Applications of studying Electrophoretic pattern of *Haemonchus contortus* as tool for vaccine designing - A Review

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Abstract: The study of electrophoretic pattern of *Haemonchus contortus* has proved to be an important tool for vaccine designing. The aim behind the review is to encourage young researchers to initiate work on this aspect. Disease biomarker discovery is generally carried out using two dimensional polyacrylamide gel Electrophoresis (2D-PAGE) to identify differences in the protein expression patterns. After 2D-PAGE fractionation and staining, the protein(s) of interest are removed, proteolytically or chemically digested and identified by mass spectrometry (MS). Although 2DPAGE separation provides excellent resolution, the need for protein staining and the subsequent sample handling limits the sensitivity of the overall approach. Protein profiling is expected to discover unexpected targets for drug design by determining the function of thousands of unidentified proteins still likely to be found in the genome of *Haemonchus contortus*. Protein profiling is expected to multiply the number of known drug targets 100-fold. This will encourage the pharmaceutical industry to develop new drugs against this economically important parasite. This review will focus on research carried out globally by the applications of electrophoresis and so far various proteins have been identified as targets for vaccine designing.
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1. Introduction

Sheep being a close grazer is regarded as museum of parasites especially for helminths. Economic losses due to helminth parasites in sheep throughout the world are considerable. There is no single requirement more crucial to the rational and sustainable control of helminth parasites in grazing animals than a comprehensive knowledge of the epidemiology of the parasites as it interacts with the host in a specific climatic, management and production environment. The availability of complete genome sequences for a large number of helminth parasites has opened the door for large-scale proteomic studies to dissect both protein expression/regulation and function. Electrophoresis is one of the innovative tools to exploit proteome - the genome operating system by which the cells of an organism react to environmental signals (Anderson and Anderson, 1996). The techniques include the development of activity-based probes and activity-based protein profiling methods to screen for pharmacological tools to perturb basic biological processes. The standard method for quantitative proteome analysis combines protein separation by high resolution isoelectric focusing, SDS-PAGE, two-dimensional gel electrophoresis (2DE) with mass spectrometric (MS) or tandem MS (MSyMS) identification of selected protein spots. Important technical advances related to 2DE and protein MS have increased sensitivity and reproducibility while creating an integrated technology. By using 2DE with extended pH range and high-sensitivity protein identification by electrospray ionization and MSyMS, we have evaluated the potential of the 2DE-MS strategy to serve as the technology base for comprehensive and quantitative proteome analysis (Steven, 2000). Two dimensional Electrophoresis (2DE) form the first generation proteome tool as host proteome responses such as post-translational modifications of host proteins (phosphorylation, glycolysylation, acetylation and methylation) in reaction to parasite invasion can be detected and identified (Patton, 2000). The present paper revises the application of electrophoresis in devising control stratigies against *Haemonchus contortus*.

2. Haemonchosis

Haemonchosis is a predominant infection in small ruminants caused by *H. Contortus* a blood sucking abomasal nematode causing severe anaemia which may be fatal particularly to young animals. Detection of infection during prepatent period has not been attempted although 4th stage larva and the immature worm are blood sucking (Soulsby, 1982). Till the infection becomes patent and the eegs appear in faeces, young animals suffer from anaemia resulting in sudden death. Therefore detection of prepatent infection is a prerequisite for effective control of infection in light of the problem of drug

resistance in *H. contortus*. Early diagnosis of the infection is essential for timely treatment with suitable anthelmintics. Diagnosis of *H. contortus* infection during prepatency utilising excretory secretory (ES) antigens in ELISA has already been reported. (Schallig *et al*., 1994). Crude ES antigen and immunoaffinity purified ES antigen have been used in dot-ELISA, a simple, easy to perform and less time consuming test for detecting early haemonchosis during prepatent period since ES antigen is considered to be a potent antigen for diagnosis and immunoprophhylaxis. (Prasad *et al.,* 2007).

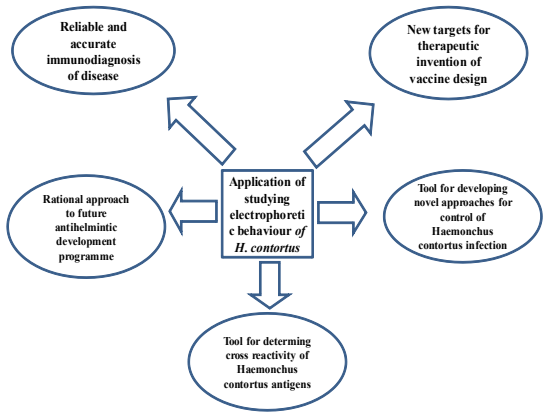

Fig. 1. Potential applications of electrophoretic study of *Haemonchus contortus*

1. Application of Electrophoretic Study of *Haemonchus contortus*

The need for basic laboratory research on Haemonchosis is stronger than ever. Recent advances in technology, particularly in the so-called post-genomics arena have created opportunities for the identification of proteins expressed by *Haemonchus contortus*. Electrophoresis is the global proven technique to open new vistas in human, veterinary, and laboratory animal medicine. It provides the increased resolution and precision in the inference of results. The new conceptual approach of electrophoresis suggested for parasite proteomics will help to increase the knowledge of immune responses to different parasite species, in addition to the creation of a proteomic database with a holistic view of host–parasite interactions, based on evolutionary concepts of host immune responses to a parasite. This new methodological approach offers a new way not only to discover drugs and vaccines but also to study host–parasite interactions, such as characterizing proteins whose function is implied in the behavioral manipulation of host in many taxa. In addition, it will open the way to reconstructing the molecular phylogeny of proteins such as those involved in the host immune response and to determine their level of conservation during evolution (Biron, 2005). Electrophoretic type of study will increase the knowledge of manipulative strategies and open the way to create protein databases of *Haemonchus contortus* based on a manipulative chart. Thus electrophoresis help to broaden the vision regarding different parameters pertaining to Haemonchosis, important ones are enlisted and discussed in Figure 1 (**Fig 1**).

3.1. Electrophoretic pattern as tool for diagnosis of Haemonchosis:

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. In electrophoresis the major peptide bands of crude antigens of uterus, intestine, cuticle, whole male and whole female of *H. contortus* have been found to be 7, 2, 6, 8 and 5, respectively at molecular weights of 15 to 110 kDa. In immunoblotting positive and negative sera have been compared and the molecular weight of specific protein bands for *Haemonchus* in sheep have been determined. Two major peptide bands belong to intestine and uterus with 35 and 40 kDa molecular weight, respectively, have been found to be specific for diagnosis of the parasite infection. (Meshgi and Hosseini, 2007). Also results have showed that a soluble fraction from adult stages of the nematode (p26/23) induces partial protection against challenge. Recombinant DNA technology has been applied to obtain a synthetic protein (rHcp26/23). Immunological assays (ELISA, Western blotting, and immunolocalization), using sera from lambs immunized with p26/23 have confirmed the identity of the recombinant protein and demonstrated that the synthetic protein is equivalent to the purified protein employed in the previous immunoprophylaxis studies. Vaccination of lambs with 300 μg of rHcp26/23 and Freund's adjuvant has been found to elicit a notable specific antibody response. (Leticia *et al*., 2010). Research has also been carried out in which dot-ELISA was performed with crude ES antigen as well as immunoaffinity purified fraction (F-1) with experimental and natural sera of sheep infected with *H.contortus* and it was proved that purified fraction was a more potent antigen. Crude ES and F-1 have also been fractioned through SDS-PAGE in which ES antigen revealed polypeptides in the range of 10 to 200 kDa of which 26, 32, 60 and 120 kDa were found prominent. F-1 fraction on SDS-PAGE analysis revealed only four polypeptides of 26, 32, 60 and 120 of which 60 and 120 kDa were found

to be most prominent. Results indicate that the purified fraction of ES antigen may be utilized for early diagnosis of haemonchosis. Further studies on cross antigenicity of this fraction with other nematode and trematode needs to be conducted. (Prasad *et al*., 2008). Isotype-specific serum antibody responses of sheep to *Haemonchus contortus* antigen has also been carried out in which sera of immune animals specifically reacted with low molecular weight proteins. In particular, a 24 kDa antigen present in adult worms appeared to be specifically recognized. (Schallig et al., 1994). Purification of this antigen by electrophoresis and its application to quantitative serologic tests will permit further analysis of its predictive value to evaluate cure. Modified Counter Immuno-Electrophoresis (MCIE) has been found to be a simple, rapid, and inexpensive test and can be used for preliminary screening of haemonchosis and other parasitic infections in sheep/goats. (Imtiaz *et al*., 2011). It may also be important to mention here that the antigen preparation of worm metabolic products confer no resistance to challenge infection with the parasite. (Neilson, 1975).

3.2. Electrophoretic pattern as tool for vaccine design

Vaccine development against *H. contortus* has been progressing for over three decades with varying results (Smith and Munn, 1990; Emery and Wagland, 1991; Newton and Meeusen, 2003). Extracts of adult *Haemonchus contortus* have been purified and used as a vaccine against the blood feeding parasite in goats and sheep. The proteins used are H11 and H-gal-GP, hidden gut antigens from the microvillar membrane of the gut of the worm and combined with Quil A as adjuvant The antigens are then administered to a group of goats kept on concrete then artificially infected with *H. contortus*. The control group receives Quil A injections and also gets infected. The trial shows that IgG levels peak three weeks after the first vaccine and remains high throughout the remaining booster series but begins to wane after artificial infection. However, the IgG levels have been found to remain significantly higher in the controls. Overall mean fecal egg counts (FEC) are significantly higher in the controls and packed cell volume levels have been found to be significantly higher in vaccinated goats compared to controls from Week 3 post infection. A booster vaccine given Week 7 pi causes a sharp increase in IgG levels, elimination of worm burdens and decrease in FEC in the vaccinated group. Ninety-six percent fewer *H. contortus* adults are recovered at necropsy in vaccinated group compared to controls and >96% reduction in FEC after booster vaccine given during established infection. This shows that the H11/H-gal-GP vaccine is sufficient in protecting goats after challenge infections but is shorter lived than when given under the same conditions in sheep as shown in previous trials. Booster vaccine given when infection levels are rising are effective in eliminating infections, reducing FEC and therefore may be used in place of an anthelmintic to control haemonchosis in goats as in sheep. (Donya Dupree Olcott, 2006). Similarly vaccination with an antigen pool from the excretory/secretory (ES) products of L3 larvae of *H. contortus* reduces faecal egg counts and worm burden drastically (Schallig & van Leeuwen, 1997). Another study has focussed on global proteomes from male and female *H. contortus* worms. This has been helpful to some extent in discovering new antigens that can be potential vaccine candidates. (Yatsuda *et al.,* 2003).

3.3. Electrophoresis as a tool for future antihelmintic development programme against *Haemonchus contortus*

Research has shown that there is anthelmintic resistance in *H. contortus* to benzimidazoles, imidothiazoles, and avermectins in sheep and goats throughout the world (Miller *et al*., 1987; Sangster, 1999; Jackson and Coop, 2000). Goats have a problem with multiple drug resistant strains of *H. contortus* particularly due to the difference in drug metabolism in goats and sheep. Goats tend to require a higher dose of certain anthelmintics than sheep for similar blood level profiles but this is often not recognized and underdosing occurs, leading to resistance at a rapid rate (Conder and Campbell, 1995; van Wyk, 2001). Studies conducted in the Gulf South region of the United States have demonstrated that ivermectin and moxidectin resistance can develop over a short period of time; even within a few years in particular herds of goats (Miller *et al*., 1994; Terrill *et al.,* 2001). General parasite control recommendations include decreasing stocking rates, keeping pastures and pen areas well drained, and periodically moving feeders or troughs to decrease transmission from heavily grazed areas. Short grasses in pens or around barns may need to be completely eliminated, as these are usually the most contaminated areas. Dilution strategies by mixing two or more livestock species on the same pasture may also be helpful as sheep/goats and cattle or horses do not share most of the parasite species. Rotating the pastures between species may also be effective as one species will “vacuum” up the other species’ parasites; therefore, reducing contamination of pastures (Smith and Sherman, 1994; Barger, 1999) (Table 1). But the prevalence of multiple drug resistant *H. contortus* is alarmingly high. Veterinarians and producers are at risk of

having no effective anthelmintics in the near future (Sangster, 1999; van Wyk, 2001; Waller, 2004).

Table 1. Anthlemitic resistance of *H. contortus* reported in different parts of the world. (Paul Millares, 2010)

Africa			
Albendazole & Levamisole & Ivermectin	*Kenya*	Waruiru *et al.*	1997
Albendazole & Levamisole	*Kenya*	Waruiru *et al.*	1998
Asia			
Fenbendazole	*India*	Yadav	1990
Benzimidazole & Levamisole	*India*	Uppal *et al.*	1992
Fenbendazole	*India*	Yadav *et al.*	1993
Fenbendazole & Levamisole	*India*	Singh & Yadav	1997
Europe			
Benzimidazole	*Netherlands*	Borgsteede *et al.*	1997
Benzimidazole	*Sweden*	Höglund *et al.*	2009
Benzimidazole	*France*	Cabaret *et al.*	1995
North America			
Benzimidazole	*U.S.A.*	Theodorides *et al.*	1970
Thiabendazole	*U.S.A.*	Miller & Baker	1980
Oceania			
Thiabendazole	*Australia*	Webb *et al.*	1979
Benzimidazole & Levamisole	*Australia*	Green *et al.*	1981
Ivermectin	*Australia*	LeJambre *et al.*	1995
Ivermectin	*New Zealand*	Vickers *et al.*	2001
South America			
Benzimidazole & Levamisole, Ivermectin	*Brazil*	Echevarria *et al.*	1996
Benzimidazole & Levamisole, Ivermectin	*Argentina*	Eddi *et al.*	1996

3.4. Novel Approaches to control and recent developments in the development of vaccines against *Haemonchus contortus* using proteins obtained as a result of Electrophoresis

Researchers worldwide have been studying new strategies and novel approaches to the control of *H. contortus* in hopes to alleviate the current dependency on anthelmintics that are becoming less efficacious (Waller, 2004). Copper-oxide wire particle boluses have shown very positive results in reducing FEC in recent work but much research is left to ensure the safety of the copper in sheep and goats (Burke et al., 2004). Condensed tannin (CT)-containing forages are another new approach to controlling haemonchosis (Paolini et al., 2003; Athanasiadou et al., 2001). Research studies have proven that when given CT-containing hay, established infections are reduced and animals are generally healthier. Condensed tannins are found in forages 2 such as *Serecia lespedeza* and chicory and the amount of the forages that must be consumed by the animal to be efficacious is still being studied. Nematophagous fungi have been researched such as *Duddingtonia flagrans*, which affects all worm larvae in feces (Fontenot et al., 2003; Terrill et al., 2004). Breeding animals for genetic resistance to parasites has been studied at length and some sheep breeds such as the Gulf Coast Native, Barbados Blackbelly and St. Croix have shown resistance to nematode infections (Miller et al., 1998; Li et al, 2001). Since these breeds are not high producers such as the Suffolk breed, there is reluctance by the sheep industry to incorporate the resistant sheep into their flocks. Although the above approaches are promising, there is still much work to be done to incorporate them into parasite control programs that do not continue to rely on anthelmintic use. Another approach being studied is the development of a vaccine against *H. contortus* that could give protection from the parasite and not lead to resistance in the future. Several methods of vaccine components such as irradiated larvae, cysteine proteases, somatic antigens, whole nematode antigen, and hidden gut antigens have been researched with variable results (Urquhart et. al., 1966; Knox et al., 2005; Alunda et al., 2003; Kabagambe, et al., 2000; Smith et al., 2001). The most consistently successful attempts have been vaccination using proteins extracted from the microvillar surface of the intestinal tract of *H. contortus* with some reductions in worm burdens higher than 90% (Knox and Smith, 2001). These antigens that are found in the gut of the parasite and are not present on the worm's surface are "hidden" from the host under normal infection conditions. The animal mounts an immune response to the antigens contained in the 3 vaccination and then the parasite is affected after it ingests blood containing the antibodies. H11 and H-gal-GP antigens are thought to be involved in the breakdown of peptides that are produced by digestion of dietary protein and therefore the mechanism of protection after vaccination could be by antibody-induced disruption of nutrient uptake (Newton and Meeusen, 2003).

3.5. Electrophoresis as tool for determing cross reactivity of *Haemonchus contortus* antigens

The adult *Haemonchus contortus* somatic antigens responsible for cross-reactivity have been analysed using serum samples from goat kids infected and reinfected with *Teladorsagia circumcincta*. Goat kids infected with *T. circumcincta* had similar serum ELISA values against somatic antigens of *Haemonchus contortus* as goats infected with *H.contortus* itself. Immunoblotting confirmed this extensive cross-reactivity particularly in the molecular weight range 105-29 kDa. However, peptides with high (195, 152 and 119 kDa) or low (23 kDa) molecular weight were only faintly recognized by heterologous sera. (Molina *et al*., 2005).

4. CONCLUSION

The electrophoretic behavioural study is the most cost-effective and simplest way in the field of parasitoproteomic to characterize proteome of *Haemonchus contortus*. Though, some studies have shown the limitations of the current approach to parasitoproteomics but no one can deny that 2DE is the most accessible and efficient proteomic tool for a laboratory. Many parasitologists are betting heavily on proteomic studies based on electrophoresis to explain protein profile of *Haemonchus contortus* and, thus, to contribute to the control of haemonchosis.

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