

PREVALENCE OF GASTROINTESTINAL NEMATODES AND ITS SUSCEPTIBILITY FOR DIFFERENT ANTHELMINTICS IN GRAZING SHEEP IN BENI-SUEF PROVINCE, EGYPT

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ABSTRACT

This study aimed to detect the prevalence and susceptibility of gastrointestinal nematodes (GIT) against three major anthelmintic drugs. Therefore, a total of 1217 sheep from ten grazing flocks was examined for the detection of gastrointestinal parasites. A salt flotation technique and larval culture were carried out. Moreover, FAMACHA system was applied on the examined animals as a guide for anemia. Among examined sheep, a number of 80 infected animals were divided into four equal groups; control untreated group, albendazole-administered group-, levamisole-administered group, and ivermectin-administered one. Meanwhile, a group of 20 animals, GIT-parasites free, were used as a control non-infected. The efficacy of anthelmintics was detected by fecal egg reduction test (FERT) 7 days post treatment. Findings showed that, the overall prevalence of gastrointestinal nematodes was 21.36%. Among them, *Haemonchus contortus* was present in 16.1%, *Trichostrongylus* spp. in 2.8%, *Strongyloides papillosus* in 1.7%, *Cooperia* spp. in 0.25%, *Bonostomum* spp. in 0.25%, and *Chabertia ovina* in 0.25%. There was a correlation between FAMACHA, PCV and fecal egg per gram. FERT was 100% by the 7th day post treatment in the three treated groups. In conclusion, *H. contortus* was the highest GIT nematodes among the examined animals, and anemia induced by them could be detected with FAMACHA. Anemia of nutritional disorders and other non-parasitic causes have to be considered parallel with the application of FAMACHA on grazing animals. Based on findings of FER post treatment, animals were susceptible for three anthelmintics, albendazole, levamisole, and ivermectin.

Key words: GIT nematodes, sheep, larval identification, anthelmintics, susceptibility.

INTRODUCTION

Indeed shortage of meat of human consumption is a huge concern. Sheep is an important source for animal protein. Gastrointestinal parasites are a major problem all over the world. Gastrointestinal nematode parasites are a major problem in the livestock. They cause significant economic losses, alter the well-being of the animals and, in heavy infections, the death of the host (Emery *et al.*, 1993). It was recorded that a total of 23 species of helminths were identified belonging to the family of *Trichostrongylidae*, with the genera of *Haemonchus*, *Ostertagia* (*Teladorsagia*), *Trichostrongylus*, *Cooperia* and *Nematodirus*; *Strongylidae* with the genera of *Oesophagostomum* and *Chabertia*; *Ancylostomidae* with *Bunostomum* and *Trichuridae* with *Trichuris*. *Teladorsagia*

circumcincta was the most common in sheep abomasum, *Bunostomum trigonocephalum* and *Trichostrongylus* spp. in the small intestine and *Chabertia ovina* and *Trichuris ovis* in the large intestine (Torina *et al.*, 2004). Among diseases that constrain the survival and productivity of sheep and goats, gastrointestinal nematode infection ranks the highest on a global index, with *Haemonchus contortus* being of overwhelming importance (Perry *et al.*, 2002). These parasites are common blood feeders that cause anemia, reduced productivity and can lead to the death in heavily infected animals (Githigia *et al.*, 2001). It has been estimated that each worm sucks about 0.05 ml of blood daily by ingestion or seepage from lesions (Urquhart *et al.*, 2000).

Anthelmintics chemotherapy is the best way to treat helminth infection, though treatment is expensive and resistance to drugs has developed in all the major parasite species (Veale, 2002). Three different classes of broad-spectrum anthelmintics (AH) are frequently used for sheep today: benzimidazoles (albendazole),

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macrocyclic lactones (Ivermectin) and imidothiazoles-tetrahydropyrimidines (levamisole) (Kahn and Line, 2005). The benzimidazoles (BZs) are usually the first choice of sheep and goats deworming because of their low cost and broad spectrum of activity. The difficult financial situation of the owners of small ruminant farms in recent years has led to their uncontrolled and often wrong use. In consequence of these and other factors some GIS are able to select a resistance against BZs. (Prichard *et al.*, 1980).

A novel system called FAMACHA was developed in South Africa, which enables clinical identification of anemic sheep and goats (Kaplan *et al.*, 2004). The main benefits of the FAMACHA system are the rational anthelmintic drenching. Therefore, treatment costs dropped by approximately 58% (Vanwyk and Bath, 2002). They estimated the correlation between FAMACHA-scores, haematocrit values, fecal egg counts, plasma albumin level and worm burden following an experimental infection with *Haemonchus contortus* in lambs. Consequently, FAMACHA reduces anthelmintic resistance by limitation of anthelmintic treatment to those with only clinical symptoms (Gauly *et al.*, 2004). There is an ongoing discussion both about when the sheep should be treated and which individuals that should be included. Out of the perspective of increasing multiple anthelmintic resistance, it would be preferable to only treat animals with clinical signs of helminth infection (Coles, 2002).

The current study aimed to investigate the prevalence of GIT nematodes in sheep and their susceptibility to different anthelmintics with the use of FAMACHA system.

MATERIALS AND METHODS

1. Animal's study

A total of 1217 native breed sheep from ten grazing flocks at Beni-Suef province was investigated for GIT nematodes infection. Fecal samples were directly collected from rectum of sheep or freshly deposited in clean polythene bags, then labeled and transferred to the laboratory. Eggs of helminths were analyzed through direct smear and salt flotation technique. By using Mac-Master technique eggs per gram (EPG) were counted while the different ova of helminths were identified by using key as mentioned by Soulsby (1982). All the examined sheep were subjected to application of FAMACHA system which obtained as

a gift from Dr Thomas Craig, Texas A&M University, (by examining the color of the inside of the lower eyelid as in (Fig. 1) to help in anemia determination.

2. Fecal cultures for larvae recovery and identification

Fecal cultures were done according to Eckert (1960) to obtain larvae. Recovery and identification of the larvae were carried out using the keys mentioned by MAFF (1977) and Gibbons *et al.* (2006). Morphological identification of L3 of most parasitic nematodes is based principally on examination of the caudal and cranial extremities, although other features such as the length or shape of the oesophagus or cranial refractile spots are important in some genera (van Wyket and Mayhew 2013).

3. Susceptibility of GIT nematode to different anthelmintics

3.1. Treatment trail

Based on FAMACHA[®], degree of anemia, PCV (blood samples were collected before treatment) and EPG, 80 GIT nematodes infected sheep (≥ 700 EPG) were selected for this trial. Besides, 20 animals were examined and free from GIT nematodes to be control uninfected sheep. The 80 animals were divided into four groups of twenty animals each. Then the used anthelmintics (albendazole, levamisole, ivermectin) were drenched for sheep groups. The drugs were administrated according to the manufacturers' recommendations (Albendazole 7.5mg/Kg, levamisole 15mg/Kg and Ivermectin 0.2mg/Kg).

3.2. Fecal Egg Count Reduction Test

Fecal examination of the treated animals was done at 7 and 14 days post treatment. FECRT was calculated according to the methods described in the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for the detection of anthelmintic resistance and efficacy (Coles *et al.*, 2006; Wood *et al.*, 1995). Percent efficacy of the various anthelmintic formulations was calculated post treatment by using the standard formula of (Varady *et al.*, 2004).

$$\frac{\text{Pretreatment EPG} - \text{Post treatment EPG}}{\text{Pretreatment EPG}} \times 100$$

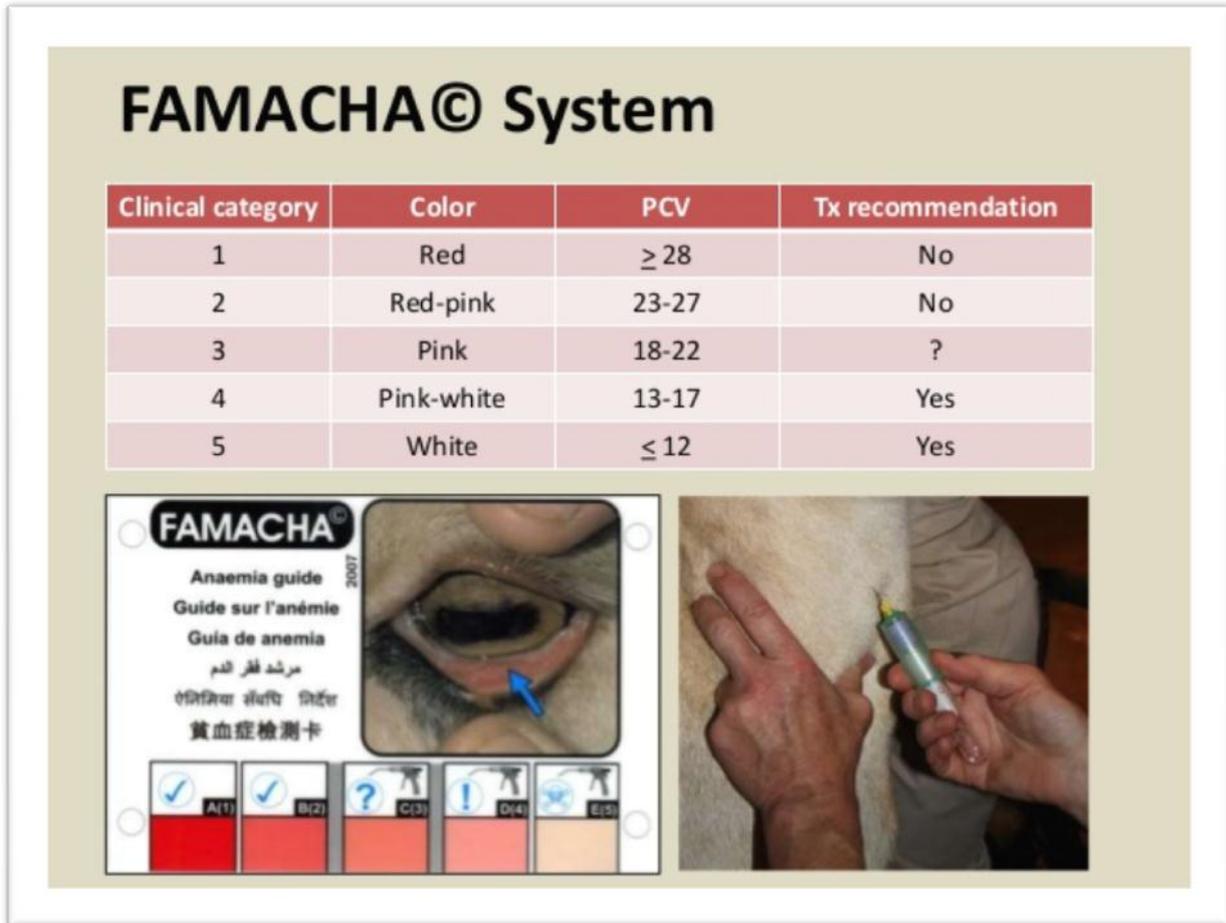


Fig. 1: FAMACHA® chart shows the different clinical categories of anemia, coloration of conjunctival mucous membrane, supposed PCV, and the treatment recommendation.

RESULTS

1. Prevalence of gastrointestinal nematodes

The fecal examination of 1217 sheep showed that 260 (21.36%) harbored gastrointestinal nematodes eggs. The infection % varied within the different months (figure 2). The highest infection % was in March (45.45%) and February (40.25%), while the lowest

infection was in June (2%). There was no infection in July and August.

Moreover, the seasonal prevalence of infection showed that, the highest infection% was in winter 37.4% (95/245) then spring 25.49% (91/375). In autumn the prevalence was 19.72% (72/365). It was obvious that the lowest % was in summer 0.82% (2/241) (table 1).

Table 1: Sesonal prevalence of GIT infection in sheep in Beni -Suef governorate.

Season	Number of examined animals	Infected animals	
		No	%
Winter	254	95	37.40
Spring	357	91	25.49
Summer	241	2	0.82
Autumn	365	72	19.72
Total number	1217	260	21.36

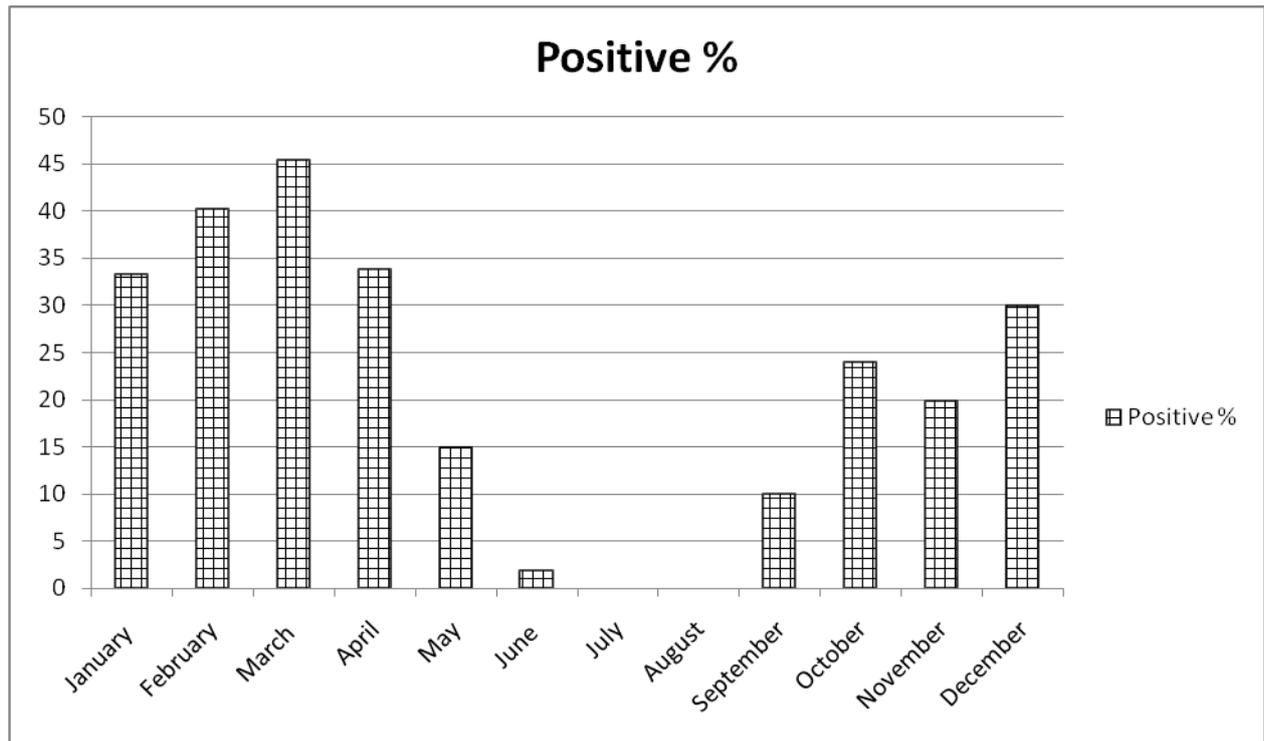


Fig. 2: Showing monthly prevalence of the GIT nematodes infection

2. Larval identification

Depending on the fecal culture; the recovered larvae of GIT nematodes of infected sheep were; *H. contortus*, *Trichostrongylus spp.*, *Cooperia spp.*, *Bonostomum spp.*, *Strongyloides papillosus*, *Chabertia ovina* (table 2). The single infection was recorded in

243 animals (93.46%) while the mixed one in 17 cases (6.53%). The mixed infection was *H. contortus* and *Strongyloides papillosus* in 15 sheep (5.76%), *H. contortus* and *Trichostrongylus spp.* in 2 sheep (0.77%).

Table 2: Identified larvae from fecal culture

	No. of infected animal/ 1217	%
<i>H. contortus</i>	196	16.1
<i>Trichostrongylus spp.</i>	34	2.8
<i>Strongyloides papillosus</i>	21	1.7
<i>Bonostomum spp.</i>	3	0.25
<i>Cooperia spp.</i>	3	0.25
<i>Chabertia ovina</i>	3	0.25
<i>Total</i>	260	21.36

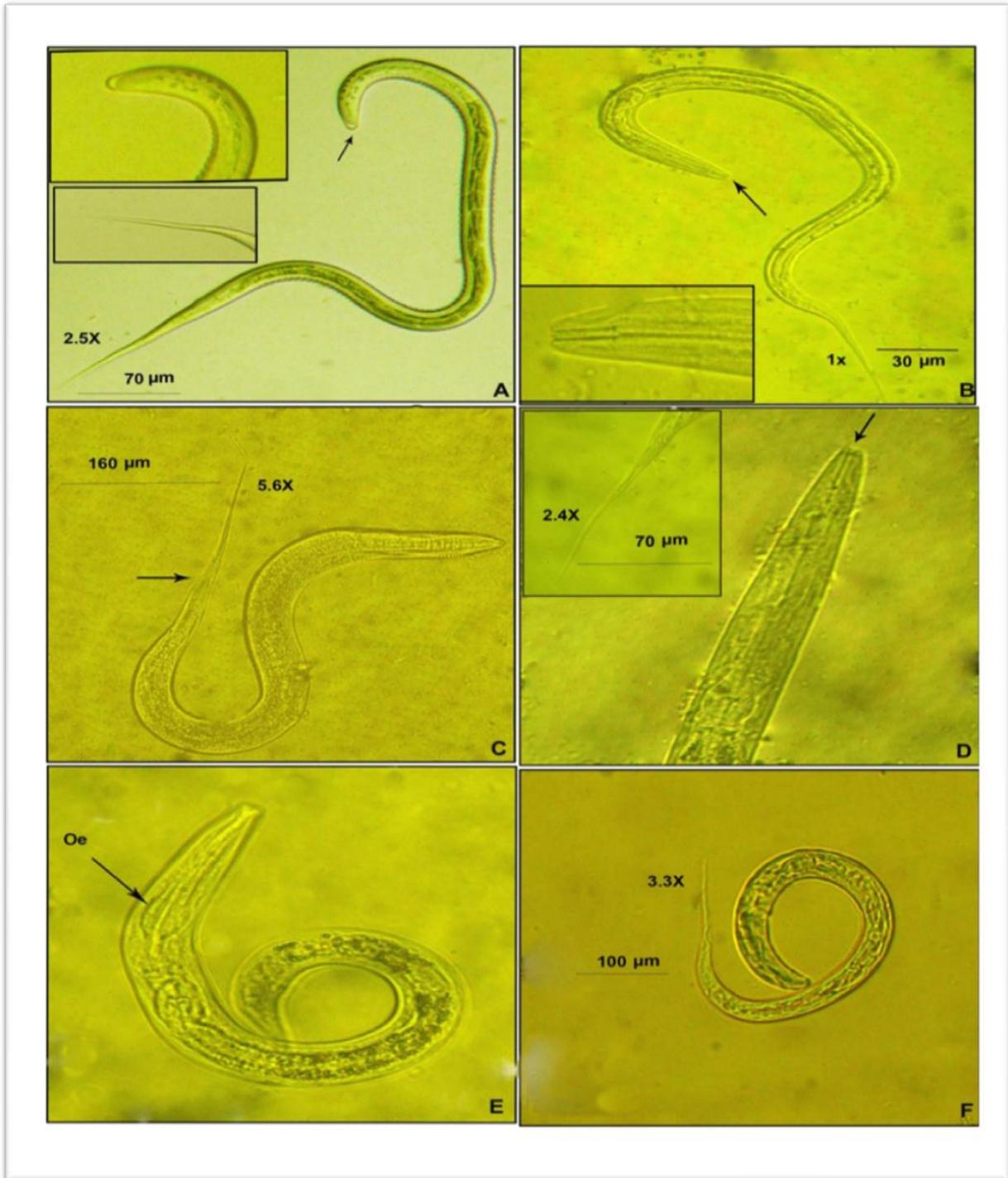


Fig. 3: A) *Haemonchus contortus* L3 with round ant end (arrow) and 70 µm (2.5 X) tails sheath. B) *Trichostrongylus* spp L3 with flat ant end (arrow) and 30 µm (1X) tail sheath. C) *Chabertia* spp with long tail sheath 160µm (5.6X). D) *Cooperia* spp L3 with 2 refractile spots (arrow) and tail sheath of 70 µm (2.4X). E) *Strongyloides papillosus* L3 with rhabditiform oesophagus (arrow). *Bunostomum* L3 very small larva with long tail sheath 100µm (3.3X).

3. Monitoring efficacy of FAMACHA to detect GIT infections

FAMACHA classified 646/1217 (53.07%) of examined animals non anemic, Meanwhile 571/1217 (46.93%) were anemic. Inside the non-anemic

animals, (12.57%) had GIT eggs but in lower than 100/EPG. In the anemic animals, 38.12% had no eggs of nematodes. In addition, 8.79% had anemia with GIT infection with variable EPG (Table 3).

Table 3: Classification of animals based on FAMACHA and EPG findings.

Item	Non anemic			Anemic with		
	EPG free	EPG up to 100	EPG free	EPG 500-700	EPG 700-1000	EPG 1000-1500
No/1217	493	153	464	61	37	9
(%)	(40.50%)	(12.57%)	(38.12%)	(5.01%)	(3.04%)	(0.74%)
FAMACHA	1-2	2	3-4	3	4	5

4. Treatment trail

At zero day of treatment, the FAMACHA findings, PCV and EPG were correlated with each other in the animals of the trial (Table 4). The FECRT of the

treated groups was 100% after 7 days post treatment while in the control infected non-treated was zero. In another words, the efficacy of the used drugs (albendazol, levamizol and ivermectin) was 100%.

Table 4: The relation between EPG & PCV & FAMACHA scoring and treatment

	Control non infected	Control Infected	Albendazole	Levamisole	Ivermectin
No. of Animals	20	20	20	20	20
FAMACHA score	1-3	3-5	3-5	3-5	3-5
Mean of EPG before treatment \pm SD	0	700 \pm 139	730 \pm 79.06	850 \pm 129.4	900 \pm 204.9
FER%	0	0	100	100	100
PCV % \pm SD	29.36 \pm 1.845	20.5 \pm 1.4*	21* \pm 3.263	18.68* \pm 1.203	19.84* \pm 2.577

DISCUSSION

In this study prevalence of GIT nematodes of sheep was 21.36%. This finding goes parallel with; Arafa *et al.* (2008) who found that, rate of *Haemonchus contortus* in sheep in Assiut Governorate was 18 %, Mahran (2009) who found that, the prevalence of *Haemonchus spp.* infecting sheep in Red Sea Governorate, Egypt was 23.62% and Abouzeid *et al.* (2010) who recorded the prevalence of infections with nematodes 27.5% in Sinai and zoo garden in Egypt. On the contrary, in Ahmed nagar District of Maharashtra, Sutar *et al.* (2010) recorded that gastrointestinal nematodes in 62.75%. This difference may be due to differ in locality, environment.

The highest seasonal prevalence of the GIT nematode was in winter 37.4% and the lowest prevalence was in summer (0.82%). Bhat *et al.* (2012) noted that the highest prevalence of G.I parasites was recorded during monsoon season (March - May) followed by summer season (June-August) and the lowest prevalence were recorded during winter season. On the contrary, Qamar *et al.* (2007) recorded that the highest seasonal prevalence in all types of sheep and goats was in summer (43.69%). Also, Chaudary *et al.* (2007) reported the peak infection level during rainy season (July-October) and low infection level was noted from December upto May. In addition, it is different to results of Khalafalla *et al.* (2011) who recorded the highest rate during autumn (15.2%) followed by summer (11.1%) and winter (9.4%) while the lowest rate was during spring (5.6%). In our

study, the high prevalence during winter is supported by the requirement of the parasites to the humidity. Moreover, and the lowest during the summer is augmented by the adverse effect of the dryness on the parasites.

In this study, different degrees of anemia and conjunctival mucous membrane coloration were recorded. Blood feeding habits of *H. contortus* can be reflected in the degree of anemia. Therefore the severity of haemonchosis and high EPG correlates with the low PCV and FAMACHA scores. Animals of EPG ≥ 700 showed lower PCV (≤ 21) and from 3 to 5 FAMACHA scored. During the course of fatal haemonchosis the color of the conjunctivae of sheep changes from the deep red of healthy sheep, through shades of pink to practically white, as a result of a progressively worsening anemia. The extent to which these changes relate to a range of haematocrit (Ht) values (chosen as the "gold standard" of anemia) (Jain, 1986). The feasibility of grading the degree of anemia clinically in conjunctival mucous membranes was investigated by both photographing the mucous membranes and determining the Ht of sheep which ranged from very healthy to extremely anemic (Bath *et al.*, 1996; Malan *et al.*, 2001). In area where haemonchosis is endemic FAMACHA is very reliable (van Wyk and Bath, 2002).

Regarding findings of FAMACHA, PCV and EPG, FAMACHA score was 3 or more with PCV ≤ 21 in animals of EPG ≥ 700 . Meanwhile animals of low EPG (up to 100) showed FAMACHA score 2. Some studies were applied on FAMACHA and they recorded that anemia due to GI strongyle infection was considered when the Hct and/or Hb values were under 27% and 9g/dl, respectively (DiLoria *et al.*, 2009). Furthermore, sensitivity of the method for detecting goats which needed a treatment was 76%, with regard to FEC of *Haemonchus contortus* (treatment cut-offs: FAMACHA ≥ 3 and FEC >300 EPG (Scheuerle *et al.*, 2010). In our findings false negative 153/1217 (12.57%) was detected in animals of low EPG (≤ 100). Similar findings showed that, percentage of false negatives was less than 11% (Reynecke *et al.*, 2011). Furthermore, false positive 474/1217 (38.12%) due to non-parasitic causes with FAMACHA SCORE 3-4 was recorded. Meanwhile, 107/1217 (8.7%) of examined animals were anemic because of GIT nematodes. Therefore reliability of FAMACHA depends on history of *Haemonchus* in the area and excluding the nutritional causes of anemia.

In the present study, FCRT was applied on GIT infected animals to detect the susceptibility of GIT parasite to three groups of anthelmintics (albendazole, levamisole and ivermectin) and they showed 100% reduction in fecal egg count. This result come in agreement Kumsa and Wossene (2006) who found

that albendazole had 100% efficacy against Ogaden isolate of *H. contortus*. Sheferaw and Asha (2010) recorded that the fecal egg count reduction for albendazole, tetramisole and ivermectin were 99.34%, 97.77%, and 98.30% respectively. On the contrary, in Mubende goats, albendazole, levamisole and ivermectin reduced FEC by 28.5%, 91%, and 98%, respectively. Meanwhile in Boer crosses, albendazole, levamisole, and Ivermectin reduced FEC by 11%, 84.88% and 78.47%, respectively (Byaruhanga and Okwee-Acai, 2013). Furthermore, reduction in EPG was only 77.63% in albendazole, 88.91% in fenbendazole and 93.21% in ivermectin (Nasreen *et al.*, 2007). Reduction in EPG was 96%, 99% and 97% respectively for albendazole, tetramisole and ivermectin (Teref *et al.*, 2013; Adediran and Uwalaka, 2015). All those differed with the present study may be referred to that they found a resistance while here there is no resistance but susceptible.

In conclusion, *H. contortus* was the highest prevalence GIT nematodes among the examined animals and its anemia effect can be detected with FAMACHA. Anemia of nutritional disorders and other non-parasitic causes have to be considered before application of FAMACHA on our grazing animals. All nematodes infected animals were susceptible for the three anthelmintics groups.

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انتشار وحساسية الديدان المعدموية في اغنام الرعى لمضادات الديدان في محافظة بنى سويف

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تناولت هذه الدراسة تحديد نسبة انتشار الديدان المعدموية في الاغنام ومدى حساسيتها واستجابتها لمضادات الديدان في محافظة بنى سويف. تم فحص عدد ١٢١٧ حيوان من ضمن عشرة قطعان اغنام. تم الفحص باستخدام اختبار التعويم وتم تحصين البيض لعمل توصيف لليرقات. وايضا تم استخدام الفاماكا لتحديد درجة الانيميا. تم اختيار ثمانين حيوان لاجراء اختبار الحساسية لمضادات الديدان. تم تقسيم الحيوانات الى ٤ مجاميع: مجموعة ايجابية ضابطة- مجموعة الاليندازول- مجموعة اليفاميزول - مجموعة الايفرميكتين. بالاضافة الى عدد ٢٠ حيوان غير مصابين تم استخدامهم كمجموعة سلبية ضابطة. كانت نسبة الاصابة بالديدان المعدموية ٢١.٣٦%. وكانت ديدان الهيمونكس اكثرهم انتشارا حيث كانت نسبتها ١٦.١%. بالنسبة كفاءة الادوية المستخدمة كانت نسبة اختزال البيض ١٠٠% فى المجاميع المختلفة. بالنسبة لاستخدام الفاماكا كان هناك ارتباط بين معدل البيض فى البراز واختبارات الانيميا والفاماكا فى الحيوانات عالية الاصابة بالديدان المعدموية. حدث تداخل فى اختبار الفاماكا بين الانيميا التى بسبب الطفيليات والتى بسبب سوء التغذية.