

## THE USE OF A SPECIFIC ANTIGEN FOR SERODIAGNOSIS OF HAEMONCHUS CONTORTUS INFESTING SHEEP

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### SUMMARY

One hundred and ninety two randomly collected sheep sera were tested serologically by indirect haemagglutination test (IHA) for diagnosis of *H. contortus*. The sensitivity of the test was 87.50%. The most antigenic fraction obtained from adult worms extract was used as a specific antigen. The highest percentage of seropositive samples (26.79%) was detected at titres of 1/128. Incidence of *H. contortus* in seropositive samples at various titres was also estimated.

### INTRODUCTION

Gastro-intestinal nematodes could be considered as one of the commonest forms of parasitic diseases in sheep causing outbreaks and severe losses.

Indeed it was not so easy to express these losses due to disturbances in nutrition and growth (depression of appetite, reduced digestibility of protein, calcium, phosphorus, and dehydration with a consequent marked and rapid loss of weights) as a result of gastroenteritis (Reid, 1976 and Tawfik and Selim, 1979)

The pathogenicity of *Haemonchus contortus* in sheep stimulated the efforts of various authors to study its antigenicity and immune effector mechanism (Adams and Beh, 1981 and Adams et al. 1982).

Faecal examinations depend mainly upon the presence of eggs which may be followed by larval identification. These examinations are changeable without reasons and in many instances as the presence of migratory larval stages, eggs were never to be found for diagnosis.

The use of crude antigenic extract lacked the sensitivity in the diagnosis (Derbala, 1993) as well as possibility of cross reactivity. So it was necessary to use a specific antigens for avoiding such of these problems.

Therefore, the present study was suggested to investigate the use of specific antigen in the serodiagnosis of *Haemonchus contortus* infesting sheep.

### MATERIAL AND METHODS

One hundred and ninety two random sheep blood samples were collected from Cairo abattoir. The sera were separated in clean dried bottles and stored at -20°C until use for serodiagnosis.

Ten serum samples were collected from sheep that proved to be free from any parasites used as negative controls. Twelve serum samples were obtained from sheep proved to be pure infected with *Haemonchus contortus* as positive controls.

*Haemonchus contortus* adult worms were collected from sheep abomasae, the worms were washed in normal saline, homogenized in phosphate buffered saline pH 7.2 and centrifuged at 10000 r.p.m. The supernatant was obtained as a crude soluble

antigen (Adams et al., 1982). Protein content was determined according to Lowery et al. (1951). Isolation of the most antigenic fraction of crude *H. contortus* antigen was performed using column chromatography technique adopted by (Ogunba, 1972). The preliminary fraction using sephadex G200 gel filtration of crude *H. contortus* antigen resulted in (F<sub>1</sub>-F<sub>5</sub>) fractions. In this connection, Derbala (1988) stated that F<sub>1</sub> fraction was the most antigenic obtained fraction from *H. contortus* antigenic extract. Therefore, F<sub>1</sub> was used in this study as a specific antigen in serodiagnosis of *H. contortus* infestation. The antigenicity had been tested by the gel diffusion and IHA tests against immune sera prepared in rabbits.

Indirect haemagglutination (IHAT) procedure was used in serodiagnosis of *H. contortus* as adopted by Kagan and Norman (1976).

### RESULTS

Serological results of *H. contortus* in (Table 1) showed that the percentage of seropositive samples was 87.50% as detected by IHAT.

Variable titres of 168 seropositive samples were ranged between 1/8 and 1/2048. The lowest percentage (5.36 & 8.93%) was obtained at titres of 1/128 and 1/512 and the highest percentage (26.79%) was detected at titre of 1/16 (Table 2).

When 12 positive serum samples were tested by IHAT, all of them showed haemagglutination patterns with titres ranging from 1/16 to 1/256, denoting 100% sensitivity of the test.

Incidence of *H. contortus* in seropositive sheep sera at titres rather than 1/8, 1/16 and 1/32 were 87.50, 73.44 and 50%, respectively while the incidence was 37.50, 32.81, 25 and 14.06% at titres 1/128, 1/512, 1/1024 and 1/2048 respectively (Table 3).

### DISCUSSION

One of the major difficulties encountered in immunological studies of parasites has been the mul-

tiplicity of antigens present in the parasite.

The complex extract of *H. contortus* has been resolved into less complex unit and partially purified after filtration through sephadex G 200 column chromatography technique

The present work described the use of the partially purified antigen (fraction) which proved to contain a high antigenic proportion in the serodiagnosis of *H. contortus* where five fractions were obtained from the parasite extract. Only F<sub>1</sub> and F<sub>2</sub> fractions showed positive precipitating and haemagglutinating antibodies beside fraction 1 proved the most antigenic one (Derbala, 1988).

Similar results were recorded with slight variation, in the fraction numbers as well as its antigenicity, Ozerol and Silverman (1970) obtained two major fractions from the third and fourth *H. contortus* larval extracts. Moreover, Neilson (1975) obtained 4 and 7 excretory & secretory and larval somatic extracts fractions of *H. contortus* larvae, respectively. The results obtained by Wedrychowicz (1984) were 6 and 5 fractions separated from 4th & 5th *O. circumcincta* larval somatic extracts. In each larval stage of *O. circumcincta*, 2,3 & 4 and 1,3 & 4 fractions revealed positive precipitating and haemagglutinating antibodies.

Further work had been done by Wedrychowicz (1980) showed that homogenized and extracted *S. papillosus* larvae obtained 3 fractions, only 2 of them (1 & 2) had immunological activities.

Previous serodiagnostic studies had been obtained by Rothwell & Griffiths (1977), Adams & Beh (1981) and Shubber et al. (1984). They proved that the *T. colubriformis* and *H. contortus* antigenic extracts gave a specific haemagglutinating antibodies response.

The results of this investigation demonstrated haemagglutinating antibodies titre ranged from 1/8 - 1/2048. Moreover, Wedrychowicz and Bezubik (1981) showed that a somatic extract of *O. circumcincta* could elicit production of haemagglutinating antibodies in infested sheep.

It has been found that several workers have dealt

Table (1): Percentage of H. contortus seropositive samples detected by IHAT.

Total numbers	Seropositive samples		Seronegative samples	
	Number	%	Number	%
192	168	87.50	24	12.50

Table (2): Titres obtained by IHAT in determination of H. contortus antibodies in 168 seropositive ovine sera:

Number of samples	Titres	Percentage
27	*1/8	16.07 %
45	1/16	26.79 %
24	1/32	14.29 %
9	1/128	5.36 %
15	1/512	8.93 %
21	1/1024	12.50 %
27	1/2048	16.07 %
Total 168		100 %

\* The threshold positive titre was recorded at 1/8

Table (3): Incidence of H. contortus at different titres by IHAT

No. of samples	> 1/8		> 1/16		> 1/32		> 1/128		> 1/512		> 1/1024		> 1/2048	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
192	168	87.50	141	73.44	96	50	72	37.50	63	32.81	48	25	27	14.06

with evaluation of serological tests (Soulsby, 1956; Varju, 1967; Stankiewicz, 1970) and Rytynska-Prill, 1980), stating that the highest CF titre always occurs within the first few weeks of infection when compared with the specific

haemagglutinating antibodies response that were observed in the 6th to 12th weeks after first infestation and persist. They also found that the IHAT was the most reliable test for diagnosis and the use of CFT, HAI, conglutination, precipitation

and Ouchterlony gel-diffusion tests were unsatisfactory. Concerning the cross reactivity, Soulsby (1956) and Oliver-Conzalez & Burga Acha (1970) discussed the marked cross reactivity between the prepared antigenic extracts prepared from *H. contortus*, *Trichostrongylus* spp. as well as *Strongyloides* spp.

The preparation of antigen of very high purity is often laborious and difficult. The IHAT showed a much greater sensitivity with highly purified picture than with crude material (Surjan & Nyerges, 1962). Such results supported the present work, the sensitivity of IHAT reached 87.50% in the diagnosis of *H. contortus*. Probably, this high sensitivity might be attributed to the use of purified antigen (specific antigen) and that the use of purified antigen gave relative satisfactory results, compared with the results obtained by Derbala, (1993) who used the crude antigenic extract in the diagnosis of *S. papillosus*. The sensitivity of the test resulted in 73.91% positive results.

Further purification might be necessary for obtaining a purified antigens and higher results.

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