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Protective Value of *Haemonchus contortus* Adult Worm Purified Antigen Against Haemonchosis in Sheep

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Abstract: The objective of this study was to characterize and purify *Haemonchus contortus* adult worm antigen and clarify its protective value against haemonchosis in sheep. Adult soluble extract of *H. contortus* was purified through gel filtration column chromatography using Sephadex G-100 resulted in 3 peaked chromatograms. Crude adult and 3peak fractions were fractionated through SDS-PAGE.The results of SDS-PAGE revealed that *H. contortus* crude antigen separated (10) protein bands of molecular weights ranging from 14.84 to 106.2, peak I of *H. contortus* partially purified antigen contained 14 protein bands ranging from 17.51 to 98.70 KDa, peak II contained 4 bands of protein ranging from 17.51 to 45.54 KDa and peak III contained one protein band observed at 13.63 K Da. The electrophoretic profile of *H. contortus* crude antigen and partially purified peaks by immunoblot using rabbit anti *H. contortus* sera was recognized. Of interest was the immunogenic component of 26 KDa expressed only by peak II in immunoblot assay. Peak II was used as a partially purified antigen in the serodiagnosis of haemonchosis using ELISA and as a vaccine. Immunization of sheep against *H. contortus* using partially purified antigen was evaluated by experimental infection in groups of lambs. Reduction in mean faecal egg count (FEC) and worm burden of the immunized group(41.4% and 84%, respectively) were recorded compared to that of the control positive group. Results of the clinical signs, the FEC, worm burden and the immunogenic evaluation of the used vaccine might indicate efficacy of the prepared vaccine and the purified fraction of 26Ka of adult worm antigen might be utilized for early diagnosis of haemonchosis. The results seemed to be promising. However, further investigations on a large scale of sheep are still needed.

Key words: *Haemonchus contortus* · Vaccination · ELISA · Lamb · Crude antigen

INTRODUCTION

Haemonchus. contortus is a major pathogen in the abomasa of sheep and other ruminants worldwide [1-3]. Blood is considered the main source of nutrients for Haemonchus spp. [4]. Infections with this parasite can cause, mainly in young animals, anaemia, weight loss that in some cases result in death [5]. The control of *H. contortus* infection is largely based on pasture management and the use of anthelmintics. However, clean pasture is not readily available under intensive grazing conditions and there is an increasing occurrence of parasites resistant to anthelmintics [6-10]. This situation has triggered efforts towards the exploration of potentially immunoprotective antigens to be used as alternative to chemotherapy [11]. The use of partially purified antigen as immunogen afforded a degree of protection to sheep against this parasite [12-14]. It has been demonstrated that a protective natural immune response is associated with the humoral recognition of low molecular weight *H. contortus* antigen, in particular, 26 kDa adult somatic or soluble extracts [5, 15-18].

Diagnosis is based on coprological methods, which are time consuming and require specially trained personnel. ELISA is a rapid and simple test with which a considerable number of samples could be processed at

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the same time. However, the detection of specific antibodies against the parasite by ELISA has produced inconsistent results because of the complex nature of the antigen extract used and the limited value of the soluble extracts as an antigen source [19]. The use of less complex antigen mixtures, has led to better results in ELISA test[20]. Many studies focused on identification of immunogenic protein antigens of *H. contortus* and the analysis of their potential to induce protective immunity by vaccination [21-23]. The immune response and the associated resistance can be modified by the type of antigen that is recognized and by such factors as age, nutrition and the number of infections [24].

The objective of this study was to characterize and purify *Haemonchus contortus* adult worm antigen and clarify its protective value against haemonchosis in sheep.

MATERIALS AND METHODS

Collection of Samples: Collection of *H. contortus* adult worms from abomasa of slaughtered male sheep at Monieb abattoir (Giza Governorate- Egypt) was applied according to Schallig *et al*.[25]. Adult worms were used for preparation of third stage larvae (L3) culture as described by Soulsby [26] and preparation of antigens.

Preparation and Purification of *H. Contortus* Antigens

Preparation of Antigen: *H. contortus* adult worms antigen was prepared according to Cuquerella *et al.* [15]. The extract was obtained by 8 cycles of freezing and thawing. Homogenization of approximate 500 adults for 15 minutes on ice followed by sonication for 5 minutes was done. The homogenates were centrifuged at 20000 rpm for 60 minutes at 4°C. The protein content of the supernatant was determined according to Lowry *et al*. [27]. The antigen was aliquoted and stored at -20 °C until use.

Preparation of Hyperimmune Sera: Hyperimmune sera against crude *H. contortus* antigen were raised in rabbits; the protocol was done according to Fagbemi *et al*. [28]. Briefly, each rabbit was subcutaneously injected with 1 ml (200µg protein content/kg b. w.) of the antigen emulsified with equal volume (v/v) of complete freund's adjuvant. Booster doses of antigen emulsified with incomplete freund's adjuvant were given one and two weeks later. One week after the last booster dose, the rabbit sera were collected and stored at -20°C.

Column Chromatography: Gel filtration chromatography of adult soluble antigen was performed by sephadex G100 column using standard laboratory methods according to Schallig and Van Leeuwen[13]. A 2ml sample containing approximately 25 mg protein was loaded on the column and protein fractions were eluted at a flow rate of 1 ml/ minute. The eluted fractions were measured at 280 nm using spectrophotometer. Peaks I, II and III from several runs were pooled separately and concentrated by sample concentrator. The protein content was determined for each peak according to Lowry *et al*. [27]. Peak II was used as partially purified antigen in ELISA and in the vaccination trial.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAG) and Western Blot Techniques Protein fractions of crude and partially purified antigens of *H. contortus* were demonstrated using 10% SDS- PAGE under reducing condition according to Laemmli, [29], with the aid of low molecular weight marker (Amersham, Co. U.S.A)

The fractionated antigens were transferred onto nitrocellulose sheet for Western blot technique. The nitrocellulose strips blotted with these antigens were allowed to react with rabbit hyperimmune sera raised against *H. contortus* crude antigen [30]. The molecular weight of recognized polypeptides was determined, with the aid of broad range molecular weight marker (Bio Rad Co. U.S.A), using software analysis gel pro-analyzer.

Immunization Trial and Seroassays

Experimental Infection: A total of nine Balady lambs of about (3 - 5 months old), were kept for 4 weeks indoor, before beginning of experiment, to acclimate together and were subjected to parasitological examination to prove their freedom from any parasitic infection [20].The lambs were divided into 3 groups, each of 3 lambs as follows: Group 1 served as (control negative group) non immunized non infected lambs. Group 2 (control positive group) non immunized infected lambs which received only 10000 L3 orally. Group 3 (immunized group) was immunized 3 times through subcutaneous injection with 50ug of the partially purified *H. contortus* antigen peak II, on days (0, 14 and 28); the first dose of the antigen was mixed with 1 ml complete Freund's adjuvant while the second and third doses of the antigen were mixed with 1 ml incomplete Freund's adjuvant according to Dominguez-Torano *et al*. [17] and challenged on day43 post immunization orally with 10000 L3. Individual anal faecal and serum samples were collected weekly to the end of the experiment. Feacal egg counts (FECs) were

made using the modified Mc. Master technique according to Soulsby [26]. All the experimental and control lambs were slaughtered at the end of the experiment (16th week) for abomasal examination and abomasal worm counts as well as the post mortem examination was carried out.

Enzym-linked Immunosorbent Assay (ELISA): Blood samples were also collected weekly from (0) week to the end of the experiment. Sera were separated and stored at -20°C until used for ELISA. They were subjected to indirect ELISA as mentioned by Schallig and Van Leeuwen [13]. In brief; ELISA plates were coated with 100 µl/well of the prepared purified *H. contortus* antigen peak II at the concentration of 5µg protein/well coating buffer. The selected dilution of sera was 1:200. 50 µl/well of O-phenylene diamine(OPD) was used as a substrate. Then 100µl /well of anti-sheep IgG alkaline phosphatase conjugate diluted at 1:5000 in PBS were added and 50 µl /well of 1 N Na OH were added for stopping the reaction. The OD was read at 405 nm with a micro-ELISA reader system. The sera were considered to be positive when the absorbance values were as or more than the cut off value (the cut off value = mean value of the negative control ± 2 standard deviation of the negative control) according to Allan *et al*. [31].

The blood samples were examined in the same day for determination of the total erythrocytic and packed cell volume (PCV) and haemoglobin content (Hb) as described by Feldman *et al* [32].

Statistical Analysis: Data were statistically analyzed adopting one way ANOVA for the general linear model (GLM) in the statistical analysis system [33].

RESULTS

Purification of Adult *H. Contortus* Crude Antigen: Three peaked chromatograms were obtained (I, II and III), as shown in Fig.1.

SDS-PAGE: As shown in Fig. 2 *H. contortus* crude antigen was separated by SDS-PAGE into 10 protein bands of molecular weights ranging from 14.8 to 106.2 KDa. While, peak I of *H. contortus* partially purified antigen contained 14 protein bands ranging from 17.51 to 98.70 KDa, peak II contained 4 bands ranging from 17.51 to 45.54 K Da and peak III contained one protein band at molecular weight of 13.63 kDa. There was one common protein band of molecular weight 17.5 KDa shared between the crude antigen and the partially purified peaks I and II.

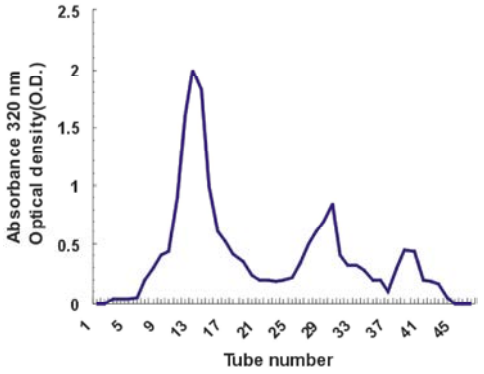

Fig. 1: Elution profile of fractionation of adult *H. contortus* somatic antigen by Sephadex G-100. Peaks I, II and III were obtained.

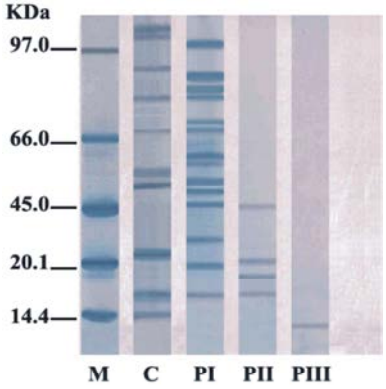

Fig. 2: Protein profile of crude *H. contortus* antigens obtained from one dimensional gel separated by SDS-PAGE 10% under reducing conditions and stained with Coomassie brilliant blue. M, Unstained low molecular weight marker (Amersham, Co., U.S.A); C, crude *H. contortus* antigen, lane 1; PI, peak I of partially purified *H. contortus* antigen, lane 2; PII, peak II of partially purified *H. contortus*, lane 3 and PIII, peak III of partially purified H. contortus, lane 4.

Western Blot Technique: The adopted Western blot technique (Fig. 3) revealed that rabbit anti *H. contortus* sera reacted with16 protein bands present in its own antigen of molecular weights ranging from 15.71 to 168.71KDa. While, the partially purified peak I revealed 14 protein bands of molecular weights ranging from 15.782 to 211.77 KDa and peak Ï revealed 9 protein bands of molecular weights ranging from 15.489 to 189.02 KDa. There were 2 common immunogenic reactive protein bands of molecular weights 30 and 15 KDa between the crude antigen and the partially purified peaks I and II. SDS-PAGE combined with Western blotting revealed that

Table 1: Mean number of the adult worms (females and males) in the control negative, control positive and immunized group ± standard error (SE).

	Mean number of adult worms			
Gender	Control negative group ± SE	Control positive group ± SE	Immunized group ± SE	Over all means of gender
Females	0	461.67±37.56	71.33±38.489	177.67 A
Males	0	281.67±26.03	47±27.513	109.56 B
Over all means of group	0 C	371.67 A	59.17 B	

LSD=58.513.

Over all means with the same letter are not significantly different (P<0.05).

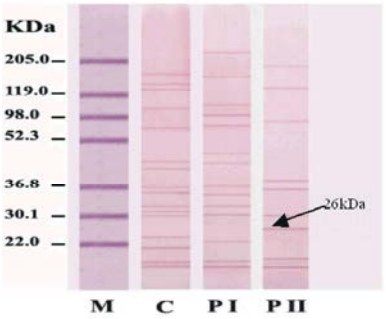

Fig. 3: Western blot showing analysis of SDS-PAGE under reducing condition for gel slab. M, prestained broad range molecular weight marker, (Bio Rad Co., U.S.A); C, crude *H. contortus* antigen, lane 1; PI, peak I of partially purified H. contortus antigen, lane 2 and PII, peak II of partially purified *H. contortus* lane 3.

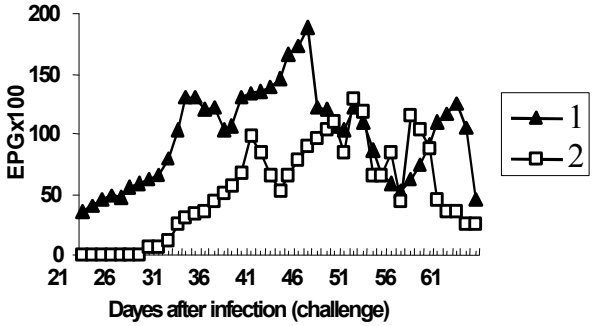

Fig. 4: Feacal egg counts 1; Mean values of FEC of the control positive group, 2; Mean values of FEC of the immunized group.

26 KDa fraction was present in the peak II of partially purified antigen (Figs. 2 and3). This peak was used for immunization and serodiagnosis of haemonchosis using ELISA

Experimental Infection

Parasitoloical Finding: Examination of faecal samples of the control negative group revealed that lambs were free

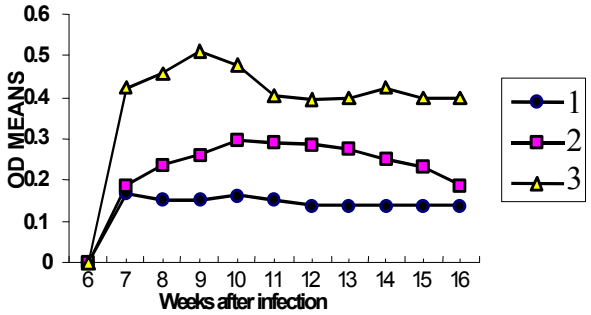

Fig. 5: Serological evaluation of the partially purified *H. contortus* antigen II as a vaccine: (1; control negative group; 2;control positive group and 3; immunized group).

from family Trichostrongylidae eggs throughout the experimental period. Reduction of mean egg per gram (EPG) was recorded in the immunized group when compared with that of the control positive group. The mean reduction of faecal egg count (FEC) was calculated to be 41.4% (Fig. 4).

The mean number of female worms was significantly higher than that of male worms. The mean reduction of worm burden was calculated to be 84% as described in Table 1.

The Clinical Sings: The clinical sings of the control negative lambs revealed that the lambs were normal till the end of the experiment, while the clinical examination of the control positive group of lambs revealed that the lambs were suffering from inappetance, anemia with pale mucous membranes, weakness, loss of wool and decrease in body weight. The immunized group of lambs revealed that lambs were apparently normal. Post mortem examination of the control positive group revealed general wetness of the tissues and presence of hundred of adult worms. The mucosa was swollen with the severe of small red shallow ulcers. While, the immunized group the abomasal mucosa appeared normal with few small shallow ulcers, but the lesions weren't severe.

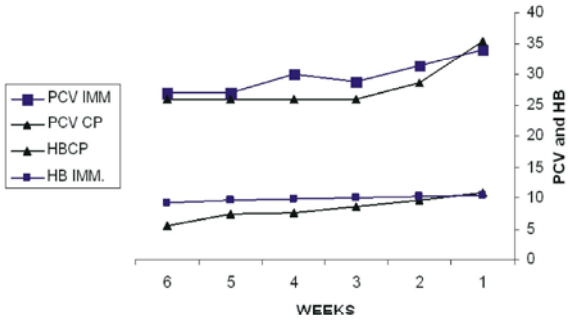

Fig. 6: PCV and Hb values of (IMM; immunized group and CP; control positive group).

The Seroassay: Antibody responses were monitored by ELISA (Fig. 5). The mean antibodies level of the immunized group was higher in comparison with the antibodies level of the control negative. The mean antibodies level increased gradually from 6th week post immunization and reached the peak on the 9th week post immunization then started to decrease but remained higher compared with the other 2 groups till the 16th week post immunization.While in the control positive group the mean antibodies level increased gradually from one week post infection and reached the peak on the 10th week of the experiment then the antibody levels decreased gradually till the end of the experiment (16th week).

The haematological changes of the control positive group on the 6th week (0 week of infection) to the 16th week (end of the experiment) revealed that significant decrease was recorded in Hb, RBCs and PCV values Fig. (6). There was decrease in Hb, RBCs and PCV values of the immunized group Fig. (6).

DISCUSSION

The present study revealed that immunization with the partially purified *H. contortus* antigen peak II might induce protective immune response against haemonchosis in sheep which was expressed by reductions in FECs and worm burden. Fractionation of *H. contortus* antigens through SDS-PAGE showed relatively large numbers of different protein bands as demonstrated in this study. These bands were varied or were of closely related molecular weights. These variations might reflect the antigenicity of these proteins [34]. The study of proteins by SDS-PAGE of crude and partially purified antigens, under reducing conditions be nearly in agreement with the results obtained by Abd El-Rahman [35] who reported that SDS-PAGE of partially purified adult somatic extract of *H. contortus* peak II contained 5 protein bands ranging from (25 to 45 kDa). Also Kaur et al. [36]who mentioned that SDS-PAGE of whole adult soluble extract of *H. contortus* revealed 11 bands with molecular weights ranging from 28.2 to 144.5 KDa. On the other hand, the presented results disagreed, Abd El-Rahman [35] who found that SDS-PAGE of whole adult somatic extract of *H. contortus* revealed 4 bands with molecular weights ranging from 20 to 72 KDa, peak I contained one protein band (70 KDa) and peak III contained 3 protein bands ranging from 19 to 30 KDa. Also, Gomez-Munoz *et al*. [37] who reported that SDS-PAGE of the partially purified *H. contortus* adult soluble extract (A1, A2, A3 and A4) were corresponding to molecular weight of > 200, 150-200, 78-150 and 42-78 KDa, respectively and Derbala and Abd El-Rahman [34] who found that SDS-PAGE of whole adult somatic extract of *H. contortus* revealed 12 polypeptides in both high and low molecular weights ranging from 14 to 216 KDa. Variations of the molecular weights and number of the obtained protein bands could be attributed to the method of preparation, denaturing and reducing conditions of *H. contortus* protein, differences in the amount of protein loaded to each lane and staining and destaining techniques used by the different authors.

In this study,the electrophoretic profile of crude and partially purified *H. contortus* antigens using rabbit anti-*H. contortus* sera via Western blot technique resembled to that obtained by Cuquerella *et al*. [15] who mentioned that some soluble proteins of *H. contortus* were recognized by sera of infected animals with reactivity ranging from molecular weights >94 to 25 KDa and exhibited a strong reactivity around two peptides of approximately 25 and 26 KDa and Kaur *et al*. [36] who found the A1 fraction displayed immune reactivity in a region over 94 KDa Besides, A2, A3 and A4 peaks reacted in a similar way with reactivities in the regions of 48-55 KDa and 27-35 KDa and reported that the A4 fraction had two immunodominant regions (48-55, 25-27 KDa) were recognized by *H. contortus* infected lambs, whereas uninfected control animals sera did not show this recognition pattern. Meanwhile, these data disagreed with Sood and Kapur [38] who reported that antisera of adult *H. contortus* prepared in rabbits revealed five bands with homologous antigen. Moreover, Derbala [39] who mentioned that the agar-diffusion and immuno-electrophoresis of *H. contortus* extracts (sodium acetate, PBS and Tris-HCl), produced 4 precipitin lines; Mous. and El-Fauomy [40]who reported that concerning the experimentally infected sheep with haemonchosis, Enzyme

linked Immunotransfer Blot (EITB) revealed 4 bands (23, 28, 32.5 and 51.5 kDa) which appeared at one to two weeks post-infection and continued till the end of the experiment (4 weeks post-infection) using crude adult *H. contortus* antigen and Derbala. and Abd El-Rahman [34] they reported that the antigenic components of somatic antigen of *H. contortus* which reacted with rabbit antisera were 234, 205, 178, 85, 48 and 14 KDa. These differences might be attributed to the method of preparation, sera dilution and stringent washing used by the different authors. Of interest, the immunogenic component of 26 KDa was expressed only through western immunoblotting assay by the peak II using rabbit hyperimmune anti *H. contortus* sera. Since the 26 KDa antigen had been reported in the diagnosis of haemonchosis in naturally infected animals [15,41] and in immunization of sheep against haemonchosis, [17,18] therefore the peak contained 26 KDa was used as antigen, in this study, to immunize a group of lambs and detect antibody response in the tested groups. It is worthy to mention that the immunization had been associated with lengthening of the prepatent periods and lowering of FEC. [5,12,42]. Immunization had delayed detection of the first *H. contortus* eggs in the immunized group a week later than the control positive group (on the day 28 post infection). The clinical examination of the control positive group of lambs revealed that they suffered from anaemia with pale mucous membranes and weakness that might be attributed to the blood sucking ability of the adult and 4th larval stage of *H. contortus* which feed on blood of the host; one worm may remove 0.05 ml blood per day [26]. The postmortem changes in the control positive group of lambs revealed general wetness of the tissues; this wetness might be attributed to the oedema resulting from loss of blood constituents including plasma proteins (a reduction in the level of albumin), which resulted in a corresponding reduction in the colloid osmotic pressure with a net outflow of fluid from blood which accumulated in the interstitial spaces, [26]. Comparing the results of the mean number of worm burden in both control positive and immunized groups revealed that reduction in worm burden had occurred in the immunized group (84%) [13, 43].

The immunized lambs suffered from frank anemia in comparison with the control negative and the control positive groups. Nevertheless, these lambs consistently had slightly higher Hb, RBC´s count and PCV´s level than the control positive group as observed by Andrews and Rolph [43]. The use of partially purified antigen as immunogen (low molecular weight antigen) afforded a degree of protection against this parasite to sheep [12]. Therefore, a 26 kDa from adult parasite could be of potential use in the diagnosis of lamb haemonchosis [15] and the peak which contained this antigen is used in this study. The immunization has been associated up challenge with high antibodies level specific for partially purified *H. contortus* antigen peak II as mentioned by Schallig and Van Leeuwen [13,41].

CONCULOUSTION

Results of the clinical signs, the postmortem examination, the FEC, worm burden and the immunogenic evaluation of the used vaccine through using ELISA of the immunized group in comparison with the control negative (non infected non immunized) and the control positive group (infected non immunized) might indicate efficacy of the prepared vaccine and the results seemed to be promising. However, further investigations on a large scale of sheep are still needed.

REFERENCES

1. Troell, K., J.G. Mattsson, A. Alderborn and J. Höglund, 2003. Pyrosequencing TM analysis identifies discrete populations of *Haemonchus contortus* from small ruminants. Int. J. for Parasitol., 33: 765-771.
2. Chagas, A.C.S., A.R. Freitas, M.R.A. Araujo, J.A. Araujo-Filho, L.S. Vieira, W.R. Araguao and A.M.C. Navarro, 2008. Anthelmintic efficacy of neem (Azadirachta indica A. Juss) and the homeopathic product Fator Vermes ®in Morada Nova sheep. Veterinary Parasitology, 151: 68-73.
3. García-Coiradas, L., F. Angulo-Cubillán, B.Valladares, E.Martínez, C. De la Fuente, T.M. Alunda and M. Cuquerella, 2010. Immunization against Lamb Haemonchosis with a Recombinant Somatic Antigen of Haemonchuscontortus (rHcp26/23). Vet. Med. Int. Article ID852166.
4. Noble, E.R., G.A. Noble, G.A. Schad and A.J. Maclnnes, 1989. Parasitology: The biology of animal parasites, 6th Ed. Lea and Febiger, Philadelphia, Pennsylvania, pp: 574.
5. Bakker, N., L. Vervelde, K. Kanobana, D.P. Knox, A.W.C.A. Cornelissen, E. Vries and A.P. Yatsuda, 2004. Vaccination against the nematode Haemonchus contortus with a thiol binding fraction from the excretory / secretory products (ES). Vaccine, 22: 618- 628.

6. Prasad, A., A. Nasir and N. Singh, 2008. Detection of anti-Haemonchus contortus antibodies in sheep by dot-ELISA with immunoaffinity purified fraction of ES antigen during prepatency. Indian J. Exp. Biol., 46(2): 94-9.
7. Prichard, R., 2001. Genetic variability following selection of *Haemonchus contortus* with anthelmintics. Trends Parasitol., 17: 445-53.
8. Cezar, A.S., G. Toscan, G. Camillo, L.A. Sangioni, H.O. Ribas and F.S.F. Vogel, 2010. "Multiple resistance of gastrointestinal nematodes to nine different drugs in a sheep flock in southern Brazil," Vet. Parasitol., 173: 157-160.
9. Bhat, S.A., M.R. Mir, S.Q. Allaie, H.M. Khan, I. Husain and A. Ali, 2011. Comparative Resistance of Sheep Breeds to *Haemonchus contortus* in Pasture Infection in Jammu and Kashmir. Global Veterinaria, 8: 222-228.
10. Fentahun, T. and G. Luke, 2012. Small Ruminant Haemonchosis: Prevalence and Associated Determinants in Randomly Selected Restaurants and Hotels of Gondar Town, Ethiopia European Journal of Applied Sciences, pp: 168-172.
11. Knox, D.P. and W.D. Smith, 2001. Vaccination against gastrointestinal nematode parasites of ruminants using gut expressed antigens. Vet. Parasitol., 100: 21-32.
12. Jasmer, D.P. and T.G. McGuire, 1991. Protective immunity to a blood-feeding nematode (*Haemonchus contortus*) induced by parasite gut antigens. Infect. Immun., 59: 4412-4417.
13. Schallig, H.D.F.H. and M.A.W. Van Leeuwen, 1997. Protective immunity to the blood feeding nematode *Haemonchus contortus* induced by vaccination with parasite low molecular weight antigens. Parasitol, 114: 293-299.
14. Piedrofito, D.P., M.J. Deveer, J. Sherrard and T. Kraska, 2012. Field vaccination of sheep with a larval-specific. antigen of the gastrointestinal nematode, *Haemonchus contortus*, confers significant protection against an experimental challenge infection.Vaccine, 30: 7199-7204.
15. Cuquerella, M., M.T. Gomez-Munoz, C. De-La-Fuente, L. Carrera and J.M. Alunda, 1993. Lamb serum recognition of infective larvae and adult *Haemonchus contortus* antigens. Vet. Parasitol., 49: 255-264.
16. Cuquerella, M., M.T. Gomez- Munoz, S. Mendez and J.M. Alunda, 1994. Partial protection of Manchego sheep against *Haemonchus contortus* after a 6-month post-priming period: Preliminary note.J. of Vet. Med. Series B, 41: 399-406.
17. Domínguez-Toraño, I.A., M. Cuquerella, M.T. Gomez-Munoz, S. Mendez, F.J. Fernández-Pérez and J.M. Alunda, 2000. Vaccination of Manchego lambs against *Haemonchus contortus* with a Somatic Fraction (P 26/23) of adult parasites. Parasite Immunol., 22: 131-138.
18. Domínguez-Toraño, I.A., F.J. Fernández-Pérez, M.T. Gómez-Muñoz, Alunda and M. Cuquerella, 2003. Humoral and cellular response in lambs vaccinated against *Haemonchus contortus* with p26/23 . Small Ruminant Res., 50(1-2): 29-37.
19. Parkhouse, R.M.E., N.M. Almond, Z .Cabrera and W. Harnett, 1987. Nematode antigens in protection, diagnosis and pathology. Vet Immunol Immunopathol, 17: 313-324.
20. Schallig, H.D.F.H., M.A.W. Van Leeuwen and W.M.L. Hendrikx, 1995. Isotype specific serum anibody responses of sheep to *Haemonchus contortus* antigens. Vet. Parasitol, 56: 149-162.
21. Vervelde, L., M.A. Van Leeuwen, M. Kruidenier, F.N. Kooyman, J.F. Huntley, I. Van Die and A.W. Cornelissen, 2002. Protection studies with recombinant excretory/secretory proteins of *Haemonchus contortus*. Parasite Immunol., 24: 189-201.
22. Knox, D.P., D.L. Redmond, G.F. Newlands, P.J. Skuce, D. Pettit and W.D. Smith, 2003. The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. Int. J. Parasitol., 33: 1129-1137.
23. Redmond, D.L. and D.P. Knox, 2006. Further protection studies using recombinant forms of *Haemonchus contortus* cysteine proteinases. Parasite Immunol., 28: 213-219.
24. Alba-Hurtado, F. and M.A. Muñoz-Guzmán, 2013. Immune Responses Associated with Resistance to Haemonchosis in Sheep BioMed Research International Volume 2013 , Article ID 162158, pp: 11.
25. Schallig, H.D.F.H.J.H., A.W.C. Boersema, A. Cornelissen and W.M. Van der Aar, 2000. The effect of oxfendazole terminated infection with *Haemonchus contortus* on the development of immunity in sheep. Vet. Parasitol., 88: 61-72.
26. Soulsby, E.J.L., 1986. Helminthes, Arthropods and Protozoa of Domesticated Animals 7th Ed. Bailliere Tindall, London.
27. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

28. Fagbemi, B.O., I.O Obarisiaghon and J.V Mbuh, 1995. Detection of circulating antigens in sera of *Fasciola gigantica* infected cattle with antibodies reactive with a fasciola- Specific 88 k D antigen. Vet. Parasitol., 58: 235-246.
29. Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of the head of acteriophage T4. Nature, 227: 680-685.
30. Towbin, H., T. Staehelin, and J. Gordon, 1979. Electrophoretictransfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceeding Natural Academic Science USA, 176: 4350-4354.
31. Allan, J.C., P.S. Craig, J. Garci, J. Noval, F Mencos, D. Liu, Y. Wang, H. Wen, P Zhou, R Stringer, M. Rojan and E. Zebjhle, 1992.Coproantigen detection for immunodiagnosis of Echinoccosis and Taeniasis in dogs and humans. Parasitol., 104: 347-355.
32. Feldman, B.F., J.G. Zinkl. and N.C. Jain, 2000. Schalm's Veterinary Hematology 5th Ed. Lippincott Williams and Wilkins, Wolters kluwer Co. Philadelphia, USA.
33. SAS. 1988. SAS, Statistics Analysis System,SAS usres guide: Inst. Inc. Cary, Nc, USA.
34. Derbala, A.E.A. and E.H. Abd El-Rahman,2001. Potency of three *Haemonchus contortus* antigens in the diagnosis of ovine haemonchosis. J. Egypt. Soci. Parasitol., 31: 701-710.
35. Abd El-Rahman, E.H., 1990. Serological characterization and partial purification of sheep Gastro-intestinal parasites *Trichostrongylus colubriformis* and *Haemonchus contortus* antigens. M. Sc. Thesis, Faculty of Science, Cairo University, Egypt.
36. Kaur, K., J. Kapur, A. Parmar. and M.L. Sood, 2002. Identification of immunodominant antigens of adult Haemonchus contortus (Nematoda : Trichostrongylidae). J. Vet. Med., 49: 260-262.
37. Gomez-Munoz, M.T., M. Cuquerella and J.M. Alunda, 1996. Identification and Partial purification of a 26 kiloDalton antigen of adult *Haemonchus contortus*. Int. J. for Parasitol., 26: 311-318.
38. Sood, M.L. and J. Kapur, 1980. Studies on the chemical composition of phenotyps of female *Haemonchus contortus*. In processing third national congress, parasitology, Haryana. Agricultural University, Hissar, April pp: 24-26.
39. Derbala, A.E.A., 1993.The use of a specific antigen for serodignosis of *Haemonchus contortus* infesting sheep.Vet.Med. J Giza, 41: 13-16.
40. Mousa, W.M. and M.M.El-Fauomy, 1994. Studies on two serological techniques for diagnosis of experimental and natural haemonchosis in sheep. J. Egyptian Vet. Med. Associ., 54: 41-47.
41. Cuquerella, M., M.T. Gomez-Munoz and J.M. Alunda, 1991. Serum IgG response of Manchego lambs to infections with *Haemonchus contortus* and preliminary characterization of adult antigens Vet. Parasitol., 38: 131-143.
42. Munn, E.A., T.S. Smith, M. Graham, C.A. Greenwood, A.S. Tavernor and G. Coetzee, 1993. Vaccination of merino lambs against haemonchosis with membrane associated proteins from the adult parasite. Parasitol., 106: 63-66.
43. Andrews, S.J. and T.P. Rolph, 1997. Duration of protective immunity against ovine haemonchosis following vaccination with the nematode gut membrane antigen H11.Res. in Vet. Sci., 62: 223-227.