Female | | | |
| | | Under 1 year | 1 year up to 2 years | Over 2 years | Total | Under 1 year | 1 year up to 2 years | Over 2 years | Total |
| Kohafa | 54 | 4 | 8 | 3 | 15 | 8 | 15 | 16 | 39 |
| Elmohami | 43 | 6 | 6 | 2 | 14 | 4 | 16 | 9 | 29 |
| Kafrelzait | 20 | 3 | 2 | 0 | 5 | 5 | 5 | 5 | 15 |
| Kotor | 20 | 7 | 0 | 3 | 10 | 7 | 0 | 3 | 10 |
| Nawag | 12 | 2 | 1 | 0 | 3 | 1 | 4 | 4 | 9 |
| Sprbay | 91 | 13 | 8 | 3 | 24 | 27 | 29 | 11 | 67 |
| Tanta | 70 | 40 | 1 | 1 | 42 | 6 | 18 | 4 | 28 |
| Zefta | 9 | 2 | 1 | 0 | 3 | 0 | 5 | 1 | 6 |
| Total | 319 | 77 | 27 | 12 | 116 | 58 | 92 | 53 | 203 |

**Table 2:** Prevalence of parasitic gastrointestinal nematodes in relation to age.

| Age | No. of examined sheep | No. of infected sheep | % |
|---|---|---|---|
| Under 1 year | 135 | 62 | 45.93 |
| 1 year up to 2 years | 119 | 86 | 72.27 |
| Over 2 years | 65 | 51 | 78.46 |
| Total | 319 | 199 | 62.38 |

Significant variation was recorded among different age groups (P<0.001)

**Table 3:** Prevalence of gastrointestinal nematodes in relation to sex

| Sex | No. of examined sheep | No. of infected sheep | % |
|---|---|---|---|
| Male | 116 | 45 | 38.79 |
| Female | 203 | 154 | 75.86 |
| Total | 319 | 199 | 62.38 |

Significant variation was recorded among different sex (P<0.001)

**Table 4:** Clinical signs in relation to degree of infection.

| Degree of infestation | No. of animals | % | Clinical signs |
|---|---|---|---|
| Severe (over 1000 Epg) | 47 | 14.73 | Severe emaciation<br>wool easily detached<br>pale mucous membrane<br>diarrhea<br>bottle jaw in 2 cases |
| Moderate (500 and 1000 Epg) | 56 | 17.55 | Emaciation<br>soft feces in some cases<br>pale mucous membrane |
| Low (less than 500 Epg) | 96 | 30.09 | Vary from apparent healthy without clinical signs to poor growth or slight emaciation and diarrhea |

**Table 5:** Relation between infestation and RBCs count

| Degree of infestation | Fecal egg count (EPG) | RBCs (mil/cmm) | Hb (gm/dl) | PCV (%) | MCV | MCH | MCHC |
|---|---|---|---|---|---|---|---|
| Low (less than 500 Epg) | 166.66±33.33* | 4.6±0.12* | 9.66±0.08 | 31.53±0.63 | 68.7±3.1 | 21.04±0.71 | 30.66±0.34 |
| Moderate (500 and 1000 Epg) | 700±57.73* | 4.3±0.1* | 8.36±0.7 | 26.8±1.7* | 62.22±2.87 | 19.39±1.34 | 31.12±1.12 |
| Severe (Over 1000 Epg) | 4133.33±2535.9* | 4.1±0.1* | 7.13±1.16 | 22.66±4.06* | 54.85±8.79* | 17.27±2.49* | 31.69±0.7 |
| Negative | -ve | 5.2±0.16 | 10.46±0.52 | 33.16±0.83 | 63±1.04 | 19.85±0.64 | 31.51±0.8 |

(*) Significant at P ≤0.05

**Table 6:** Relation between infestation and serum biochemical analysis

| Degree of infestation | Fecal egg count (EPG) | Serum total protein (g/100ml) | Serum albumin (g/100ml) | Serum globulin (g/100ml) | Albumin globulin ratio (A/G ratio) (%) |
|---|---|---|---|---|---|
| Low (less than 500 Epg) | 166.66±33.33* | 9.4±0.05* | 3.22±0.02* | 6.17±0.07* | 0.52±0.01 |
| Moderate (500 and 1000 Epg) | 700±57.73* | 8.8±0.17* | 3.06±0.12* | 5.73±0.23* | 0.53±0.03 |
| Severe (Over 1000 Epg) | 4133.33±2535.9* | 8.3±0.32* | 3±0.17* | 5.3±0.15* | 0.56±0.01 |
| Negative | -ve | 11.2±0.2 | 3.6±0.14 | 7.53±0.33 | 0.49±0.03 |

(*)Significant at P ≤0.05

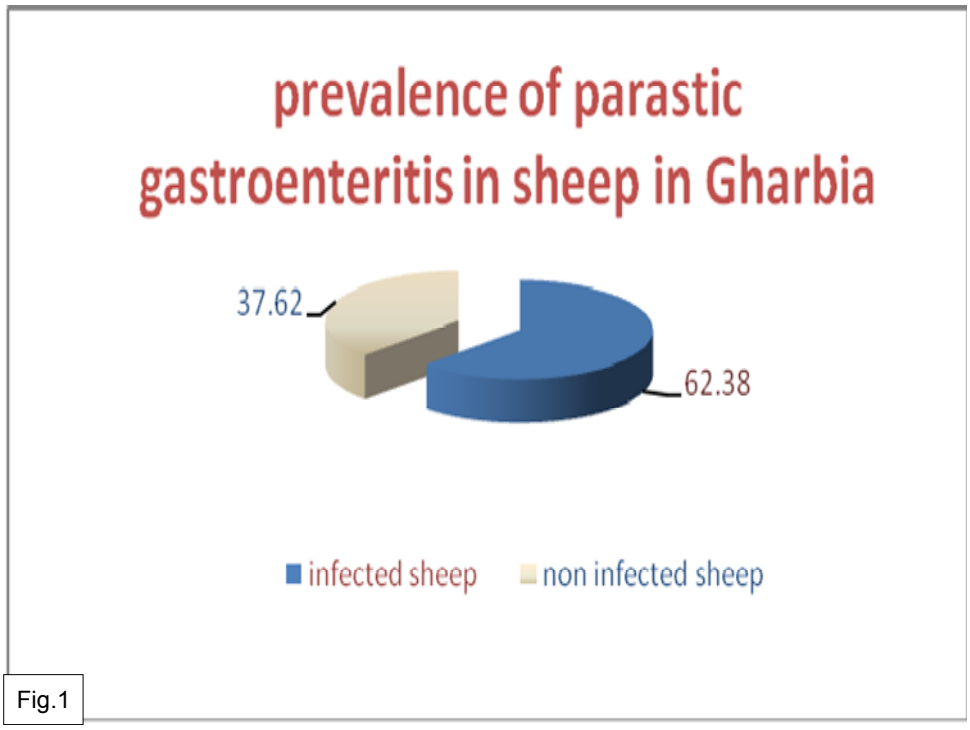


**Fig. 1:** Prevalence of parasitic gastrointestinal nematodes in sheep in Gharbia.

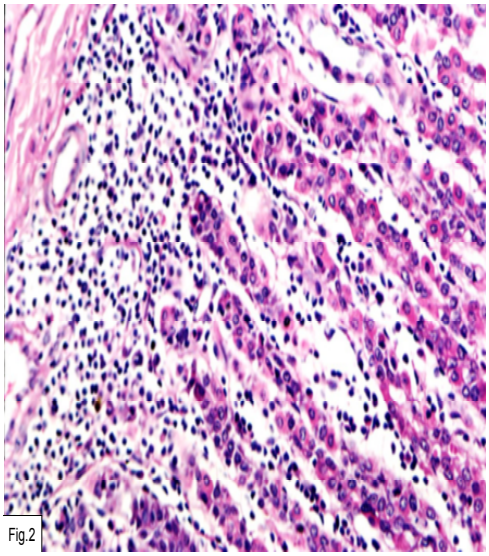


**Fig.2:** Mononuclear cell infiltration in between mucosal glands in *Haemonchus Controtus* infested abomasa. H.&E. X = 400.

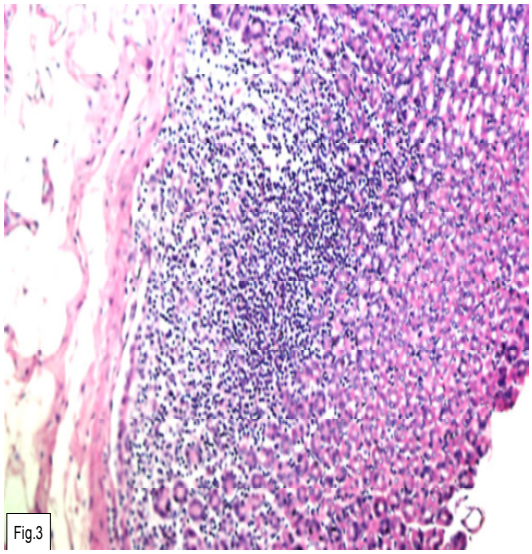


**Fig.3:** Mononuclear cell infiltration in between lamina propria in *Haemonchus contortus* infested abomasa H.& E. X = 100.

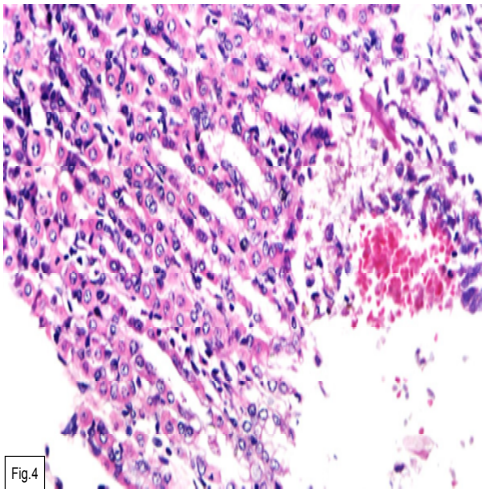


**Fig. 4:** Hemorrhage at abomasal wall in *Haemonchus contortus* infested abomasa. H.&E. X=400.

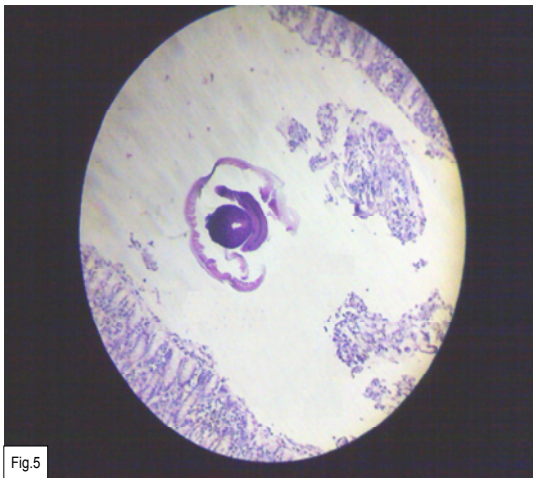


**Fig. 5:** Cross section of adult Haemonchus worm within the abomasal wall. X = 100.

## DISCUSSION

Sheep is considered as one of the important sectors in livestock production. Sheep may be affected with many diseases which decrease production. Parasitic gastroenteritis is one of them causing many economic losses (Miller and Horohov, 2006).

Concerning the prevalence of parasitic nematodes, out of 319 examined sheep, 199 (62.38%) were proved to be infested with parasitic gastrointestinal nematodes via flotation concentration technique. Nearly similar prevalence was reported by El-Fayoumi (1989), (65.83%). Higher prevalence was reported by Al-Gaabary *et al.* (2007), (71.69%). Lower prevalence (42.66%) was reported by Abdel-wahed and Salem (1999). The variations between the prevalence in different studies may be attributed to the type of rearing, hygiene and control measures where, sheep raised on high protein diet developed resistance to parasitic gastroenteritis (Knox and steel, 1999). In addition, the changes in climatic condition may affect the degree of infection where rain may lead to increase infection with parasitic gastroenteritis.

Highest infestation was recorded by *Trichostrongylus* species (59.25%) and the lowest one recorded by *Nematodirus* (0.63%). Similar results were recorded by Hashem and El-Sayed (1997) and Al-Gaabary *et al.* (2007) who found that *Trichostrongylus* was the predominant species in percent of 46.4% and 64.92% respectively.

Our results differed from that recorded by Gharib (1998) who found that *Trichuris ovis* was the most common species of gastrointestinal nematode.

Concerning the seasonal distribution of parasitic infestation, the highest prevalence was recorded in spring (71.59%) and the lowest one was recorded in summer (43.01%). Similar result obtained by Altaif and Issa (1983); who recorded that the peaks of worm egg counts occurred in spring and in autumn. Our results differed from the results of Aly *et al.* (1994) who found lowest infestation during autumn and spring and Khalafalla *et al.* (2011) who reported lowest infestation during spring.

The prevalence of the recovered 3rd stage nematode larvae from fecal culture revealed that the predominant species in winter, spring and autumn were *Haemonchus* (30%) while in summer *Bunstomum* (30%). Nearly similar results were obtained by Reynecke *et al.* (2009) who recorded that *Haemonchus* species was the predominant during wet seasons from October to March. Our results differed from that obtained by Horak (2003) who recorded *Haemonchus* species as the predominant nematodes in summer season.

Concerning age predisposition, parasitic gastrointestionl infestation was significantly different ($P<0.05$) among age groups, where the prevalence was higher in the animals over 2 years (78.46%) followed by animals from 1 to 2 years (72.27%) and lastly in that below 1 year age (45.93%). Similar results were recorded by Ramadan *et al.* (1992) who recorded highest worm burden in adult and old sheep. On the other hand, our results were different from that obtained by Khan *et al.* (2010) who found high nematode infestation in young animals than adults. Vlassoff *et al.* (2001) attributed these results to development and increase in immunity during increase of age. On the other hand Bonfoh *et al.* (1995) observed no relation between animal age and prevalence of parasitic gastroenteritis.

Concerning sex predisposition, significant variation was recorded within different sex where the prevalence rate in female animals was 75.86% and in male animals was 38.79%. Similar results were obtained by Khan *et al.* (2010). These

results may be attributed to pregnancy and lactation that cause rising in fecal egg count (Vlassoff *et al.,* 2001). The rise in fecal egg count in female with specific physiological status may be attributed to relaxation of immunity and resistance (Valderrábano *et al.,* 2006).

Some parasitologically positive animals appeared clinically normal. The clinical signs were severe emaciation, easily detached wool, pale mucous membrane and diarrhea in addition to bottle jaw in 2 cases. Our results were similar to those obtained previously by Yacob *et al.* (2009) who noticed that the severity of the clinical signs was related to the intensity of infection.

These clinical signs may be attributed to the decrease in the levels of serum total protein, albumin and globulin which lead to bottle jaw (Radostits *et al.,* 2010). Beside that there was significant decrease in serum calcium (Hasan *et al.,* 1986) and alkaline phosphatase. Moreover, there was significant reduction in bone mineral density (Thamsborg and Hauge, 2001). All these factors may lead to poor growth rate and emaciation. In addition, ulceration in abomasum and inflammation in intestinal wall interfere with digestion and absorption leading to diarrhea, emaciation and detached wool (Radostits *et al.,* 2010). Severe anemia recorded by Yacob *et al.* (2009) caused pale mucous membrane.

The hematological changes associated with parasitic gastrointestinal nematode in sheep showed negative correlation between the degree of infestation and the levels of RBCs, Hb and PCV. There were significant decrease in levels of RBCs and PCV and a significant decreases in serum total protein, albumin and globulin levels. In addition, there were in all infested animals. These results agree with the results obtaind by Radostits *et al.* (2010). In addition, there was a significant decrease in levels of MCV and MCH in severely infected animals only. Our results nearly similar to results obtained by Yacob *et al.* (2009) who found that low level of infestation with *Haemonchus contortus* leading to normochromic normocytic anemia. These results may be attributed to the effect of the hemolytic factor that released from adult *Haemonchus contortus* on the surface of sheep RBCs lead to hemolysis (Fetterer and Rhoads, 1998). Moreover significant decrease in serum total protein, albumin and globulins levels were recorded. This may be attributed to the inflammatory enteropathy that occurred in the gastrointestinal tract and, in turn, the alteration of the intestinal micro-circulation, permeability and motility leading to albumin lossing enteropathy (Nesheim, 1993). Moreover, Rhodes *et al.* (1978) claimed that albumin might serve as a nutrient for the growing parasites and this might be responsible for the recorded hypoalbuminemia.

Post mortem examination of abomasum of sheep infected with *Haemonchus contortus* revealed thickening, hyperemia and small ulceration in addition to petechial hemorrhage at the site of worm attachment. The abomasal contents were dark in color and the adult worm was noticed grossly. Histopathologically, there were mononuclear cell infiltration in between mucosal glands and lamina propria beside necrosis and sloughing of mucosal epithelium and presence of adult worm. Similar results were previously obtained by Hertzberg *et al.* (2000).

Finally, it can be concluded that, the prevalence of gastrointestinal nematode among examined sheep was high especially in females during spring and in age group over 2 years. *Trichostrongylus* spp was the most detected nematode. Infected sheep showed anemia and decrease in serum total proteins, albumin and globulins levels.

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