

Original Article

Evaluation of Different Antigens in Western Blotting Technique for the Diagnosis of Sheep Haemonchosis

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Abstract

Background: To use different methods for serodiagnosis of ruminants' haemonchosis is important because detection of egg in the faeces is not so reliable.

Methods: Peptide bands of 5 different crude antigens of intestine, uterus, cuticle, whole male and whole female of *Haemonchus contortus* were determined using SDS-PAGE and western blotting. Five lambs were infected with 10,000 third stage larvae of *H. contortus* and 2 parasites free were kept as control. Positive and negative sera collected from infected and no infected animal were tested using western blotting for immunodiagnostic antigens.

Results: In electrophoresis the major peptide bands of crud antigens of uterus, intestine, cuticle, whole male and whole female of *H. contortus* were 7, 2, 6, 8 and 5, respectively at molecular weights of 15 to 110 kDa. In immunoblotting positive and negative sera were compared and the molecular weight of specific protein bands for *Haemonchus* in sheep was determined. Two major peptide bands belong to intestine and uterus with 35 and 40 kDa molecular weight, respectively, were specific for diagnosis of the parasite infection.

Conclusion: Our findings suggest the use of these antigens with different molecular weights for immunodiagnosis of haemonchosis in sheep as a primary screening test is promising.

Keywords: *Haemonchus contortus*, Antigen, Western blotting, SDS-PAGE

Introduction

Haemonchus contortus is a very common nematode parasite of ruminants, with a world- wide distribution (1). Its prevalence in ruminants of Iran varies from 49% to 12% in sheep and cattle, respectively (2). Subclinical form is not uncommon in ruminants and could have some economic impacts.

Although coprological examination is more practical for diagnose of haemonchosis but accurate diagnosis needs fecal culture and at least 2 weeks time producing 3rd stage larvae. Therefore in urgent cases and in the prepatent period of infection other methods could be used.

The aim of this study was to determine specific protein bands of different parts of *H. contortus* in experimentally infected sheep using western blotting.

Materials and Methods

Experimental infection

Seven one-year old parasite free male lambs, were obtained from a local lamb's producer were housed under conditions precluding helminth infections and fed with commercial pelleted food and tap water. Five of them were inoculated with 10,000 exsheathed L3 and 2 were kept as control. All animals were examined for nematode infection using McMaster technique on day 20 (PI). During the experiment, individual blood samples were taken once a week. Blood samples were allowed to clot at room temperature.

Positive sera samples were taken from lambs with 5,000 EPG. All lambs at the end of experiment were necropsied and adult *H. contor-*

tus were collected from abomasa. The sera were labeled and stored at -20 °C until use.

Antigen preparation

Adult *H. contortus*, males and females, obtained from the abomasa of experimentally infected lambs, were washed in cold phosphate buffer saline (PBS) and stored at -20 °C.

Crude antigens of uterus and gut isolated from large female, cuticle and adult female and male of *H. contortus* were obtained as described by Klesius *et al.* (3) with slight modification. Briefly, five antigens including uterus, gut, cuticle, whole male (MAg) and whole female (FAG) antigens were obtained. They were homogenized at 4°C using a tissue-glass homogenizer and PBS contains protease inhibitors, 0.05 mM phenylmethylsulphonyl fluoride (PMSF). The extracts were centrifuged at 30,000g for 30 min at 4 °C and their supernatants from five were aliquoted and stored in -70 °C until used. Protein concentration was measured as stated earlier (4). In addition, somatic antigens from *Parabronema skrjabini*, *Camelostrongylus mentulatus*, *Nematodirus oiratianus* and *Marshallagia marshalli* based on these methods were prepared and stored.

SDS- PAGE and Western blotting

The prepared antigens were run on SDS-polyacrylamide gels composed of 5% resolving gel and 10% stacking gel under reducing conditions using the discontinuous buffer system (5). For size estimation a prestained protein marker at range 15-160 kDa Molecular weight (SM-0671) were used from Fermentase-Chemical.

The transfer of proteins were performed (6) in a Bio-Rad Trans-Blot Cell for 12 h in a constant current of 30 V. Blocking in 3% dry skimmed

milk in PBS containing 0.1% Tween 20 was carried out 1 h at room temperature. Strips were washed three times with PBS containing 1% Tween 20 for 10 min and incubated for 1 h at room temperature with first antibody diluted in PBS 0.1% Tween 20. The strips were washed again for 3×10 min and incubated in secondary antibody conjugated to horseradish peroxidase (1:1000, Koma Biotech Company) for 1 h at room temperature. After incubation the strips were washed as before in PBS 0.1% Tween 20. 3, 3'-diaminobenzidine tetra hydrochloride (sigma) substrate was in 25 ml of PBS with 10 µl of H₂O₂ 30% was added for exactly 2-5 min at room temperature. Finally, the reaction was stopped by the addition of distilled water to strips.

Results

Our findings using SDS-PAGE showed that 7, 6, 2, 8 and 5 were the major peptide bands of uterus, cuticle, intestine, whole male and whole female of *H. contortus*, respectively with molecular weights of 15 to 110 kDa. In western blotting a large number of protein bands with 45-80 kDa molecular weights were observed. It is worth mentioning that the 35 and 40 kDa band were specific for intestine and uterus, respectively (Fig.1).

The sera and the conjugated antibody were used at 1:100 and at 1:1000 dilutions, respectively.

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Results obtained from western blotting of somatic antigens of *Parabronema skrjabini*, *Camelostrongylus mentulatus*, *Nematodirus oiratianus* and *Marshallagia marshalli* with positive sera against *H. contortus* found are shown in Fig. 2 which lack any protein bands.

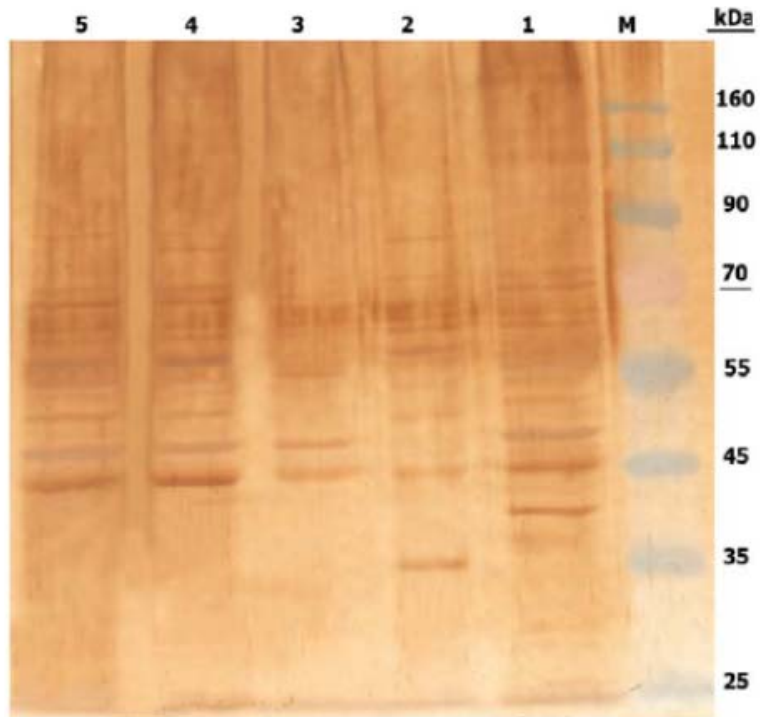


Fig.1: Western blot analysis of different antigens of *H. contortus* against positive sera. (1); Uterus (2); Intestine (3); Cuticle (4); Whole male (5); Whole female; (M) Prestained Protein Ladder-Fermentas SM 0671

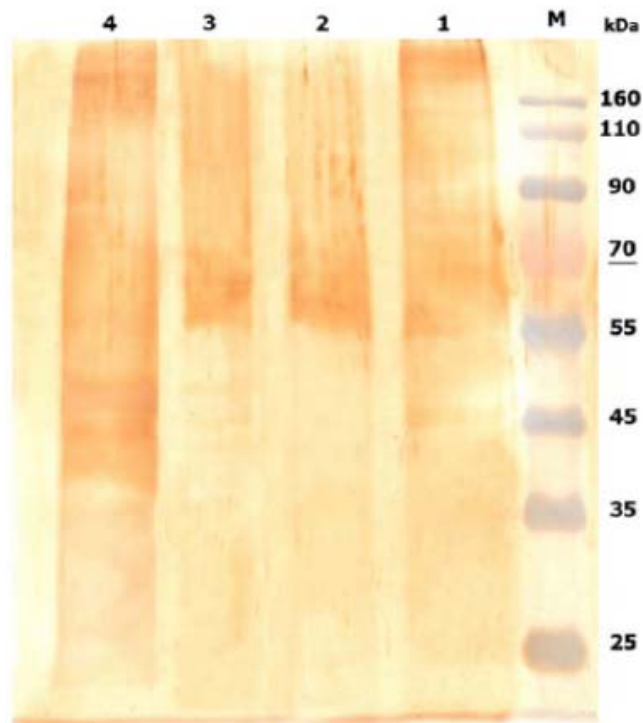


Fig.2: Western blot analysis of somatic antigens of other nematodes against positive sera. (1); *Parabronema skrjabini* (2); *Camelostongylus mentulatus* (3); *Nematodirus oiratianus* (4); *Marshallagia marshalli*

Discussion

H. contortus a helminth of ruminants of Iran (2) produces mainly sub clinical forms and reduces animal production (1). Although diagnosis of this disease during the patent period can be done through coprological examination but in a differential diagnosis with other *Trichostrongyle* infections, fecal culture is needed and this take at least one week. To overcome this deficiency especially in the patent period of the infection and to some extent in producing vaccine, serological methods based on identification of different antigens can be used. In a number of investigations, various serological methods were used and different antigens were reported for diagnosis of haemonchosis (7-9).

In the recent years, efforts have been focused into producing potential vaccine for the haemonchosis (1). Two types of antigens consisting natural and hidden antigen have been recognized in *H. contortus* (10). The natural antigens which can be recognized by the host although can induce an immune response but, of a somatic fraction (p26/23) of hidden antigens that are not exposed to the host immune system, can elicit a significant protection in vaccinated lambs (11, 12). Somatic antigens of adult *H. contortus* (56, 39 and 18.5 kDa) were used to vaccinate lambs against this nematode (13). Lambs vaccinated with 39 kDa showed reduction in both abomasal worm burdens and eggs per gram counts.

In the present study, several worm antigens were used to detect anti-*Haemonchus* antibody in sera of experimentally infected sheep using western blotting. Western blotting carried out on the SDS-PAGE gel of different antigens preparation demonstrated the transfer of protein bands to the nitrocellulose that were recognized by sheep polyclonal antisera. The immunoblotting analysis showed that total IgG presented in the serum of the primary infected sheep commonly reacted against intestine, uterus, cuticle, whole male and whole female crud antigen

proteins of 35-160 kDa, but intestine and uterus antigen with 35 and 40 kDa respectively, were specific for serodiagnosis of *H. contortus* infection in sheep.

Previously, a 26 kDa antigen of adult wire worm was suggested as specific for the diagnosis of *H. contortus* infection (8) and 91.2 kDa antigen was identified as immunodominant antigen in this helminth (9) which was not in line with our finding. This could be due to the differences in preparation method of antigens, characteristics of parasite species or even breed of sheep. A 66 kDa adult *H. contortus* excretory secretory antigen was identified in western blot by reaction with sera from the infected goats (10). A *H. contortus* antigen that is expressed on the cuticle of the mature nematode was detectable in faeces of infected animals. Investigators showed presence of polypeptides 122, 56 and 49 kDa molecular weight of *H. contortus* as surface antigen in faeces of infected sheep (7).

In conclusion, the present study suggests that the peptide bands of 35 and 40 kDa molecular weight of intestine and uterus, respectively, are specific and therefore, could be used as a reliable serodiagnostic potential for sheep haemonchosis. In addition further studies should be done to verify cross reactions with other nematodes such as *Trichostrongylus* spp., *Chabertia* spp., trematodes and cestodes of ruminants.

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