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HAEMATOLOGICAL, BIOCHEMICAL, SEROLOGICAL AND HISTO-PATHOLOGCAL CHANGES IN EXPERIMENTALLY INFECTED SHEEP WITH HAEMONCHUS CONTORTUS

By

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SUMMARY

The present study was designed to infect lambs with *H. contortus*. The eggs appeared in feces of experimentlly infected lambs 21 days post-infection and the highest level of eggs/gram feces "EPG" was recoreded on day 38 post-infection (p.i.).

Experimentally infected sheep with *H. conortus* showed significant decrease in Hb%, R. B. Cs count and PCV accompanied with leucocytosis. Differential leucocytic counts revealed neutrophilia and eosinophilia with decrease in lymphocytic counts, while basophils and monocytes were not affected. After treatment (45 days p.i. with levamizole), the blood picture had improved and reached the normal level 35 days after treatment.

Biochemical analysis of serum samples revealed decrease in total protein, albumin and A/G ratio, while serum globulin level was increased. There was also decrease in serum calcium and phosphorus levels. The biochemical changes improved and returned back to their normal levels 35 days post treatment (p. i.).

Sera from experimentally and naturally sheep haemonchsis were used to evaluate the diagnostic sensitivity of egg and crude *H. contortus* antigens using ELISA as compared to IHA. The present study revelaed that, the crude antigen gave the highest sensitivity value with ELISA 100% and IHA 91.67%. This result indicated that the crude antigen was the most suitable one for diagnosis of sheep haemonchosis especially with ELISA.

Histopathological changes of abomasum from experimentally infected lamb cleared dilatation and engorgement of serosal blood vessels together with submucosal leucocytic aggregation as well as the mucosal epithelium was distructed and sloughed.

INTRODUCTION

We must offer veterinary care for sheep to

overcome the problems and diseases to which they are exposed, especially in the field of endoparasites due the great lossses occurring. The gastro-intestinal parasites are very common in sheep due to their grazing and watering habits (Godbole et al., 1988).

H. conttortus is one of the predominant gastro-intestinal parasites of sheep in many areas of the world (Beck et al., 1985). It is distinguished by its blood sucking ability, due to a prominent buceal tooth presumed to function as lancet for slitting blood vessels (Noble et al., 1989), and also the ability of parasites to secrete cathepsin-L-like cystcin proteases that can facilitate the parasite to suck host blood(Rhoads and Fetterer, 1995).

H. contortus adult worms and fourth-stage larvae cause considerable damage of abomasal mucosa, extensive haemorrhage, greater changes in the blood picture, sever anemia, poor growth rate, weight loss or even death (Schalling et al., 1994 and Roads and Fetterer, 1996).

The diagnosis of haemonchosis is not confirmatory unless accompanied by the appearance of *H. contortus* eggs in the feces, which occurs about three to four weeks p. i. (Soulsby, 1982). Therefore haematological and biochemical changes as well as serological procedures are needed to increase sensitivity of detection and confirmation of the disease in tis early stages.

So, the aim of present work was planned to study haematological, biochemical changes, evaluation of some serodiagnostic techniques (ELISA and IHA) and histopathological changes from sheep experimentally infested with *H. controtus* as well as the ability of two serodiagnostic techniques for diagnosis of naturally sheep haemonchosis.

MATERIALS AND METHODS

1- Collection of H. controtus larvae:

Infective third stage larvae were obtained from fecal cultures of eggs which were collected from a lamb that was found naturally infected with *H. controtus* only, which was ensured by slaughtering and identification of adult *H. contrtus* worms according to Soulsby (1982).

2- Experimental infection:

Seven lambs (6-8 month old) were used in this study. Five lambs were orally infected; each with a single dose of 10000 larvac of *H. contortus* according to Mousa and El-Fauomy (1994). The remainder tow lambs were kept as non-infected control. Meanwhile, the fecal samples were collected from infected lambs at regular intervals (every two days) to detect the patent period. Also, the feces of control lambs were examined routinely throughout the whole experiment.

Four from the infected lambs were treated on day 45 p. i. (using levamizole HCL, 10% injectable solution, 5-mg/kg. b. w. s/c.). They were left alive till the end of the experiment i. e. 101 days p.i. Meanwhile the fifth infected lamb was slaughtered 45 day p. i. to study the histopathological changes occurred in the abomasum.

3- Haematological study:

Blood samples on anticoagulant from both infected and control lambs were collected during a period of 101 days p.i. (viz; 0,3,7,10,17, 24, 31, 38, 45, 52, 59,66,73,80,87,94 and 101 days). The samples were examined on the same day to determine erythrocytic count, haemoglobin (Hb) content and packed cell volumel (PCV) according to Bezubic et al,. (1980), as well as to determine leucocytic counts and differential leucocytic counts according to Shawkat et al., (1991).

A- Biochemical study:

Serum samples from both experimentlly infected and control lambs were obtained during the same previously mentioned period for the haematological studies The serum total protein, albumin and globulin were determined as that described by Rahman and Collins (1990). Also the serum clacium and phosphorus levels were determined acording to Ayeni and Adepetu (1985).

5- Serological study:

5a-Serum samples:

Sera of experimentally infected sheep:

Serum samples from both infected and control lambs were collected on days mentioned for the haematological studies to detect and evaluate the specific antibodies.

Sera of naturally infested sheep:

Serum samples were obtained from slaughtered sheep in Beni-Suef abattoir. Parasitological and meat inspection were carefully performed to determine any parasitic infestation. Fecal examination as well as fecal cultures of these animals were also performed. Twenty-four serum samples were obtained from naturally infected sheep with *H. comorus* as well as 9 serum samples from parasitc free sheep as controls.

5b-Preparation of antigens:

Crude antigen:

H. contortus adult worms were obtained from the abomasum of sheep. The worms were washed carefully in 0.01 M PBS, pH 7.4. The antigen was prepared using the method described by Mousa, 1994.

Egg antigen:

The obtained *H. contortus* eggs were washed carefully in PBS. The egg antigen was prepared using the method described by Mousa et al., 1996.

5c- Serodignostic techniques:

Enzyme linked immunosorbent assay:

As described by Iacona et al., (1980) with some modifications. The optimal reaction conditions as regards sensitizing antigen concentrations, antibody and conjugate dilutions were chosen for use with micro-ELISA after preliminary checkerboard titration. In the present study, the optimun conditions were 10 Mg/ml coating buffer antigen concentration, 1:50 serum dilution, 1: 1000 horseradish peroxidase labeled rabbit anti-sheep IgG as conjugate and ABTS (Sigma) as substrate. All incubation steps were carried out at 37°C in a moist chamber. The positively threshold value was determined as double fold the

mean cut off value of negative sera.

Indirect haemagglutination (IHA) assay:

Fresh turkey red blood cells were coated with the egg and crude *H. contortus* antigens as described earlier (Weir, 1979). The tested sera were diluted at 1:10 in PBS and added to 50 ul of antigen-cells diluted 1:80 in PBS , pH 7, 4 in V shaped wells. The reaction was considered positive when complete spread of cells was observed at the bottom of the wells.

6- Histopathological study:

Tissue specimens from the abomasum of both experimentally infected sheep 45 day p.i. and from control non-infected sheep were fixed in 10% formaline and histologically prepared. sections of 5-7 microns in thickeness were prepared and stained with haematoxylin and eosin (H&E) according to Carleton 1967). The sections were examined and photographed.

RESULTS

The present study revelaed the first appearance of *H. controtus* in the feces was on day 21 p.i. The number of EPG was (3200-4500) and that was continued to increase to reach (10100-13300) on the day 45 p. i. . On administration of levamizole Hcl 10% s/c, once for the first and third experimental lambs at a dose of 5-mg/kg b. w. eggs of *H. contortus* were dropped to zero after 2 days p.t., Continuos weekly examination of the treated lambs till the end of the experiment (101 days p.i.), revealed absence of *H. controtus* eggs in their fecal samples.

The haematological change occurred in blood constituents of experimentally infected sheep with *H. contortus* were displayed in table (1). It was cleared that the mean values of haemoglobin

Table (1): Blood picture of experimentally infected sheep with *H. contortus* before and after treatment.

Blood Constituents	Control Group	Days after infection				Days post - treatment				
		10	17	24	38	7	14	21	28	35
Hb(gm/100ml)	1.41±10.87	2.51±8.97	2.23±8.33	1.64±6.75	*1.29±5.52	1.58±5.82	1.82±6.43	1.42±7.91	1.65±9.20	2.10±9.35
RBCs (x106/ml)	1.65±11.32	2.35±10.0	2.40±8.75	1.63±7.12	*1.71±6.75	1.87±7.03	2.34±7.75	2.11±9.21	2.35±10.34	2.63±10.81
PCV%	2.73±34.69	2.471±27.34	2.11±24.37	1.88±22.41	*2.27±20.94	2.53±21.37	2.70±24.25	2.75±28.82	3.10±30.81	3.22±31.92
WBCs (x106/ml)	1.21±10.10	1.65±10.95	1.42±11.95	1.85±12.93	**2.13±14.75	2.35±14.20	2.21±13.18	1.93±12.49	1.88±11.35	1.73±10.75
Neutrophils(%)	3.21±30.55	3.21±36.22	3.55±39.91	4.03±40.46	*4.70±42.42	4.65±41.46	4.82±36.94	3.40±34.63	3.85±33.19	3.28±21.83
Eosinophils(%)	0.70±2.64	0.65±2.84	0.93±3.42	1.02±4.21	**1.21±4.72	1.35±4.65	1.26±4.25	1.02±3.20	1.41±3.02	1.24±2.80
Basophils(%)	0.02±0.91	0.09±0.92	0.05±0.88	0.08±0.86	0.03±0.85	0.03±0.89	0/04±0.88	0.03±0.89	0.03±0.89	1.20±0.90
Monocytes (%)	0.61±2.73	0.63±2.73	0.85±2.67	0.73±2.66	0.82±2.61	0.75±2.64	0.61±2.68	0.65±2.63	0.78±2.65	0.71±2.65
Lymphocytes(%)	4.31±63.17	3.62±57.3	3.75±52.21	3.92±51.81	*3.65±51.35	3.42±52.36	3.55±55.25	4.22±58.65	4.63±60.25	4.94±61.82

*** Significant at (P <0.001**)** **(Significant at (P<0.05).**

Fig (1): Sensitivity of egg and crude antigens for diagnosis of experimentally infected sheep with H. contortus using ELISA.

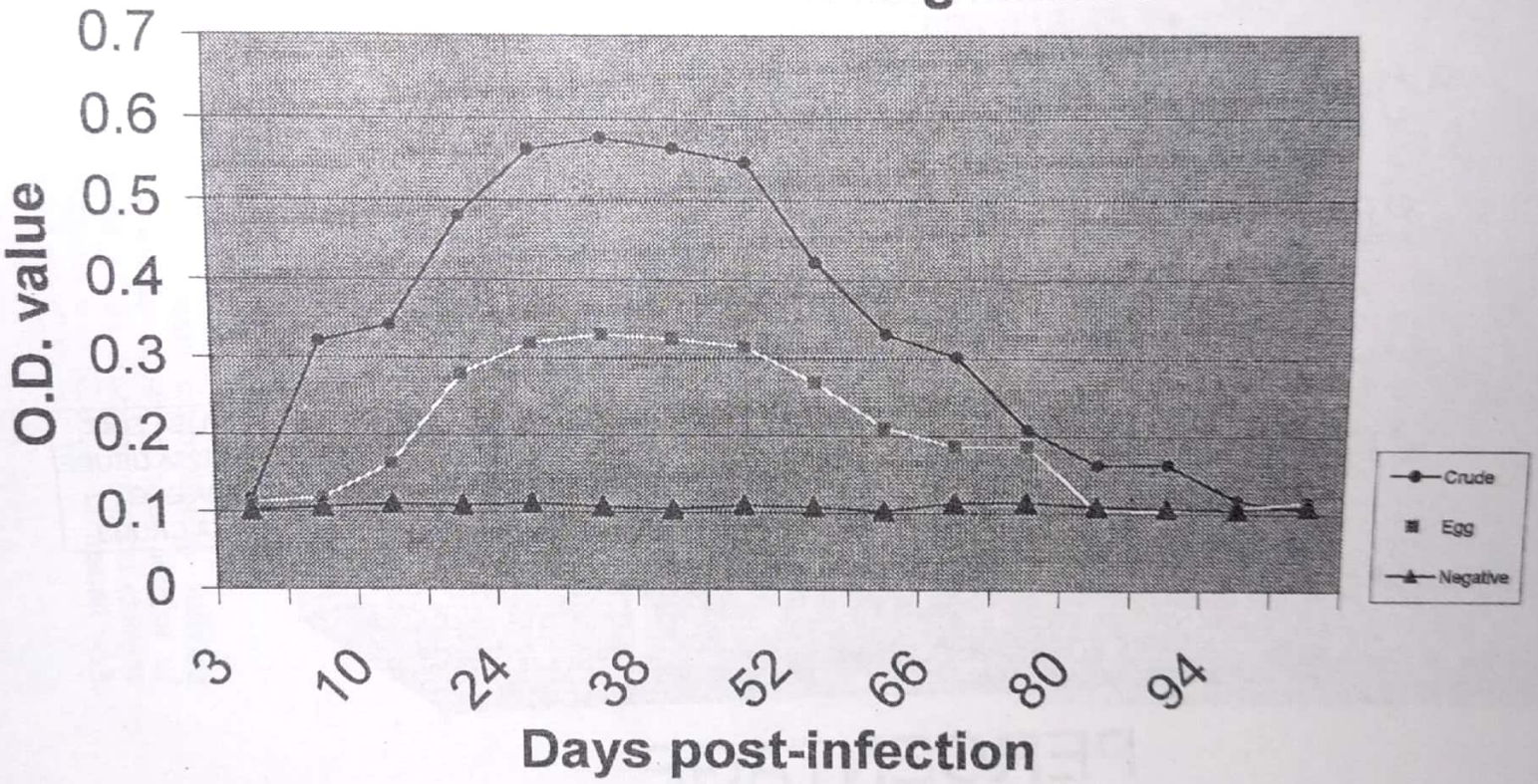
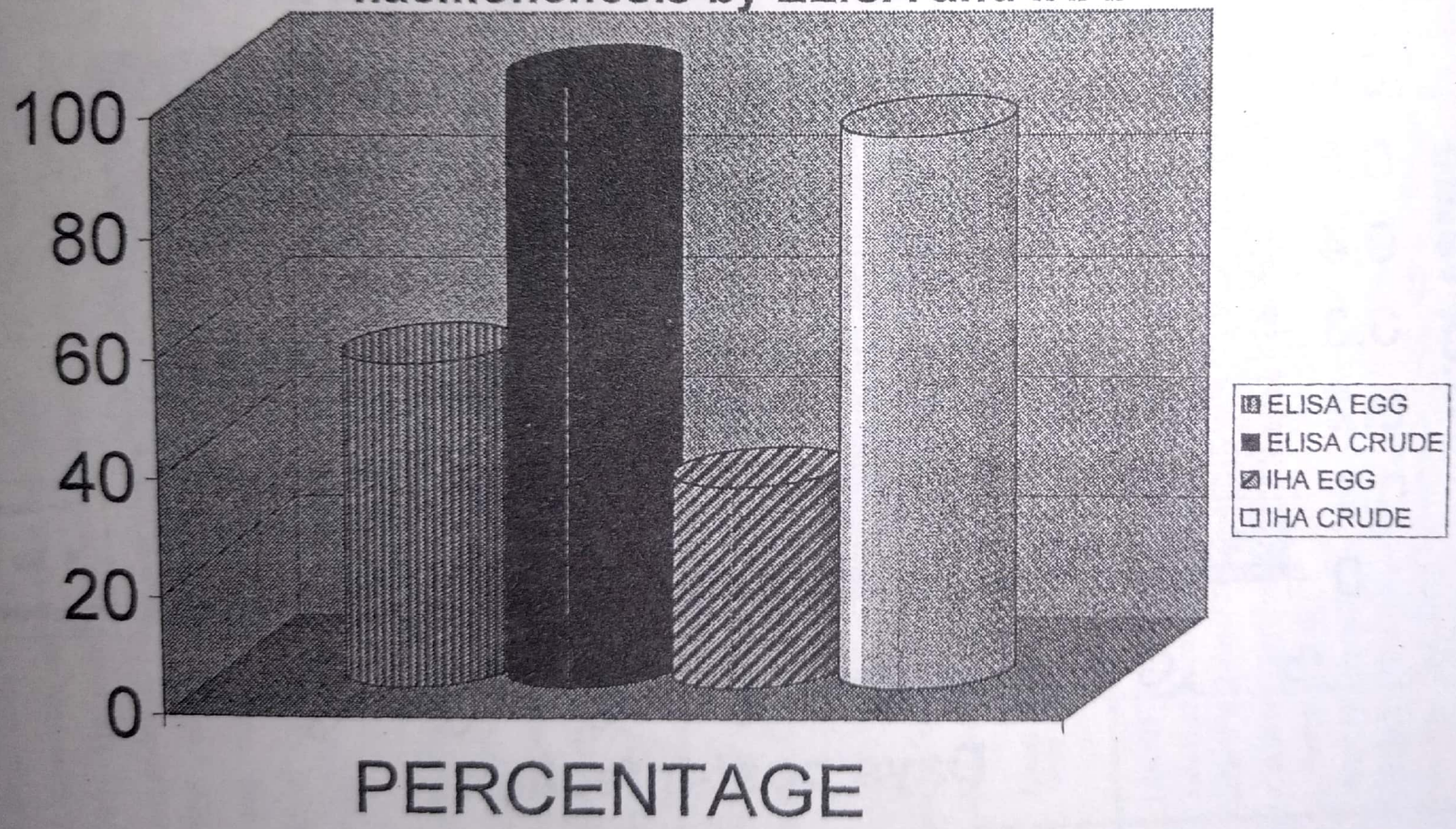
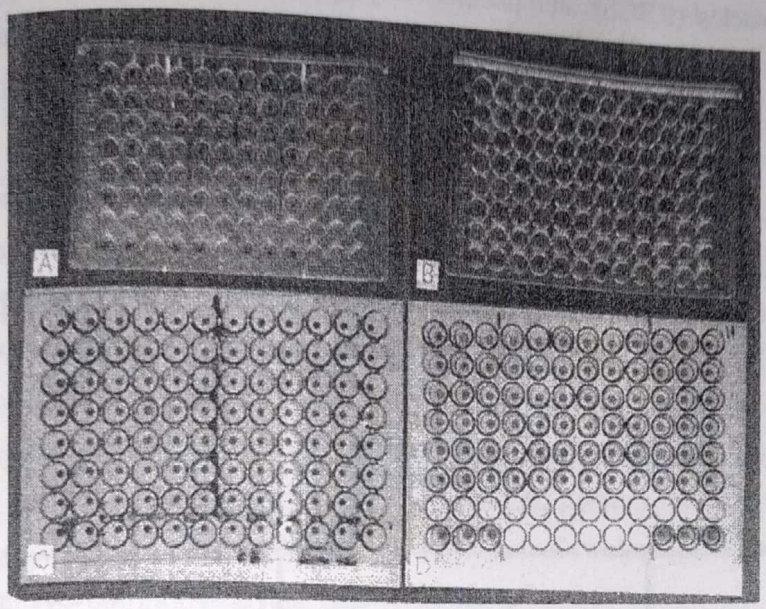
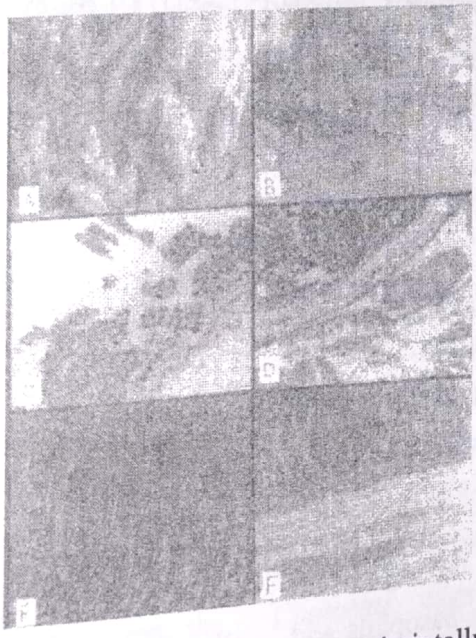


Fig (2): Sensitivity of egg and crude antigens with sheep haemonchosis by ELISA and IHA.





Plate(1): The sensitivity of egg & crude *H. contortus* antigens for diagnosis of Experimintally (A,B) and naturally (C,D) sheep haemonchosis by IHA



Plate(2):Abomasum of lamb experimintally infected with *H. contortus* showing large number of adult worms, (A,B). Histopathological changes in the abomasal wall (C,D), as compared with non-infected one (E,F).

(Hb), red blood corpuscles (RBCS) and packed cell volume (PCV) were significantly decreased (P<0.001), on the day 38 p.i.. Meanwhile, the mean value of W. B. Cs. was significantly increased (P<0.05). At the same time, there was higly significant increase in neutrophils (P<0.001) and eosinophils (p<0.05). On the contrary, there lymphocytes were significantly improved, where they reached again to the normal values 35 days p. i. (table 1).

Table (2) displayed the results of biochemical chances of serum samples collected from experimentally infected sheep with *H. contortus*.

Table (2): Biochemical parameters in serum of experimentally infected sheep with *H. contortus* before and after treatment.

Variables units	Control Group	Days after infection			Days post - treatment			
		17	24	38	14	21	28	35
Total protein (Gm/dl)	1.58±6.41	1.36±5.64	1.41±5.13	**1.34±5.13	1.28±4.65	1.18±4.82	1.22±5.37	1.45±5.83
Albumin (Gm/dl)	0.92±3.85	0.67±2.73	0.49±2.10	**0.21±1.24	0.39±1.52	0.49±1.86	0.64±2.52	0.83±3.12
Globulin (Gm/dl)	0.54±2.56	0.78±2.91	0.52±3.00	**0.63±3.15	0.62±3.13	0.39±2.96	0.32±2.85	0.39±2.71
A/G ratio	0.07±1.50	0.13±0.94	0.09±0.70	**0.07±0.39	0.09±0.49	0.08±0.61	0.07±0.88	0.08±1.15
Calcium) mg/dl)	1.88±9.78	1.76±7.72	1.85±6.55	*1.67±5.90	1.59±6.62	1.52±6.94	1.48±7.64	1.55±8.12
Phosphorus (mg/dl)	1.45±7.11	1.65±6.23	1.33±5.39	*1.38±4.43	1.43±6.65	1.62±6.75	1.75±6.82	1.83±7.08

*** Significant at (P <0.001**)** **(Significant at (P 0.05 >).**

was highly significant decrease (P<0.001) in lymphocytes, while there were no significant changes in the mean values of the basophils and monocytes cells, on the day 38 p. i. (table 1).

Concerning the blood picture of experimentally infected sheep after treatment, it was cleared that the mean values of Hb, R. B.Cs. PCV, total leucocytic counts, neutrophils, eosinophils and

It was proved that there was significant decrease (P<0.05) in the mean values of the total protein, albumin and albumin/globulin (A/G) ratio. Meanwhile, the level of globulin was significantly increased (P<0.05). Concerning the mean values of calcium and phosphorus, it was cleared that they were signifiantly decreased (P<0.05) till they reached to theri maximum decrease on the day 38 p.i.

On the other hand, the mean values of studied parameters were improved after treatment; where the total protein, albumin, globulin, calcium and phosphorus reached to their normal level, 35 days after treatment, (table 2).

The sensitivity of egg and erude *H. contortus* antigens using sera from experimentally infected sheep was studied by ELISA and IHA. The analysis of the obtianed ELISA data were illustrated in fig. (1), which showed significant antibody, levels at 17 days p.i. with egg and crude antigens, respectively. The antibody levels had increased gradually till 24 days p.i. and nearly remained in constant level till 45 days p.i. (the time of treatment) with both antigens. The antibody level had decreased gradually after treatment till 14 and 28 days p.i. with egg and crude antigens, respectively. After that time, the optical density (OD) of antibody level from inftected sera appeared as normal control sera.

On the other hand, the analysis of the obtained IHA data were illustrated in plate 1 showed significant antibody levels at 17 and 10 days p.i. with egg and crude antigens, respectively. The antibody level remained in the sera of infected sheep until 14 and 21 days after treatment with both antigens, respectively. After that time, the antibody level had disappeared from the sera.

The sensitivity of the egg and crude *H. contortus* antigens for diagnosis of naturally sheep haemonchosis using ELISA and IHA was studied. Sera from natural sheep haemonchosis (n=24) as well as normal control sera (n=9) were tested against both antigens. In ELISA, The sensitivity of egg and crude antigens was 54. 17% and 100%, respectively. Meanwhile, the sensitivity of the same two antigens was 33.33% and 91.67%, respectivley using IHA. (fig. 2).

Macroscopic examination of the abomasum of slaughtered experimentally infected lamb (45 days p.i.) revealed the presence of large numbers of adult *H. contors*, reddish brown fluid ingesta and mucosal swollen (plate 2A.B.)

Microscopic examination of histopathological sections of the abomasum revealed the presence of dilatation and engorgement of serosal blood vessels, together with submucosal leucocytic aggregations. The epithelium was distructed and sloughed as shown in plate (2C,D.).

DISCUSSION

The present study cleared that *H. contortus* eggs had firstly appeared on the day 21 p.i. with maximum appearance on day 45 p.i. .These data are in agreement with Bennett et al., (1980), Mousa and El-Fauomy (1994) and Schalling et al., (1995) who reported the presence of *H. Contortus* eggs in feces of experimentally infected lambs after 21-23 days p.i. . Concerning the number of EPG recorded for experimentally infected sheep, out results are in agreement with Silva-Vieira et al., (1989) and albera et al., (1990) who reported that the maximum egg count (EPG)

had appeared after 42 days p.i. respectively.

Studying the blood picture of experimentally infected lambs with *H. contortus*, the present study revealed a significant decrease in R. B. Cs. count, Hb content and PCV in infested lambs compared with non infected control group. These results go parallel with those of Bezubic et al., (1980); Ahmed and Ansari (1989) and Yadav et al., (1993) who observed nearly the same result.

Concerning the leucocytic and differential leucocytic counts, there was a significant increase in the total leucocytic counts, neutrophils and eosinophils with decrease in lymphocytic counts, but the basophils and monocyte counts were not affected. These result are in agreeement with those mentioned by Shawkat et al., (1991); Mottelib et al., (1992); Adams (1993) and Yadav et al., (1993) who reported significant increase in total leucocytic counts, neutrophils and eosinophils among sheep infected with *H. contortus*..

On the other hand, the biochemical study of blood serum constituents of experimentally infected lambs with *H. contortus* revealed significant decrease in the mean vlaues of serum total protein, serum albumin and A/G ratio, beside increase in the serum globulin level. These results are in agreement with those observed by Rahman and Collins (1990); Shawkat et al., (1991) and El-Sayed (1993). Also, there was significant decreased in the calcium and phosphorus levels in infected group compared with non infected one; with maximum decrease on the day 38 p.i. These results were in agreement with those recorded by Hamid et al., (1981) and ayeni and Adepetu (1985).

In the present study, egg and crude *H. contortus* antigens were used to capture anti-haemonchal antibodies in sera from experimentally and naturally sheep haemonchosis by ELISA and IHA, the antibody level of experimentally infected sheep with *H. contortus* appeared at 17 days and 7 days p.i. with egg and crude antigens respectively, then gradually increased till 24 days p.i. and nearly remained in constant levels till 45 days p.i. with both antigens (the time of treatment with levamizole). The antibody level decreased gradually from the day of treatment till 14 days and 28 days p.i. wit both antigens, respectively. Such data were in agreement wit those desceibed by Mousa and El-Fauomy (1994) and Schalling et al., (1995) who reported that the antibody elvel of experimentally infected sheep with *H. contortus* appeared one week after infection. Concerning the IHA, our result revealed that, the antibodies were observed at 17 days and 10 days p.i. with egg and crude antigens; respectivley. The antibody level remained in the sera till 14 and 21 days p.i. with both antigens, respectively. These data were in agreement with Adams and Beh (1981) who reported the titre of haemagglutinating antibodies specific for *H. contortus* rose in the serum during the course of primary infection. Also, Akulin et al., (1985) and Jan et al., (1993) reported that the antibody level appeared after days till 42 days p.i. using IHA.

Also the present study cleared that, crude antigen demonstrated a higher degree of sensitivity (100% & 91.67%) as compared with the egg antigen (54.17% & 33.33%) using ELISA and IHA, respectively. These result were in agreement with that reported by Mousa and El-Fauomy (1994) who reported the sensitivity of crude *H. contortus* antigen was 100% for diagnosis of sheep haemonchosis by ELISA. They were also in agreement with Ahmed et al., (1995) who reported that the sensitivty of erude *H. contortus* antigen is the most suitable one for diagnosis of sheep haemonchosis with ELISA.

Histopathological changes occurred in experimentally infected lambs with *H. contortus* (45 days p.i) showed the appearance of ditatation and engorgement of serosal blood vessels, leucocytic aggregations and destruction of epithelium; these result were strongly supported by Hunter and Machenzte (1982) and Jones and Hunt (1983).

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