Some studies on haemonchiosis in sheep and goats in New Valley Governorate. Egypt.

Osman. F.A*., Gaadee, H. I. M. ** and Sameria sanosi*.**
* Parasitological unit, Animal health research institute. El-Kharga lab.
**Biochemistry unit, Animal health research institute. Assiut lab.
*** Biochemistry unit, Animal health research institute.,EL-Dakla lab

Abstract;

Haemonchuosis is a serious health problem which causes lower production host and hazard economic effect due to high morbidity, mortality, cost of treatment and control measures . The paper carried to study the prevalence of haemonchus species parasite infection a mange sheep and goats in New-Valley Governorate .Egypt, beside evaluated the efficiency of different diagnostic methods (Postmortem examination, fecal examination and ELISA technique) and determined the relationship between some hematological and biochemical parameters with the disease. Study were carried on total of 530, (225 sheep and 305 goats) and 100 clinically and laboratory healthy animals (50 sheep and 50 goats), as a control animals, from May 2014to May 2015. Adult worm was collected by opening the abomasum's in slaughterhouse in normal saline for Preparation of crude somatic antigen. Two blood sample collected randomly from each animal admitted to slaughterhouse and control animal for evaluated of hemato-biochemical parameters and ELIZA test study .Fecal sample collected randomly from each sheep and/or goats admitted for slaughter and control animal for fecal examination. ELIZA technique was carried with the preparation of crud somatic antigen, hematological and biochemical analysis was carried on to comparisons the parameters changes in the diseased and healthy sheep and goats. The study revealed that, 82(15.47%),75(14.15%) ,71(13.39) out of 530 of sheep and goats were found to be positive by ELISA, postmortem and fecal examination respectively, where the sensitivity of ELISA assay was 98.97%, while specificity was 98.24% in compared with postmortem examination and 96.47% ,96.96% ,respectively, in compared with fecal examination. A range of hemato-biochemical measures were revealed significantly different between infected groups and controls group, where the serum packed cells volume (PCV),Erythrocyte sedimentation rate (ESR), hemoglobin and total RBCs were significantly decreased ($P<0.05$), while the concentration of eosinophil, Lymphocytes were significantly increased ($P<0.05$) . The hematological studies indicated that, The percentage of neutrophils was low while lymphocytes count, number of basophiles and monocytes number in percentage were high, while biochemical studies revealed that, total serum proteins, Albumin/ Globulin Ratio (A/G Ratio) were significantly decreased ($P<0.05$) in infected animals . **Conclusion**; ELIZA test is more efficacy and reliable test in diagnosis of haemonchus parasite infection in sheep and goats because the test can detect the infection with the pre-patent stat and before the parasite attain to sexual maturity. Also the change in hemoglobin concentration, total serum proteins, total RBCs and A/G ratio were important indicators of haemonchuosis diagnosis and control in sheep and goats.

Keyword; Sheep and goats-Haemonchuosis-ELIZA-Hematological and biochemical studies;

Introduction;

Small ruminants play a great role in the economy of the world where they are a source of meat, milk, wool, skin and generate cash income, Fraser (1991).Parasitic infestations exert adverse effects on the health and productivity of animals. These effects are varied and more pronounced in sheep and goats compared to those seen in other species of livestock, Iqbal *et al* (1993). Many species of parasites are seen in sheep and goats and usually include. *Haemonchus, Oesophagostomum, Ostertagia, chabertia, Nematodirus, Trichuris, Moniezia* and *Fasciola*. The most important of these is *Haemonchus parasites.* That is the cause of haemonchiosis, Husnain and Usmani (2006). It is an important blood sucking parasite of the ovine and causes an insidious drain on production, Ijaz *et al* (2009). *Haemonchus species* parasite is known as the barber's pole worm, very common parasite and one of the most pathogenic nematodes of ruminants where adult worms attach to abomasa mucosa and feed on the blood, clinical signs associated with parasitic infection, loss of appetite, diarrhea anemia, edema, and death of infected sheep and goats, Bowman (2009).Mainly infection always occur during summer months in warm, humid climates , Okaiyeto *et al(* 2010).The disease caused by this parasite (haemonchuosis) is prevalent wherever sheep and goats are raised, but it exerts the greatest economic losses in temperate and tropical regions, Raza *et al* (2007). Githigia *et al* (2001). They estimated that each worm of Haemonchus species parasite sucks about 0.05 ml of blood per day by ingestion or seepage from lesions, where these parasites are common blood feeders that cause anemia and reduced productivity and can lead to death in heavily infected animals. Haemonchuosis is primarily a disease of tropical and sub-tropical regions, where high humidity, at least microclimate of the feces and the herbage is also essential for larval development and their survival. It is a serious health problem, which causes high economic loss due to high morbidity, mortality and cost of treatment and control measures, frequency and severity of the disease largely depends on the rainfall in any particular area. Surveys in countries around the world have shown that amongst domestic animals, sheep and Goats suffer more frequently from haemonchuosis, Nwosu *et al.*,(2007) and Tariq *et al.,* (2008).The diagnosis of haemonchuosis is usually based upon clinical signs and fecal examinations and ELISA assay, but ELISA enables detection of sub clinical infection. Furthermore immunodiagnostic studies in large groups of animals benefit from ELISA which is more reliable in contrast to postmortem examination, fecal examinations and less time-consuming. Almazan *et al.*, (2001)
The present study was designed to evaluated the sero-prevalence of haemonchuosis among sheep and Goats in New-Valley Governorate (Egypt),the associated hemato- biochemical changes in haemonchuosis and attempt to bridge the gap in knowledge of these aspects. The data thus obtained was helpful in diagnosing the disease at early stage as well as it was helpful for developing strategy for the control of haemonchuosis in sheep and goats.

Materials and Methods;

1-Animal;

Study carried on total of 530 animals (225 and 305 of sheep and goats respectively) , admitted for slaughter in El-Kharga abattoir and Bedouin slaughter point and 100 clinically and laboratory healthy control animals (50 sheep and 50 goats).Where The control animals was treated with anti-parasitic agent (Albendazoal) twice at one week interval and fifteen days following the last treatment fecal examination applied to exclude the infected animals.

2-Samples collection;
2,a-Fecal samples;

2 gram fecal sample were randomly collected from each animal (sheep and/or goats) admitted to abattoir or Bedouin slaughter point in El-Kharga city directly from the rectum of the animals in labeled plastic page and send for parasitological laboratory for examination..

2,b-Blood samples;

Two blood samples collected from each animal admitted for slaughter in both abattoir and Bedouin slaughter point in El-Kharga city,

1-5ml blood from jugular vein in test tube with EDITA for hematological examination. 2-5ml blood from jugular vein in test tube without anticoagulant f or preparation of serum by centrifuged at 3500 rpm for at least 5minutes and store until used with ELIZA test and biochemical examination;

2,c-Post mortem inspection;

Across-sectional study was conducted from May 2014-May 2015, to determine the prevalence of Haemonchus parasitic infection in sheep and goats at El-Kharga city. The animals were examined randomly and the abomasums were removed from the abdominal cavity and opened along their greater curvature. Close visualization was made for the presence of adult Hemonchus parasites.Adult worms were collected in normal saline for identified according to Soulsby (1982), and preparation of crud antigen according to. Johnson *et al*.,(2004).

3-Identification the parasites;

Dissect the tract wall and take the contents in clean physiological saline, then let it stand for 15 minute where worms precipitate occur, then a tightly sealable container that contain the worm as it is expanded and fixation with 70% ethyl alcohol and take a sample that has been fixed in a Petri dish and make a two-fold dilution of lacto-phenol solution with water. Add the solution to the Petri dish until the worm is immersed completely. Minami,(2001). Then with use the light microscope, the parasites were diagnosed depended on some morphological characters as length, vulvar flap, anterior end, posterior end, and presence barber pole in females of *Heamonchus* worm. Soulsby, (1982), in addition, *Haemonchus* parasite is probably the only nematode parasite of sheep and goats that can be accurately diagnosed without the aid of laboratory testing
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4- Preparation of antigen;

For preparation of crude somatic antigen (CS-Ag), adult worm of Haemonchus parasite from the abomasum's of freshly slaughtered sheep and goats were collected according to .Johnson *et al*., (2004), briefly, adult worm was collected in a Petri dish containing 0.15 M phosphate buffer saline (PBS), pH - 7.2. The worms were washed 3 times in the same buffer, and finally 200 worms were homogenized in 10ml of cooled 0.15 M PBS (pH - 7.2) containing 25 ml. M phenyl

methyl sulfonic fluoride (PMSF) and 24 ml methyl diamine tetra acetic acid (EDTA) in a glass tissue homogenizer followed by sonication (Soniprep-150). The disintegrated parasite extract was centrifuged at 4^{o}C at 10000 g for15 min and the supernatant was collected as the CS-Ag with a protein concentration of 3.82 mg/ml specified according to Lowry *et al*. (1951). The antigen was stored at -20^{0}C for use in the assay.

5- Parasitological techniques*:*

- Fecal samples were examined by direct smear, flotation and sedimentation techniques for the presence of *Haemonchus species* eggs according to Martin *et al.*, (1990). Haemonchus eggs were identified on the basis of morphology according to Valderrabano et *al.*,(2001) . Fecal pools were carried out for the copro-cultures to isolate the larvae by Barman's technique, identification of the third stage larvae (L3) were carried according to modified method described by Martin *et al.*, (1990).

5-Standardization of the assay;

Plate ELISA was performed in 96 wells polystyrene micro-titer plates with the antibody detection was performed according to Voller *et al*. (1976) with some modifications. The optimal concentration of ELISA reagents including the concentration of the coating antigen (5 µg/well), dilution of the positive and negative reference sera (1:100) as well as rabbit anti-goat IgG-horseradish peroxides (HRP) conjugate (1:1000) and the optimal test conditions, respectively were determined by checkerboard dilution assay using flat-bottom 96-well micro-ELISA plate. The absorbance (optical density; OD) of the wells was measured at 492 nm by an ELISA reader (Biorad II).The mean OD plus three times the standard deviation of the negative control sera was taken as the cut-off value for considering a sample as test positive .

6-Performance of the assay;

The sensitivity, specificity and accuracy of the ELISA were determined according to, Thrusfield, (1997), using 96 serum samples of sheep and goats where the parasitological status of animals with regard to nematodes, Trematode and Cestodes parasites was carefully examined.

7. Biochemical analytical procedures:

The clean non hemolysis sera was prepared after blood coagulation and kept in clean vials at -200C until used. The serum sample was used for quantitative determination of total serum proteins, serum albumin, serum albumin/ globulins ratio .Benjamin, (1981); Coles (1986), while calcium and Phosphorus by chemical kites.

8. Hematological analytical procedures;

Blood samples from all animals were collected in vials containing ethylene diamante tetra acetic acid (EDTA), as anticoagulant to carry of hematological analysis. Red blood cells (RBC), white blood cells (WBC), Hemoglobin estimation (HB), packed cells Volume (PCV) and Erythrocyte sedimentation rate (ESR) by the methods as described by Coles (1986) and Coffin

(1995).

9-Statistical analysis;

Statistical analysis using spas software and Chi-square test was applied for the statistical analysis of the data. Petrie and Watson,(1999).

Results;

A-Postmortem examination;

A total of 530 abomasums animals ,(225 sheep and 305 goats) were examined on post mortem for the presence or absence of Haemonchus species parasite adult worm. Examination revealed that, 33 sheep out of 225 (14.67%) and 42 goats out of 305 (13.77 %) were positive respectively while the overall prevalence of the positive parasite in this study was 75 out of 530, (14.15%). as illustrated in table 1.

***B-Parasitological* examination**;

Total of 530 fecal sample animals (225 sheep and 305 goats) were examined parasitological for the presence or absence of *Haemonchus*.spp egg. The result indicated that,31 sheep out of 225(13.78%) and 40 goats out of 305 (13.11%) were positive respectively, hence the overall prevalence of the positive parasite in this study was 71 out of 530 (13.39%) sheep and goats were positive as illustrated in table 1.

C-Sero-diagnosis of haemonchosis:

Total of 530 of serum sample animals, (225 sheep and 305 goats) were examined serologically for the presence or absence of *Haemonchus*.spp antibodies. The study revealed that,37 sheep out of 225 (16.44%) and 45 goats out of 305(14.75%) were positive respectively while the overall prevalence of the positive parasite in both sheep and goats was 82 out of 530 (15.47%), as illustrated in table 1.

D-Hematological analytical procedures:

A range of hematological measures were significantly different between infected and controls groups in both sheep and goats where the study revealed marked reduction in hematocrit(PCV), hemoglobin (HB) and RBC Count. While eosinophil and neutrophil measures were significantly increase by parasite infection in relative to uninfected control, also lymphocytes numbers were suppressed in the infected group with no evidence of a differential effect of parasite upon host responses Monocytes., as given in (Table 2, 4).

E-Biochemical analytical procedures:

The serum sample was used for quantitative determination of total serum proteins, serum albumin and serum albumin/ globulins ratio, Calcium and Phosphorus in both sheep and goats are given in(table 3 and 5).Where the study revealed that significantly lower in total serum

protein concentration (P<0.001) compared to control group animals and significant decrease in albumin/globulin ratio, also we found significant decrease in calcium and phosphorus values in compared to control group.

Fig 1;haemonchus controtus in sheep abomasum.

Animals. / Test.	Number of examined animals.			Number of positive animals.					
	Sheep	Goats	Total.	Sheep	%	Goats	%	Total.	%
Abattoir examination	225	305	530	33	14.67%	42	13.77%	75	14.15%
Fecal examination.	225	305	530	31	13.78%	40	13.11%	71	13.39%
ELIZA.	225	305	530	37	16.44%	45	14.75%	82	15.47%

Table 1.Comparsion between different used methods of diagnosis of *Hemonchus* parasite infection;

Test. / Animals.	Abattoir examination.	ELIZA test.	False positive.	False negative.
Sheep.	33	37	5	1
Goats.	42	45	3	--
Total	75	82	8	1

Table 2.Sensitivity and specificity of ELIZA in comparison to postmortem examination;
Sensitivity=true positive/true positive +false negative=82/1+82=98.97%.
Specificity True negative/True negative +false positive=448/448+8=98.24%.

Test. / Animals.	Fecal examination.	ELIZA test.	False positive.	False negative.
Sheep.	31	37	8	2
Goats.	40	45	6	1
Total	71	82	14	3

Table 3.Sensitivity and specificity of ELIZA in comparison to fecal examination;
Sensitivity=true positive/true positive +false negative=82/3+82=96.47%.
Specificity =True negative/True negative +false positive=448/448+14=96.96%.

Animals. / Parameters.	Control animals.	Infected animals
RBCs (x10/µl)	3.3± 1.02	1.74±0.94 ↓*
PCV %	30.75±5.26	10.32±3.56 ↓**
Hb (gm/dl)	15.8± 2.26	8.6± 1.48 ↓**
ESR mm /hour	3.5± 1.27	4.8± 2.21 ↑*
TLC (103/µl)	7.9± 1.23	8.67± 2.24
Neutrophile %	31.46±5.32	42.39±4.43 ↑*
Lymphocytes%	63.25±5.62	31.5± 3.49 ↓**
Eosianophile%	1.2± 0.09	22.92±2.69 ↑**
Basophiles%	1.89± 0.26	0.49±0.08 ↓*
Monocytes%	2.2± 1.01	2.7± 1.21

Table 4.Mean values of hematological parameters in heamonchiosis and control sheep.

Animals. / Parameters.	Control animals	Infected animals
Total serum protein.(gm/dl)	7.2± 0.32	6.21±0.20↓*
Albumin.(gm/dl)	3.35± 0.24	2.16±0.12
Albumin/globulin ratio .(gm/dl)	0.95± 0.02	0.25±0.02↓*
Calcium (gm/dl)	11.17± 1.13	8.73 ± 1.44↓**
Phosphorus (gm/dl)	6.92± 0.21	4.26 ± 0.85↓**

Table 5.Mean values of biochemical parameters in heamonchuosis and control sheep.

Animals. / Parameters.	Control animals	Infected animals
RBCs ($x10^6$/µl)	3.35± 1.01	1.89± 0.89 ↓*
PCV %	31.5± 5.52	15.34±5.2↓**
Hb (gm/dl)	14.1± 2.64	9.71± 1.98 ↓**
ESR mm /hour	3.5± 1.23	4.5± 1.56 ↑*
TLC (103/µl)	8.2± 2.19	10.2± 1.52

Neutrophile %	32.29±4.5	45.98±8.7↑*
Lymphocytes%	62.5± 6.41	32.25±4.5 ↓**
Eosianophile%	1.5± 0.15	20.17±2.9 ↑**
Basophiles%	1.91± 0.15	0.35± 0.04↓*
Monocytes%	1.8± 0.08	1.25± 0.07

Table 6; Mean values of hematological parameters in heamonchuosis and control goats.

Animals. / Parameters.	Control animals	Infected animals
Total serum protein.(gm/dl)	6.14± 0.08	6.34± 0.19↓*
Albumin.(gm/dl)	2.41± 0.12	2.65± 0.17
Albumin/globulin ratio .(gm/dl)	1.6± 0.11	0.71±0.01↓*
Calcium (gm/dl)	10.12± 0.31	8.59 ± 1.46↓**
Phosphorus (gm/dl)	6.26± 0.35	4.33 ± 0.65↓**

Table 7; Mean values of biochemical parameters in Hemonchuosis and control goats.

DISCUSSION;

Haemonchuosis is one of the most economically disease of sheep and goats caused by Haemonchus parasitic infection which inhibits in the abomasa of sheep and goats, bores its walls and cause great economic losses including decreased weight gain, decreased milk yield. Maqsood *et al.*,(1996).Where the parasitic cause of disease is blood feeders, cause blood loss and decrease in hemoglobin that can lead to death in heavily infected animals, where each worm sucks about 0.05 ml of blood by seepage or ingestion from lesions per day. Githigia *et al.*, (2001) -The present study revealed that the overall prevalence of *Haemonchus parasite* was. 75 out of 530 (14.15%) , 71out of 530 (13.39%) and 82out of 530 (15.47%) in sheep and goats by postmortem examination, fecal examination and serological diagnosis respectively. While in sheep signally the study revealed that the prevalence of Haemonchus parasite was, 33 out 225 (14.67%) ,31out of 225(13.78%),37out of 225 (16.44%) but in goats was, 42 out of 305 (13.77%) ,40 out of 305 (13.11%), 45 out of 305(14.75%), by postmortem examination, fecal examination and Serological diagnosis respectively. This result was contrary to the previous studies reported by researchers. El-Azazy (1995) 47.9% from Judah., Saudi Arabia., Kumsa and wassene (2007), reported., 91.1% and 80% prevalence on sheep and goats respectively and Thomas (2007) reported 81.1% in Awasa. Ethiopia. This difference may be due to different in natural resistance of the host and management practices of the animal owners, also the present finding was higher than the previous findings but higher than the previous finding, 7.9% in Egypt., Khalid et al.,(2010).Where ,Mandonnet et al.(2003), attributed that to the different management practices such as regular deworming, intensification housing and feeding management practice but Chaudhary et al, (2007), attributed that for local geo-climatic factors , nutrition, natural resistance of the host, during treatment schemes season.
-The study revealed that no significant difference in the prevalence of Haemocnhus parasite in sheep and goats by the different three diagnostic methods, postmortem examination, fecal examination and ELIZA technique but though there was high prevalence rate in sheep 14.67%

(n=33), 13.78% (n=31), 16.44% (n=37) than in goats, 13.77% (n=42) ,13.11% (n=40) , 14.75% (n=45).This attributed by. Urquhart, *et al*., (1996), due to sheep is more grazing in pasture than these of goats and environment factors.

-The indirect ELISA was evaluated on field sera and the results were compared with the post-mortem findings and parasitological examination indicated higher results occur by ELIZA, Postmortem and fecal examination respectively, This may be attributed to serological diagnosis can be detected *Haemonchus* antibodies as early as one week post infection and before sexually maturity of worm but detection of worm eggs by fecal examination occur after sexually maturity of worm. So ELISA can detection of pre-patent and patent stag of haemonchuosis in sheep and goats, where the wide variations in the antibody level in the necropsy positive samples, fecal examination samples and indirect ELISA, may be attributed to immune evasion mechanisms of the parasite. Spinelli *et al*.,(1996), where false negative results in the present assay might be due to low worm burden or poor immune response of the host, Gasser *et al*., (1994). Besides, host nutritional status, physiological and environmental factors like re-infection or co infection with other parasites might also have an impact on the antibody levels. Jenkins *et al*.,(1991). False positive result of the assay with postmortem negative samples might be due to the persistence of antibodies to the past infection, which might have been eliminated by anthelmintic medication. False positive result is probably due to cross reactivity of haemonchus parsite with other helminthes. Cross reactivity amongst different helminthes is a common and limiting factor in the development of serological tests against helminthes infection. Cuquerella *et al*., (1994) and Molina *et al*.,(1999).

-Hematological studies revealed decreased values of haematocrit (PCV), hemoglobin (HB), and RBC counts in sheep and goats in relation to Hemonchus parasitic infection. The reduced RBC counts, HB and PCV values in infected groups may be attributed to the bleeding of abomasums due to the injuries caused by the Haemonchus parasites similar to that described by. Abdel (1992).Also the present study revealed significantly increase of eosinophil, Lymphocytes and neutrophil by parasite infection.The result agreement with the findings of. Bhat and Sharma(1990) who concluded that eosinophilia is associated with antigenic stimulation or parasite burden, also agreement with Terefe *et al.* (2005), who concluded eosinophil's are considered to be important elements in the response against Haemonchus parasite infections, also concluded that increased lymphocyte count may be due to proliferation lymphocytes due to excretory secretary product of Haemonchus parasite.

-The development of systemic and local tissue eosinophilia is of the host immune response towards helminthes infection. However, opinion is divided on the role of eosinophils during infection, in terms of both their protective effect and their ability to mediate inflammation. Lee and Lee,(2005).There is also some evidence suggesting that eosinophil's may contribute to pathogenesis during parasitic infection. Moreover, it has previously been shown that a number of ovine parasitic gastrointestinal nematodes produce a factor(s) that promote eosinophil migration in vitro Wildblood *et al.*,(2005).This raises the possibility that helminthes may actively promote eosinophil recruitment and activation and utilize resulting tissue damage to aid their survival within the host. They assume that the mucosal cellular effectors components in *Haemonchus parasite* were seriously affected by activated and concentrated eosinophil's in the mucosal environment. Yacob *et al.*,(2008).

-Significantly increase in ESR agreement with. Mir *et al.*, (2007) who concluded that decreased values of HB, RBC, PCV, The inhibition of monocytes seems an important defense strategy devised by the parasite.
-Biochemical studies revealed decreased concentrations of total protein in sheep during *haemonchosis*. Decrease in total serum proteins in the present study may be attributed to heamo-dilution, a compensatory mechanism for the abomasa hemorrhages caused by the invading larvae and later on due to loss of large quantities of serum proteins into the gut and consequent increased fractional catabolic rate of albumin. Also albumin/ globulin ratio was subnormal in infected animals where globulin has been shown to contain immunoglobulin which is necessary for defense against parasitic infection and it disagreement with. Mir *et al.* , (2007). Demonstrated an increase in both cellular and humeral response following parasitic infection which go on to suggest the increase in the serum globulin and attributed that to an immunological response against the *haemonchus parasite* challenge.Decrease in level of calcium and phosphorus in sheep and goat heamonchuosis may be attributed to decrease absorption of feed mineral from gut and undernourishment of animal.

Conclusion;

-According to our study we can concluded that ELIZA was a useful technique for the diagnosis of *Haemonchus* parasitic infection in sheep and goats compared to carpological examination and postmortem examinations, because ELISA can detected prep taint and patient animal also ELIZA is more sensitive than any other tests which is one of the most pathogenic and economically important parasites of sheep and goat, Also hematological and biochemical changes in parameters with hemonchiosis can aid in diagnosis and therapeutic tool.

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