Global Veterinaria 13 (5): 672-679, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.05.85189

Evaluation of Coprological, Postmortum and Seroassay Techniques for the Diagnosis of Sheep Haemonchosis in Taif, KSA

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Abstract: This Study was carried out on *Heamonchus contortus* infection among sheep at Taif Governorate abattoirs during the period extended from May 2013-April 2014; the study included the examination of 561 abomasa and revealed that the incidence of *H. contortus* infection among sheep was 15.15%. Monthly high incidence of *H. contortus* infection percentage was during February (55.90), while the lowest percentage was recorded during November (6.45). To use different methods for serodiagnosis of ruminants' haemonchosis is important because detection of egg in the faeces is not so reliable. So uses of SDS-PAGE of the crude adult *H. contortus* antigen in this study revealed that, *Heamonchus contortus* crude antigen separated (10) protein bands of molecular weights ranging from (14.84 to 106.2 kDa), peak I of *H. contortus* partially purified antigen contained (14) protein bands of molecular weights ranging from (17.51 to 98.70 kDa), peak II contained (4) protein bands of molecular weights ranging from (17.51 to 45.54 kDa) and peak III contained one protein band observed at level (13.63 kDa). There was one common shared protein band of molecular weight (17.5 kDa) between the crude *H. contortus* antigen and partially purified antigen *H. contortus* peak I and peak II. ELISA revealed that (59.3%) faecal samples were positive to Trichostrongylidae eggs. Also (55.9%) abomasal samples contained *H. contortus* worms while(57.6%) serum samples were found positive.

Key words: *Haemonchus contortus* · Antigen · Western blotting · SDS-PAGE · Eliza and Electrophoresis

INTRODUCTION

Sheep play an important role in animal production in Taif Governorate. Sheep are raised for mutton, milk, hide and wool production. They are suseptable to the nematode parasites that cause serious morbidity and mortality among them [1]. The infection with gastrointestinal parasites is very common in sheep in many parts of the world due to their grazing and watering habits [2]. These parasites are considered one of the most common affections and a major animal health constraint to livestock production which cause great economic losses [3].

Haemonchus contortus is a major pathogen in the abomasa of sheep and other ruminants worldwide and blood is considered the main source of their nutrients [4 and 5]. Infection with this parasite can cause mainly in young animals, anaemia and weight loss that in some cases result in death in infected animals [6].

The control of *Haemonchus contortus* infection is 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 [7, 8 and 9]. Therefore increasing attention had been given to the development of alternative control methods, such as the use of vaccines [10]. A degree of protective immunity could follow parental vaccination with crude preparation of parasite [11], or proteins purified from such extracts [12]. The use of partially purified antigen as immmunogen afford a degree of protection to sheep against this parasite [13].

Diagnosis of *Haemonchus contortus* is based on evaluation of clinical signs and faecal examination, however, the development of a reliable serological assay (e.g. ELISA) which enables the detection of subclinical infection and, or early detection of infection [14, 15].

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The aim of the present study, in field and laboratory on *Haemonchus contortus* among sheep at Taif abattoir, KSA is through isolation and identification of *Haemonchus contortus* worms from sheep abomasa and preparation of *Haemonchus contortus,* application of ELISA test on sera of infected and non infected sheep using partially *Haemonchus contortus* antigen.

MATERIALS AND METHODS

Abomosal Examination: Examination of 579 sheep abomasa and collection of *Haemonchus contortus* adults worms were applied according to [16, 17] and [18].

Identification of Haemonchus Contortus Adults Worms: Collected worms were mounted according to [19]. Identification of the worms was applied using electric binocular microscope according to [19] and [20] and scanning electron microscope (SEM) by [21], using a computer image analysis system. QW in 500, Cambridge, England).

Coprological Examination: Faecal samples were collected in polyethylene plastic labeled bags and were examined during the same day of collection by the concentration floatation technique according to [22, 19, 23] and [24], [25] and [26].

Preparation of Serum Samples: Serum samples were prepared according to Schalling and Van 1997 [13].

Preparation of Crude *Haemonchus Contortus* Antigen (CHCA): It was done according to [27].

Determination of the Total Protein: It was applied according to [28] and [23].

Purification of (CHCA) Through Gel Filtration, Dialysis and Concentration: The purified *Haemonchus contortus* antigen was obtained by gel filtration according to [29] and [13].

Determination of the Molecular Weight: Antigen was fractionated using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis according to [30].

Enzyme Linked Immunosorbent Assay (ELISA): Fifty-nine random faecal, abomasa and serum samples of sheep were examind individually for detection of Family Trichostrongylidae eggs and *H. contortus* worms as described by [19]. In addition, sera samples from these animals were subjected separately to Enzyme linked immmunosorbent assay (ELISA) following the method adopted by [27] and [13] and according to [31]. Sensitivity, specificity and accuracy of the test were calculated according to [32].

RESULTS AND DISCUSSION

The infection with gastrointestinal parasites is very common in sheep in many parts of the world due to their grazing and watering habits [33]. Among gastro-intestinal nematode, the blood sucking abomasal parasite *H.contortus* is considered the most prevalent genus and most pathogenic worm in sheep [7, 34] and [35]. Infections with this parasite can cause, mainly in young animals, anemia and weight loss that in some cases result in death [6]. The parasite secrets cathepsin L- like cystein proteases that can potentially facilitate the parasite access to the host blood [36], the adults worms feed on blood and are distinguished by their blood sucking ability [5]. *H. contortus* is responsible for about (30%- 50%) of mortality in lambs which causes great economic losses [35], it also includes decreased production (meat, wool and milk), the costs of prophylaxis and treatment and deaths among the infected adults animals [37].

In the present study *H. contortus* adult worms were identified macroscopically; adult male has an even reddish color, while in adult female white ovaries are spirally around red intestine, producing the appearance of a barber's pole. Macroscopically, the anterior end of the adult worms (male and female) has 2 cervical papillae at the same level, which resemble spine- like (x100) as shown in Fig. (1,1-1) and also contains a small buccal cavity with dorsal lancet tooth (x400) as shown in Fig. (1,1-2).

The posterior end of the male bursa has elongated lateral lobes supported by long, slender rays, while the small dorsal lobe is asymmetrically situated against the left lateral lobe and is supported by inverted Y-shaped dorsal ray. Also it has 2 long spicules, each provided with a small barb near its extremity and the gubernaculums has curved stick shaped (x100) as shown in Fig. (1,1-3). the vulva of the female is covered by an anterior flap, which is usually large and very prominent (x100) as shown in Fig. (1,1-4), but may be reduced to a small knob- like structure in some specimens (x100) as shown in Fig. (1, 1-5). The female has thin slender end (x100) as shown in Fig. (1, 1-6), the anus is present before the end

Fig. (1-1): Anterior end of adult *H. contortus*.(dorsal lancet tooth) *X400

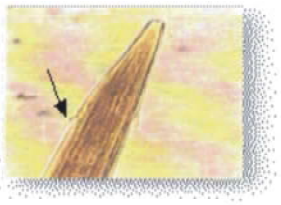

*Fig. (1-2): Anterior end of adult *H. contortus*. *X100

*Fig. (1-3): Posterior end of *H. contortus* (male bursa). *X100

*Fig. (1-4): Prominent Vulvar Flap. (Tongue shape). *X100

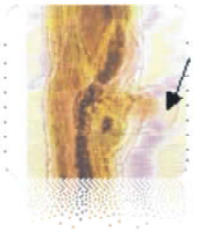

*Fig. (1-5): Valvular femal flab (knob shape) *X100

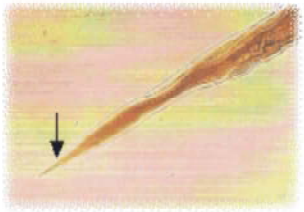

*Fig. (1-6): Femal Posterior end

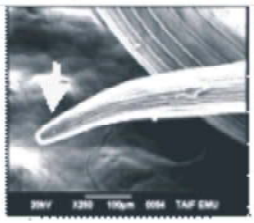

*Fig. (1-7): Anterior end of adult *H. contortus* by SEM.

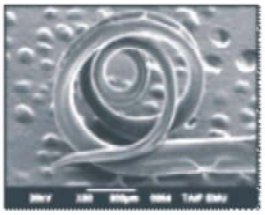

*Fig. (1-8): Adult *H. contortus* by SEM.

Fig 1: Collection of adult worms *H. contortus* pictures for main characters
*Fig(1.1-1.8): Picture, *X: Magnification times and SEM : scanning electron microscope.

by short distance, these characteristic features distinguish *H. contortus* adult worm from the other gastric nematodes. These results agree with [20] and [38].

In the present study, the incidence of *H. contortus* among sheep at Taif abattoir (table 1) was investigated through examination of 561 abomasa (May 2013- April 2014) and revealed that (15.15) of sheep were infected with *H. contortus* ; this result is shown in Table (1). This result is agreement with *that* recorded by [39] in Spain, in Egypt [40] and in Taif [41]. Menwhile this data disagrees with *that* recorded by [42] in Srilanka (90%); [43] in India (56.38%); [44] in Poland (25%) and [7] in Burkina Faso (88.3%). These differences may be attributed to the different localities, species of sheep and the number of samples in addition to the different of climatic condition in different localities.

The study of protein by SDS-PAGE of crude and partially purified *H. contortus* antigens (Fig 2), under reducing conditions showed that, *H. contortus* crude antigen separated protein bands of molecular weights ranging from (14.84 to 106.2 kDa) [10], peak I of *H. contortus* purified antigen contained (14) bands of protein ranging from (17.51 to 98.70 kDa), peak II of *H. contortus* purified antigen contained (4) bands of protein ranging from (17.51 to 45.54 kDa) and peak II of *H. contortus* purified antigen contained one protein

Table 1: Monthly incidence of *Haemonchuscontortus* infection among sheep in Taif abattoir through abomasal examination

Month	The number of examined abomasal samples	The number of positive samples	The percentage of positively
May	21	6	28.571
June	33	3	9.09
July	35	6	17.14
August	19	3	15.79
September	37	3	8.108
October	57	4	7.02
November	31	2	6.45
December	74	6	8.11
January	36	8	21.5
February	59	33	55.9
March	55	4	7.27
April	17	5	29.411
Total	561	85	15.15

P□ 0.05 for monthly prevalence and total worm burden.

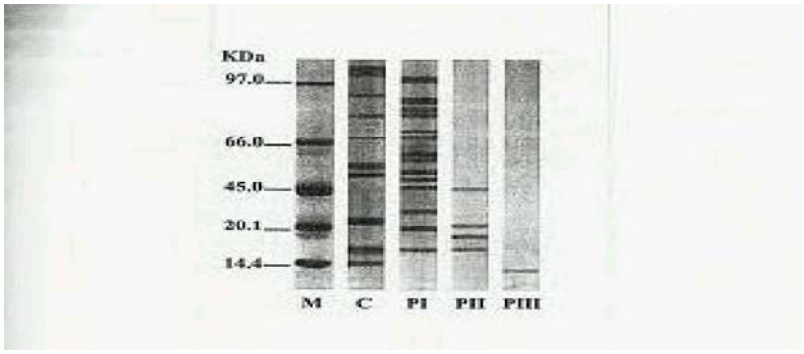

Fig. 2: Protein profile of the crude and the partially purified adult *H. contortus* antigens obtained from one dimensionalgel separated by SDS-Page 10% under reducing conditions and stained with Coomassie brilliant blue. M ,unstained low molecular weight marker (Amersham ,Co., U.S.A.); C , crude adult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

band observed at level (13.63 kDa), there was one common shared protein band of molecular weight (17.5 kDa) between crude and partially purified *H. contortus* fractions (peak I and peak II); these results are shown in Fig (2). These may be nearly in agreement with the results obtained by [29], 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 [22] 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 disagree with those obtained by [45], 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).Variation of the molecular weight 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.

The diagnosis of haemonchosis usually based upon evaluation of clinical signs and faecal examination, have their limitations. Clinical signs usually become apparent when the infection is serious. Eggs are found in faeces after the prepatent period of approximately 3-4 weeks in which infection is firmly established and damage has already been done. So a reliable serological assay such as ELISA, which enables detection of subclinical infection and early infection, is needed [35]. Fifty – nine random faecal, abomasums and serum samples of sheep at Taif abattoir were collected during February 2014 and examined separately. Results of faecal examination revealed that the incidence of Family trichostrongylidae was (59.3%) as demonstrated in Table (2 and 3) This findings nearly coincides with that of [46] in Egypt (68.39%); [47]Dhanalakshmi *et al*. in India (82.2%) and

Table 2: ELISA results compared with those of faecal and abomasal examinations for diagnosis of haemonchosis.

Number of samples	Faecal examination	Abomasal examination	ELISA results (Absorbance Value(OD)
1	+ve	+ve	+ve 0.308
2	-ve	-ve	-ve 0.187
3	+ve	-ve	+ve 0.233
4	+ve	+ve	-ve 0.151
5	+ve	-ve	+ve 0.230
6	+ve	+ve	+ve 0.230
7	+ve	+ve	+ve 0.257
8	+ve	+ve	+ve 0.245
9	+ve	+ve	+ve 0.291
10	-ve	-ve	-ve 0.218
11	-ve	-ve	-ve 0.222
12	-ve	-ve	-ve 0.164
13	-ve	-ve	-ve 0.160
14	-ve	-ve	-ve 0.165
15	-ve	-ve	-ve 0.190
16	-ve	-ve	-ve 0.150
17	+ve	+ve	+ve 0.249
18	+ve	+ve	+ve 0.239
19	+ve	+ve	+ve 0.288
20	-ve	-ve	-ve 0.110
21	+ve	+ve	+ve 0.343
22	-ve	-ve	-ve 0.110
23	-ve	-ve	-ve 0.153
24	+ve	+ve	+ve 0.322
25	-ve	-ve	-ve 0.175
26	-ve	-ve	-ve 0.185
27	-ve	-ve	-ve 0.175
28	+ve	+ve	+ve 0.285
29	+ve	+ve	+ve 0.258
30	-ve	-ve	-ve 0.162
31	-ve	-ve	-ve 0.174
32	+ve	+ve	+ve 0.286
33	+ve	+ve	+ve 0.269
34	+ve	+ve	+ve 0.244
35	-ve	-ve	-ve 0.130
36	+ve	+ve	+ve 0.278
37	+ve	+ve	+ve 0.253
38	+ve	+ve	+ve 0.283
39	+ve	+ve	+ve 0.259
40	-ve	-ve	-ve 0.174
41	+ve	+ve	+ve 0.294
42	+ve	+ve	+ve 0.254
43	+ve	+ve	+ve 0.291
44	-ve	-ve	-ve 0.153
45	-ve	-ve	-ve 0.170
46	+ve	+ve	+ve 0.301
47	+ve	+ve	+ve 0.230
48	+ve	+ve	+ve 0.286
49	+ve	+ve	+ve 0.262
50	+ve	+ve	+ve 0.265
51	+ve	+ve	+ve 0.267
52	-ve	-ve	-ve 0.162
53	+ve	+ve	+ve 0.244
54	+ve	+ve	+ve 0.243
55	-ve	-ve	-ve 0.135
56	-ve	-ve	-ve 0.112
57	-ve	-ve	-ve 0.123
58	+ve	+ve	+ve 0.237
59	+ve	+ve	+ve 0.265

Table 3: Number and percent of positive samples during February 2014 in different diagnostic techniques.

Total number of the examined samples	Number of the positive samples	Percentage of positively
59	35 Faecal samples	59.3
59	33 Abomasal samples	55.9
59	34Serum samples	57.6

[48] in Southern Nigeria (65%). The abomasal examination revealed that the incidence of *H. contortus* in examined samples was (55.9%) as, demonstrated in Table (2 and 3). This results is in agreement with the result of [49] and [8]. This variation in the results may be attributed to different number of examined samples, countries and localities. Serodiagnostic investigation was carried out also for evaluating sensitivity of the partially purified *H. contortus* antigen peak II in diagnosis of natural haemonchosis through the examination of 59 random serum samples, which were collected and examined individually by ELISA. The results revealed that *H. contortus* incidence in using the seroassay was (57.6%) as demonstrated in Table (3). This results is nearly in agreement with those of [35] who examined 153 field sheep serum samples using the His- Es 24 (cloned ES24kDa H. contortus antigen) ELISA, found (53.6%) of them were positive to H. contortus. Meanwhile, this result differs from those of [50] who found that the seroprevalence of *H. contortus* was (between 70 and 100%) in sheep using ELISA. This variation in results of ELISA with the other authors may be due to the difference in the examined number of samples, antigen and serological assay used. It could be concluded from the presented work that, the incidence of *H. contortus* infection among sheep at Taif abattoir increases the attention for the importance of veterinary care considering the seasonal incidence of haemonchosis, identification of diagnostic and immunogenic proteins of adults *H. contortus* using SDS-PAGE show relatively large number of different protein bands in both high and low molecular weights and also ELISA is concedered, a reliable serological tool in the diagnosis of haemonchosis.

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