

## Immunodiagnostic Potency of Different *Haemonchus contortus* Antigens for Diagnosis of Experimentally and Naturally Haemonchosis in Egyptian Sheep

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**Abstract:** Parasitic gastroenteritis caused by *Haemonchus contortus* (*H. contortus*) is the major constrain in Egyptian sheep industry and their impact on production, animal health and welfare is likely to increase. Different *H. contortus* antigens in their native form; crude somatic antigen (CSA), crude larval antigen (CLA) and excretory/secretory antigen (ESA) or partially purified through gel filtration using Sephadex G-100 resulted in 3, 3 and 2 peaked chromatograms, respectively or in a recombinant form as recombinant *H. contortus* protein 26/23 (rhcp26/23) were prepared, electrophoretically characterized through SDS-PAGE and immunologically evaluated through indirect ELISA for diagnosis of haemonchosis in experimentally infected sheep. Analysis of resulted bands in SDS-PAGE demonstrated that the specific bands were 23, 26, 37, 43, 60 and 76 kDa for adult antigens but 32, 38 and 66 kDa for larval antigens and only 15, 24 kDa for excretory/secretory antigens. The result of indirect Enzyme linked immunosorbent assay (ELISA) of different antigens revealed that peak I of CSA were sensitive for early detection of haemonchosis in experimental animals (after 1<sup>st</sup> week of infection) even in low antibody titer. So, this antigen conducted in ELISA assay to detect *H. contortus* antibodies in the slaughtered animals in comparison with presence of worms in their abomasal content showed 97.24% sensitivity, 65.11% specificity with 69.09% diagnostic value which mainly due to cross reactivity with other parasitic infections. The cross reactivity of this antigen judged with antigens and hyperimmune sera of *Fasciola* and *Moniezia* using both ELISA and immunoblotting ensuring our hypothesis. The present study demonstrated that peak I of CSA had the most immunodiagnostic potency and the capability to elicit humoral immune response in experimental and natural haemonchosis in sheep.

**Key words:** *Haemonchus contortus* • Diagnosis • ELISA • Immunoblotting • Sheep • Egypt

### INTRODUCTION

The nematode *H. contortus* is one of the most pathogenic and economically important parasites of sheep. The adult worms affect the abomasum of the host and feed on blood resulting in severe damage such as; poor growth rate, weight loss, anemia and occasionally, death [1]. The diagnosis of haemonchosis is usually based upon evaluation of clinical signs and fecal examination. These two methods have their limitations. Clinical signs usually become apparent when the infection is heavy. Eggs are found in the faeces after the prepatent period of

approximately 3-4 weeks at which time the infection is firmly established and damage is already done [2]. More recently, a potentially useful soluble antigen for diagnosis of *H. contortus* infections in lambs has been reported [3] and later also a 24 kDa component of excretory/secretory products of adult parasites [4]. The identification and partial isolation of a 26 kDa antigen of adult *H. contortus* by means of gel filtration also used [5]. The partially purified antigens were examined for diagnosis of haemonchosis in lambs. It has been demonstrated that a protective natural immune response is associated with the humoral recognition of low weight

*H. contortus* antigen in particular 26 kDa adult somatic or soluble extracts [6] and also adult soluble extract of *H. contortus* was purified through gel filtration column chromatograms resulted in 3 peaks chromatograms. Many studies focused on identification of immunogenic protein antigens of *H. contortus* and the analysis of their potential to induce protective immunity [7, 8]. ELISA has been considered the most commonly used serological methods based on the detection of an immune response in an infected animal [9]. Such test is usually in contrast to fecal examination, less time consuming, more sensitive and specific for detection of subclinical infections [10, 11]. The use of immunoassays based on easily obtainable total somatic adult worms or larvae for the detection of anti-*H. contortus* specific circulating antibodies in infected sheep have yielded conflicting results and especially the detection of a primary infection appears to be difficult [12, 13]. The specificity of the ESA ELISA was 87.2%, where as the specificity of the CSA ELISA was 82.7% [14]. Western blot act as a preliminary step for selecting target protein candidates for diagnosis of haemonchosis and evaluation of its efficacy [15].

Considerable progress has been made in identifying the different *Haemonchus* antigens and exploration of potentially immunodiagnostic one for diagnosis of haemonchosis in sheep. A variety of native antigen preparations including; CSA, ESA and CLA were developed, but as a result of lack sensitivity of such antigens [16], the use of partially purified antigens and further purified recombinant antigens are needed for sensitive and specific diagnosis of haemonchosis [17]. Therefore, the present study attempted to characterize native and partially purified antigens of *H. contortus*, evaluate the immunodiagnostic potency of different prepared *H. contortus* antigens through an indirect ELISA to be used for detection of antibody titer in experimentally and naturally infected sera beside immunological characterization of the most specific and sensitive antigen through western blotting analysis.

## MATERIALS AND METHODS

**Worm Collection:** Adult worms of *H. contortus* were collected from abomasa of slaughtered sheep at El-Bassatin, El-warrak and El-Monie abattoirs in Egypt. Worm recovery was carried out according to standard procedures as described by MAFF [18].

**Blood Samples:** Blood samples were collected from 880 slaughtered sheep at different abattoirs in Egypt.

Serum of each sample was collected after centrifugation at 2000 rpm for 15 minutes. All samples were stored at -20°C in small aliquots labeled as infected or apparently healthy animals. One hundred and nine sheep sera were apparently positive.

**Experimental Infection:** Six balady male lambs of about 6 months old were kept for 4 weeks indoor, before beginning of experiment, to acclimate together and were subjected to parasitological examination to prove their clearance from any parasitic infection [14]. Three sheep were kept as control negative non-infected lamb. Other lambs were kept for experimentally mono-infection per-os with 20,000 L3 of *H. contortus*. Sera were collected at weekly intervals.

**Preparation of Crude Antigens:** CSA of *H. contortus* was prepared from adult worms according to Johnson *et al.* [19] or from their excretory/secretory products (ESA) as described by Prasad *et al.* [20] or from third larval infective stage L3 (CLA) as determined by Tariq *et al.* [21].

**A Rhcp 26/23 Protein:** A recombinant protein (rhcp26/23) was kindly obtained from Prof. Dr. Omnia Kandil, parasitology and animal diseases department, National Research Centre.

**Antigen Purification:** The partially purified antigens were obtained by gel filtration according to Schallig *et al.* [22]. Sephadex G-100 was suspended in 0.01 M PBS. The CSA, CLA and ESA (30mg /3.5ml 0.15M PBS, pH 7.2) was loaded on Sephadex G-100 column (1.6x 95cm) equilibrated and eluted with the same buffer, fractions of 2.5 ml were collected at a flow rate of 30 ml/h, the absorbance of each fraction was recorded at 280 nm. The protein content of different crude antigens and their isolated fractions were measured according to Lowry *et al.* [23].

**Preparation of Hyperimmune Sera:** Twelve healthy white New Zealand males rabbits were divided into Four groups, each group had three rabbits, each group was immunized with different antigens; CSA, Peak I adult *H. contortus* antigen (PIAA), crude *Fasciola* antigen (CFA) and crude *Moneizia* antigen (CMA) in order to prepare their specific hyper immune serum according to Fagbemi *et al.* [24]. The last group used as control negative rabbit. Briefly, each rabbit was subcutaneously injected with 200 µg of each prepared antigens/ kg B. Wt. emulsified with an equal volume of complete Freund's adjuvant. Two weeks

later, two Booster doses of antigens emulsified with incomplete Freund's adjuvant were injected subcutaneously within week interval.

**Sodium Dodecyl Sulphate -Polyacrylamide Gel Electrophoresis (SDS-PAGE):** Seventy µg of CSA, CLA, ESA and its peaks with rhcp 26/23 were electrophoresed using 10% SDS-PAGE under reducing condition according to the method of Laemmli *et al.* [25].

**Enzyme Linked Immunosorbant Assay (ELISA):** ELISA was applied to determine the diagnostic value of different *H. contortus* crude and isolated fraction antigens tested with both serial double fold dilution of parasitologically-positive monospecifically infected sera from 1:50 to 12800 and with weekly interval experimentally infected sera from zero day until 6<sup>th</sup> week PI for evaluation of immunogenicity. The most immunogenic antigen was evaluated for its sensitivity, specificity, accuracy, reliability and agreement according to Schallig *et al.* [14]. The cross reactivity of chosen antigen was assayed with the aid of reference sera and antigens of *Moniezia* and *Fasciola*. The optimal concentration of antigen, antibody and conjugate dilutions were chosen after preliminary checker-board titrations. The wells were coated with 100 µl of each diluted antigen at the concentration of 2 µg/well in carbonate-bicarbonate buffer, pH 9.6 and incubated overnight at 4°C, then washed three times with PBS-Tween (PBS-T). After blocking with 200 µl/well of 5 % dry skimmed milk in PBS, pH 7.2. A 100 µl of diluted Serum samples in PBS-T containing 0.5 % dry skimmed milk at different dilutions for parasitologically-positive monospecifically infected sera with controlled negative sera and at dilution of 1:200 for the weekly interval experimentally-infected sera, natural sera and hyperimmune sera against CSA, CFA, CMA and PIAA. One hundred µl appropriate peroxidase conjugated Anti-species used at dilution 1:1000. One 20mg O-phenylene diamine tablet was dissolved in 50 ml of 0.1M citric acid and 0.2M sodium dibasic phosphate, pH 5 as substrate buffer and 25 µl 30% H<sub>2</sub>O<sub>2</sub>. The optical density values were read at 450nm with ELISA reader. Positive values were assigned as those values with absorbance reading greater than the cut-off value which was calculated from mean OD value of the negative control sera plus three fold standard deviations. Data analysis parameters were calculated for peak I of CSA according to Tabouret *et al.* [26] and Bauer *et al.* [27].

**Western Blotting Technique:** The immunogenicity of partially purified PIAA and CSA were evaluated for the

cross reactivity between *H. contortus*, *Fasciola* and *Moniezia* antigens by immunoblot. After SDS-PAGE, protein bands were electroblotted on nitrocellulose paper according to Towbin *et al.* [28] in a blotting system and their immunogenicity was evaluated against hyperimmune serum.

## RESULTS

**Purification of CSA, CLA and ESA Antigens:** Three peaks chromatograms (I, II and III) were obtained from CSA and CLA, while ESA gave two peaks (I and II).

**Electrophoretic Profile of CSA, CLA, ESA and Their Peaks:** Electrophoretic profile of the tested antigens was resolved by SDS-PAGE and showed that bands at both high and low molecular weight ranges. SDS-PAGE of different antigens revealed that 16 individual protein bands with molecular weight ranging from 22-160 kDa were resolved from CSA while 10 bands (22-121 kDa), 7 bands (22-93 kDa) and 7 bands (29-76 kDa) were resolved from PI, PII and PIII of CSA, respectively. One fraction 60 kDa was common between four antigens (Fig. 1). While CLA was separated into 5 protein bands with molecular weight ranging from 32-75 kDa while 3 bands (32, 38 and 66 kDa), only one band at 66 kDa and 2 bands (66 and 38 kDa) were resolved from PI, PII and PIII of CLA, respectively. One epitope 66 kDa was similar between four antigens (Fig. 2). Seven protein bands with molecular weight ranging from (14-93 kDa) revealed from Excretory/Secretory antigen (ESA) while 6 bands (14-77 kDa) and 3 bands (15, 24 and 63 kDa) were resolved from PI and PII of ESA, respectively. Two fraction 15, 24 kDa were similar bands for Excretory/Secretory antigens (Fig. 3) and also the electrophoretic and blotting analysis revealed that rhcp 26/23 protein was detected at 26 kDa using anti-His antibody (Fig. 4).

**Optimization of the CSA, CLA, ESA and rhcp26/ 23 ELISA:** The checker board assays were evaluated by calculating the differences between OD values measured for the reference sera at each of the antigen, sera and conjugate dilutions tested. This evaluation determined the concentration of *H. contortus* antigens to be optimal at 0.2 µg protein per well. The optimal dilution of the primary antibody was found to be 1:200 and the conjugate to be 1:1000. The cut off value of the CSA (0.272) and its peaks I, II, III (0.202, 0.459 and 0.392 respectively), CLA (0.419) and its peaks I, II, III (0.427, 0.253 and 0.271 respectively), ESA (0.339) and its peaks I, II (0.256 and 0.368) and rhcp26/23 (0.124).

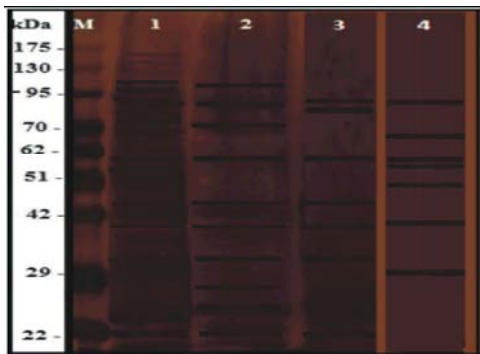


Fig. 1: SDS-PAGE of separated antigens of CSA and its peaks obtained from gel filtration chromatography

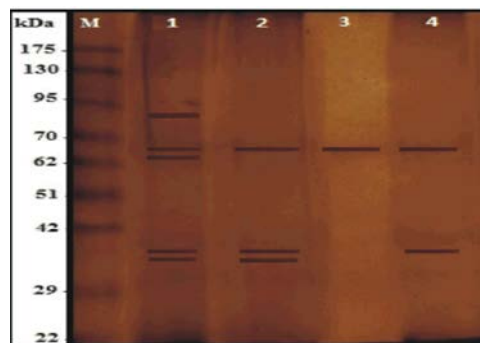


Fig. 2: SDS-PAGE of separated antigens of CLA and its peaks obtained from gel filtration chromatography

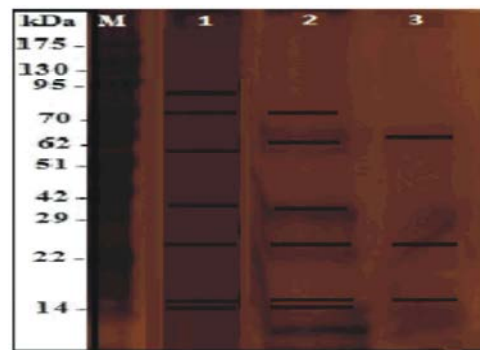


Fig. 3: SDS-PAGE of separated antigens rhcp 26/23, Lane 1: Bovine Serum filtration chromatography

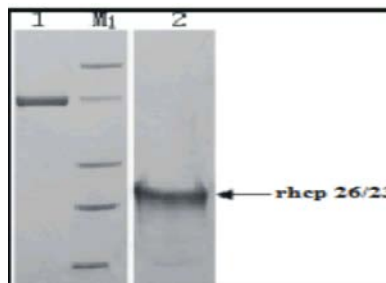


Fig. 4: SDS- PAGE of separated antigens of CSA and its peaks obtained from gel Albumin, Lane 2: rhcp 26/23

#### Evaluation of Immunodiagnostic Potency of Different *H. Contortus* antigens by Indirect ELISA Technique (ELISA):

Immunogenicity of different antigens evaluated based upon experimentally infected sera at different dilution revealed that ESA antigen sensitive till 1:400 dilution, PI ES till 1:800 dilution, PII adult till 1:1600, CSA & PIII adult and PII ES till 1:3200, CLA and its peaks till 1:6400, but peak I chromatogram of CSA and rhcp 26/23 appeared more sensitive than other antigens in detecting of anti-*Haemonchus* antibodies with significant difference in antibody level between controlled positive and negative sera in the least serum dilution at 1:12800 (Fig. 5).

**Early Detection of *H. contortus* Infection:** Immunogenic evaluation based upon experimentally infected sera at different weeks post infection (PI) revealed that adult antigens were earliest than rhcp 26/23, ES antigens and larval antigen in the detection of anti-*Haemonchus* antibodies during pre-patent periods. Both peak I and peak II adult antigens were able to detect of subclinical infections after 1<sup>st</sup> week PI (Table 1).

**Sensitivity of ELISA:** According to the results of *H. contortus* antigens immunogenicity in ELISA, Peak I adult *H. contortus* antigen was the best immunogenic antigen and among other antigens as diagnostic antigen for haemonchosis in sheep. Eight hundred and eighty sheep's sera were tested by ELISA assay used peak I adult antigen to detect *H. contortus* antibodies in the slaughtered animals compared to the presence of adult worms in their abomasal content. The sensitivity of ELISA compared with post-mortem examination is shown in Table (2) abomasums examination revealed 12.36% infected cases while 42, 61% positive cases were detected by ELISA. The low, moderate and high degree of infection occurred in 22, 61%, 7.15% and 12, 84% of examined samples, respectively. From the previous results we concluded that the sensitivity of peak I of CSA against their corresponding antibodies was 97.24% and specificity of this antigen was 65.11%, while positive predictive value 28.26, negative predictive value 99.4 and diagnostic value of this antigen was 69.09%.

#### Evaluation of Cross Reactivity Between Different Antigens:

Cross reactivity of PIAA, CMA and CFA assessed through reaction with hyper immune sera; HIS-PIAA, HIS-CMA, HIS-CFA, HIS-CSA and Control negative rabbit sera at different dilution from 1:50 to 1:3200. The data revealed that PIAA cross reacted with anti-*Moniezia* antibodies till 1:800 serum dilution, while no cross reaction could be recorded versus anti-*Fasciola*

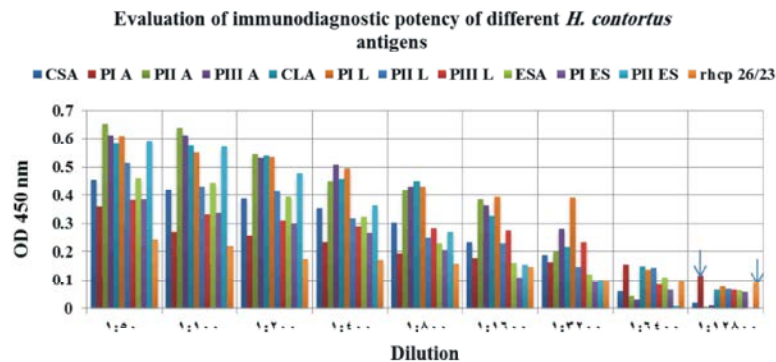


Fig. 5: Serological evaluation of immunogenicity of different *H. contortus* antigens; CSA, CLA and ESA with their peaks obtained from gel filtration chromatography in addition to rhcp 26/23.

Table 1: Time sensitivity of different antigens at different weeks post experimental infection

DAY	CSA				CLA				ESA			
	CSA	P I	P II	P III	CLA	P I	P II	P III	ESA	P I	P II	rhcp 26/23
Zero day	0.410	0.436	0.263	0.120	0.189	0.238	0.293	0.398	0.320	0.200	0.276	0.102
1 <sup>st</sup> week	0.481	0.482	0.342	0.149	0.214	0.212	0.340	0.384	0.336	0.223	0.338	0.106
2 <sup>nd</sup> week	0.493	0.522	0.425	0.277	0.253	0.230	0.352	0.390	0.360	0.255	0.383	0.122
3 <sup>rd</sup> week	0.597	0.587	0.616	0.504	0.201	0.248	0.358	0.368	0.416	0.332	0.413	0.136
4 <sup>th</sup> week	0.634	0.501	0.576	0.699	0.306	0.255	0.397	0.433	0.422	0.334	0.417	0.114
5 <sup>th</sup> week	0.636	0.529	0.509	0.661	0.365	0.353	0.524	0.462	0.495	0.335	0.463	0.188
6 <sup>th</sup> week	0.544	0.533	0.392	0.725	0.317	0.280	0.420	0.505	0.527	0.293	0.391	0.169
Control negative	0.487	0.493	0.374	0.303	0.356	0.332	0.387	0.425	0.339	0.256	0.425	0.124

Table 2: Detection of anti-*Haemonchus* antibodies in sera from slaughtered sheep compared with examined abomasums.

ELISA														
Positive														
Abomasum examined						OD Means								
Positive		Negative		Total		Low		Moderate		High		Total negative		
No. of examined sheep	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
880	109	12.38	771	87.61	375	42.61	199	22.61	63	7.16	113	12.84	505	57.39

antibodies even in 1:50 serum dilution. On the other hand, the crude somatic antigens displayed reactivity with anti-*Moniezia* antibodies till higher dilution (1:3200) and with anti-*Fasciola* antibodies till 1:200 serum dilutions. ELISA plates coated with *Moniezia* antigen or *Fasciola* antigen, the reaction could occur with anti-*Haemonchus* antibodies till 1:400 serum dilutions. None of all tested antigens showed positive reaction when testing versus the control negative rabbits' sera.

**Immunoblotting Analysis of Peak I adult *H. contortus* Antigen as Immunodiagnostic Antigen:** The western blot analysis of HIS-PIAA serum demonstrated relatively high

number of immunogenic reactive bands against its specific Peak I adult *H. contortus* antigen. These noticed 20 bands at molecular weights ranged from 10.5-175 kDa (Fig. 6). Moreover, this antigen contained 16 and 19 immunogenic reactive proteins of molecular weights ranged from 10.5-105.5 kDa presented when it reacted against experimentally and naturally infected sheep sera, respectively (Fig. 6). On the other hand, 8 cross reactive bands resulted from reaction of PIAA against both *Fasciola* hyper immune sera at molecular weights ranged from 10.5-175 kDa and *Moniezia* hyper immune sera at molecular weights ranged from 14.2-81.9 kDa. Otherwise, the zero-day rabbit serum and control negative sheep serum showed 5 and 15 non-specific bands (Fig. 6).

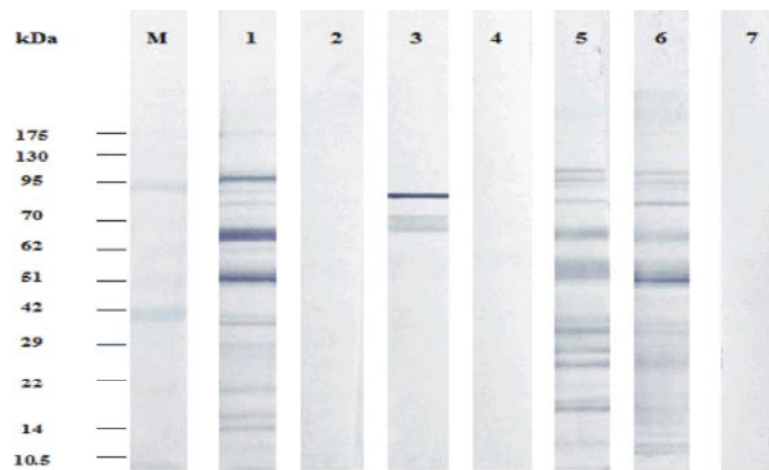


Fig. 6: Western Blot analysis showing reactivity of Peak I adult *H. contortus* antigen against different sera. Lane M: Prestained molecular weights protein marker, Lane 1: Hypeimmune sera of Peak I adult *H. contortus* antigen (HIS-PIAA), Lane 2: Hypeimmune sera of Crude *Fasciola* (HIS-CFA), Lane 3: Hypeimmune sera of Crude *Moniezia* antigen (HIS CMA), Lane 4: Control negative rabbit serum, Lane 5: Experimentally infected sheep serum, Lane 6: Naturally infected sheep serum and Lane 7: Control negative sheep serum.

The results obtained through immunoblotting of Peak I adult *H. contortus* antigen using different sera revealed that there were 6 regions around 11.8-18.3, 23.0-26.8, 45.0-63.4, 78.6-93.7, 102.4-105.5 and 120.6-153.8 kDa were strongly recognized by WB, whereas two peptides having around 23 and 26 kDa were the most common and specific immunogenic reactive bands as shown in (Fig. 6).

## DISCUSSION

*Haemonchus* species are the most predominant nematodes causing parasitic gastroenteritis with major economic losses to the small ruminants industry worldwide. The present study aimed to identify, purify and characterize the different *H. contortus* antigens with evaluation of their immunodiagnostic potency through an indirect ELISA assay to apply the most sensitive one in diagnosis of haemonchosis. *H. contortus* possess a huge variety of antigens and there is limited information on which stages and antigens are actually responsible for eliciting immune responses [29]. In this study, different antigen identified in native form as CSA, CLA and ESA but because of their complex nature and limited value as an antigen source, the use of less complex antigens mixture as partially purified antigens resulted from gel filtration chromatography has led to better result in diagnosis [14]. Further purification and recombinant DNA technology was applied to obtain a synthetic protein (rhcp 26/23) from CSA.

Purification of *H. contortus* antigens was done from CSA, CLA and ESA using gel filtration chromatography revealed three peaks from CSA & CLA and two peaks from ESA. These results appeared to be in line with the results obtained by Abd El-Rahman [30] who obtained three major fractions from CSA using gel filtration by sephadex (G-200), in addition to the results obtained by Arab *et al.* [31] who obtained three peaked chromatograms from adult *H. contortus* somatic extract through gel filtration chromatography by sephadex (G-100), while the results disagreed with those obtained by both Derbala [32] who obtained five fractions from CSA using gel filtration column chromatography by sephadex (G-200) and Go'mez-Mun'oz *et al.* [5] who obtained 4 peaks from adult *H. contortus* somatic extract through gel filtration chromatography by Sephacryl (S-200). Variations of the obtained fractions could be attributed to the method of preparation, grade of sephadex and the protein content of crude antigens used by different authors. Few attempts have been made to clone proteins from *Haemonchus* species as a diagnostic tool, except the expression of excretory/secretory proteins, particularly ES24 by Li *et al.* [17] who evaluated His-ES24 for use in a diagnostic ELISA of infection in sheep. Two studies [33, 34] proved that such antigen showed a notable immunoprophylactic value against lamb haemonchosis without any information about its diagnostic value which investigated in the present study.

The electrophoretic profile of CSA and its partially purified antigens using SDS-PAGE under reducing conditions revealed 16 protein bands of molecular weights ranged from 22-160 kDa, while 10 bands (22-121 kDa), 7 bands (22-93 kDa) and 7 bands (29-76 kDa) were resolved from PI, PII and PIII of CSA, respectively. The bands of molecular weights 23, 26, 30, 37, 43, 60, 66, 76 and 93 kDa were relatively identical or cross related to each other. These results were in the range previously described by Derbala and Abd El-Rahman [35] who found that SDS-PAGE of CSA revealed 12 polypeptides in both high and low molecular weights ranging from (14 to 216 kDa), Kaur *et al.* [36] who mentioned that SDS-PAGE of CSA revealed 11 bands with molecular weights ranging from 28.2-144.5 kDa and Yan *et al.* [37] who found that SDS-PAGE of CSA showed proteins at molecular weights ranged from 11 to 170 kDa, in addition to the prominent bands at 26, 33, 43 and 66 kDa which were relatively similar with the result of Tak *et al.* [38] who found four prominent bands of the molecular weights of 26, 33, 40 and 66 kDa. The results of fractionation of CSA and its peaks obtained from gel filtration chromatography through SDS-PAGE were nearly in agreement with the results of Arab *et al.* [31] who found that peak I of *H. contortus* partially purified antigen by sephadex G-100 gel filtration column chromatography 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 KDa. On the other hand, the presented results disagreed with those obtained by Go'mez-Mun'oz *et al.* [5] who reported that SDS-PAGE of partially purified *H. contortus* adult soluble extracts by gel filtration chromatography using Sephacryl S-200 revealed 4 chromatographic peaks (A1, A2, A3 and A4) were corresponding to molecular weights of (<200, 150-200, 78-150 and 42-78 kDa) respectively. Moreover, the CLA was separated into 5 protein bands with molecular weight ranging from 32-75 kDa, revealed three peaks with gel filtration chromatography with 3 bands (32, 38 and 66 kDa), only one band at 66 kDa and 2 bands (66 and 38 kDa) were resolved from PI, PII and PIII of CLA, respectively. The obtained results of Siamba *et al.* [39] were in agreement with the present results. The common bands between CLA and its peaks were 32, 38, 66 kDa. Additionally, ESA fractionated to 7 protein bands at 14, 15, 24, 35, 60, 77 and 93 kDa agreed with result of Schallig *et al.* [40] who found that a protein profile of total ES materials revealed 7 bands from 15 to 80 kDa. By

contrast, these results were dissimilar to Rathore *et al.* [41] who recorded at least 20 polypeptides ranged from 15 to 150 kDa after staining the gel with Coomassie blue stain and Prasad *et al.* [20] who found that ESA separated to polypeptides ranging between 10-200 kDa with prominent polypeptides at 15, 26, 60 and 120 kDa with some faint bands between 30-40 kDa and two bands above 120 kDa were also observed. The ESA revealed two peaks with gel filtration chromatography with 2 common and specific bands with molecular weights of 15 and 24 kDa which similar to the results obtained from purification process of Schallig *et al.* [40]. Furthermore, the expressed recombinant protein (rhcp 26/23) was characterized by only one band at 26 kDa [33, 34].

Indirect ELISA is known for its potential to detect anti-*Haemonchus* antibodies at quite an early stage of the infections. In experimentally infected sheep, both peak I chromatogram of CSA and rhcp 26/23 appeared more sensitive than other antigens in detecting of anti-*Haemonchus* antibodies in the highest serum dilution. These results relatively agreed with Schallig *et al.* [14] who found that the use of less complex antigens mixture as partially purified antigens resulted from gel filtration chromatography has led to better result in diagnosis and Li *et al.* [17] who mentioned that recombinant protein-based serological tests might achieve high sensitivity and specificity because of the high concentration of the immunoreactive antigen and the lack of host protein components from the crude antigen preparations, made such antigen was suitable for the diagnosis of *H. contortus* infection. Both peak I and peak II of CSA were enabled early detection of infection after 1<sup>st</sup> week post infection. Similar finding were previously reported by Mousa and El-Fauomy [42] who enabled the detection of anti-*Haemonchus* antibodies in experimentally infected sheep sera using CSA after 1<sup>st</sup> week post infection (PI) while Schallig *et al.* [14] who stated that both CSA and ESA ELISAs found all infected sheep examined positive 1 week earlier than fecal examination. This suggested that peak I chromatogram of CSA was sensitive, would be useful for early detection of haemonchosis in experimental animals and could be applied under field condition. The indirect ELISA was evaluated on field sera and the results were compared with the post-mortem findings with regard to the actual parasitological status. The results showed that out of 880 serum samples tested against partially purified antigens; 375 serum samples gave positive ELISA results (106 serum samples were true positive and 269 serum samples were false positive), while



the other 505 serum samples gave negative ELISA results (502 serum samples were true negative and 3 serum samples were false negative). The assay proved high sensitivity (97.24%) with three false negative results which might be due to low worm burden or poor immune response besides, host nutritional status or physiological and environmental factors like reinfection or coinfection with other parasites [43], while specificity (65.11%) with 269 false positive results of the assay with postmortem negative samples which is probably due to the persistence of antibodies to the past infection or worms might have been eliminated by anthelmintic medication or presence of immature worms in abomasum or recent infection or cross reactivity with other parasitic infection [43]. Regarding to sensitivity and specificity of ELISA in our study were in agreement of Gowda [44] who found the sensitivity and specificity of Indirect-ELISA by CSA was 100 and 67.18 %, respectively. These results disagreed with Mousa and El-Fauomy [42] who found the ELISA gave 100% sensitivity and specificity using CSA; Schallig *et al.* [14] who had higher specificity reached to 87.2% for ES ELISA and 82.7% for CSA ELISA and Li *et al.* [17] whose ELISA results with His-ES24 was 100% specific. In contrast, our results were higher than the results of Abdel-Gawad [45] who revealed that the sensitivity of ELISA by CSA was 54.17%; Lone *et al.* [43] who found sensitivity 80% and specificity 21.42% using CSA and Razzaq *et al.* [46] who reported that there were 100% and 92% sensitivity using ES antigen and CSA, respectively with 42% and 43.33% specificity using ES antigen and CSA, respectively. The variation in the results may be attributed to type, nature and quality of antigens used by different authors.

In this study, we reported that the cross reactivity not occurred only among related nematodes to *H. contortus* [47, 4] but also with unrelated helminthes whereas the PIAA cross reacted with *Moniezia* antigen but not with *Fasciola* antigen similar to the result obtained by Li *et al.* [17] and the crude somatic antigens displayed reactivity with anti-*Moniezia* antibodies and anti-*Fasciola* antibodies which in agreement with El-Bahy *et al.* [16]. The prevalence of infection in 880 samples rise from 12.38% based on postmortem examination to 42.61% depending on ELISA test. The reactivity of peak I adult antigen judged by immunoblot analysis revealed two peptides having around 23 and 26 kDa were the most common and specific immunogenic reactive bands as a result obtained by El-Bahy *et al.* [16].

In conclusion, partially purified peak I of CSA induced high immunodiagnostic potency than other antigens with high sensitivity & specificity and would be the best choice for coating antigen of indirect ELISA. The CSA of *H. contortus* had cross reactivity with anti-*Moniezia* and anti-*Fasciola* antibodies, while peak I adult antigen only reacted with anti-*Moniezia* antibodies. The reactivity of peak I adult antigen judged by immunoblot analysis revealed two peptides having around 23 and 26 kDa were the most common and specific immunogenic reactive bands.

## ACKNOWLEDGEMENTS

The authors thankfully acknowledge the financial assistance of STDF which support our study through project "Genetic characterization and diversity patterns of *Haemonchus* species populations isolated from small ruminants in Egypt".

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