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Standardization and Application of Modified Counter Immuno-Electrophoresis for the Detection of Antigenic Response against *Haemonchus contortus* in Rabbits

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Abstract

Present paper reports the standardization and application of modified counter immuno-electrophoresis (MCIE) technique to detect the antigenic response of *Haemonchus (H.) contortus* in rabbits. Sonicated somatic antigen prepared from the freshly collected worms was used to raise hyper immune sera in Albino rabbits. Optimum conditions for MCIE were determined by using hyper immune sera in comparison with the negative control. Optimum time for MCIE was 30 minutes at which visible precipitation band(s) were observed with hyper immune sera samples. A flow of 3 volts for 30 minutes was best for obtaining clear results. The quality of the band(s) was dependent upon the concentration of the antigen; and the amount of antigen and antibody on each side of MCIE tube. MCIE is a simple, rapid, and inexpensive test and can be used for preliminary screening of haemonchosis and other parasitic infections in sheep/goats.

Keywords: *Hemonchus contortus*, Modified counter immuno-electrophoresis, somatic antigen, rabbits

Introduction

Haemonchus (H) contortus is the most pathogenic nematode parasite of sheep and goats that cause marked decrease in productivity due to its voracious blood sucking habit and has been reported to result in 23.8 and 40.0 per cent reduction in meat and wool production, respectively (Mortensen et al., 2003). The adult worms affect the abomasum of the host and feed on blood, resulting in severe damage, such as poor growth rate, loss of weight, anemia and, occasionally, death (Githigia et al., 2001; Vatta et al., 2001). Haemonchosis is prevalent throughout the world where sheep and goats are raised but it exerts greatest economic losses in the temperate and tropical region (Wakelin, 1996). The economic losses due to haemonchosis in sheep and goat at Faisalabad (Pakistan) have been estimated to be Rs. 32 billion per anum (Iqbal et al., 1993). Most frequently described protective immune response against *H. contortus* in sheep is due to the self/spontaneous reaction where expulsion of already established worms is participated by the intake of ineffective larvae (Wakelin, 1996). However, other forms of acquired immunity may regulate the number of *H. contortus* in sheep. For instance, by preventing the establishment of infection in the host and lowered worm burden followed by re-infection in sheep due to series of the previous infections (Koski and Scott, 2001).

The diagnosis of haemonchosis is usually based upon clinical signs/symptoms and coprological examinations. Clinical signs usually appeared during serious infection and eggs passed in feces after the prepatent period of approximately 3–4 weeks in which infection firmly established and damage has already been done. Under the circumstances, any reliable serological assay, which enables the detection of subclinical infection has paramount importance (Almazan et al., 2001). In this regard, several techniques, including enzyme linked immunosorbent, radio-immuno and fluorescent antibody techniques are being used to demonstrate the various parasitic infections including *H. contortus* (Charely et ai., 1981; Eysker et ai., 2000) although all these methods have been reported to be expensive and cumbersome. Present paper describes the standardization and application of modified counter immuno-electrophoresis to detect the antigenic response against *H. contortus* in rabbits.

Materials and Methods

The Parasite

Adult *H. contortus* worms were collected from the abomasums of the sheep from the local slaughter house, Faisalabad, Pakistan. Collected worms were given several washings with phosphate- buffered saline (PBS; pH 7.2).

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Preparation of sonicated somatic antigen

Antigen was prepared following the method described by Jan (1993) with minor modifications. Worms (5gm/100mL) were subjected to ultra-homogenization for 3 minutes (1x 3) at 4°C followed by sonication for 60 seconds (30 seconds x2) in a jacketed vessel containing cool water. The sonicated suspension was centrifuged (5,000 ×g/ 15 minutes). Supernatant thus collected was used as antigen and its protein concentration was measured (Bradford, 1976). The antigen was stored at -20°C until further use.

Experimental Animals

A total of 20 male Albino rabbits were purchased from local market and maintained at experimental station, Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. Before experimentation, all the rabbits were subjected to deworming using Oxfendazole (0.5 ml/ rabbit) to make them parasite free.

Rabbits were divided into two equal groups A and B. Rabbits in group A were injected with *H. contortus* antigen to raise hyper immune sera (Gomez-Munoz et al., 2000) and those in group B served as control and injected with PBS.

Collection of sera samples

Blood was collected from the rabbits of both the groups on day 14th post last injection of antigen and serum was separated by slant method. Serum thus separated was stored at -20 °C for further use in modified counter immuno-electrophoresis.

Modified Counter Immuno-electrophoresis (MCIE)

Modified counter immuno electrophoresis was standardized and applied to detect the antigenic response against *H. contortus* (Tahir et al., 1998). Breifly, two mL of 1% melted Agarose (Sigma Inc., USA) in barbiturate buffer (pH 8.6) was dispensed in U-shaped glass tubing. Gel was allowed to solidify and then glass tubing was fixed vertically in a groove. A total of 10 µl of each serum and *H. contortus* antigen were dispensed in anodal and cathodal arms of U-shaped glass tubing (in triplicate) that was connected with aluminum wire. One end of the aluminum wire was pierced down into the gel about 0.5 cm in both arms of U-shaped glass tubing while the other ends were connected with battery cells (1.5 volts each). A constant current was applied upto 30 minutes and then it was switched off.

Observations were recorded after every five minutes upto 30 minutes. Development of circular precipitation band(s) was considered positive in comparison with the control in which there was no reaction between antigen and negative control serum.

The optimum test conditions were standardized with the help of positive and negative sera samples, making different dilutions of antigen with PBS (50µg, 100µg, 150µg, 200µg, 300µg and 350µg), supplying variable current at different time intervals.

Results and Discussion

Various serological tests have been developed and used for the accurate and early diagnosis and serological screening of haemonchosis in sheep and goats but each with certain drawbacks (Li et al., 2007; Ndao et al., 2009). MCIE is not used for parasitic infections although it is a frequently used test for the detection of viral antigens (Tahir et al., 1998). In the present study, MCIE was used for the detection of antigenic response of *H. contortus* in rabbits.

Based upon the findings of present study, MCIE was found to be a rapid test for the detection of *H. contortus* antigen. It was a time saving technique in which the reactants move towards each other. At pH 7.8, the antibodies/immunoglobulin molecules carrying a slight positive charge, move toward the cathode. On the other hand, the *H. contortus* antigens having a negative ionic charge at alkaline pH displaced toward the anode (Barrett, 1988).

Serological testing is an important component of any programme to control the disease in epidemiological surveillance, vaccine testing and in the selection of an appropriate strain because of considerable antigenic variations. Techniques reported in the literature have their own merits and demerits. It is reported that MCIE is more sensitive and the specific than agar gel precipitation test (Obi and Patrick, 1984) and this could successfully be used under the field conditions to screen out a larger number of sera samples. Keeping in view the merits of the assay, MCIE was standardized with the following conditions:

i. It gave rapid and clear results within 30 minutes.
ii. Positive response was obtained in the form of visible precipitation band(s) when hyper immune sera samples were used while no precipitation band(s) were observed with control sera samples.
iii. The flow of current was considered a vital step in the MCIE. It was observed that precipitation band(s) were more obvious when 3 volts current was allowed to pass for 30 minutes. Although, the results were achieved within 20 minutes by passing the current of 6 volts but the bands were not clearly demarcated.
iv. It was also observed that the quality of the band(s) depend upon the concentration of the antigen; and the amount of antigen and antibody (serum) on each side of MCIE.
v. During the present study, different concentra- tions of antigens (50µg, 100µg, 150µg, 200µg, 300µg and 350µg) by diluting in PBS were used. It was observed that bands were much more clear when 150µg concentration of *H. contortus*

antigen was used. It was also observed that an amount of 50µl each of antigen and serum on each side of the MCIE gave good results.

All the sera samples obtained from the experimental group processed through MCIE gave positive results in comparison with the control samples that showed no precipitation line(s)/band(s). Among ten sera samples, seven samples showed a thick precipitation band of antigen and antibody complexes while three samples showed two precipitation lines that indicated that *H.contortus* somatic antigen used in the study was complex in nature and contained two distinct antigen(s). These observations are contrary to the results of other workers (Munn et ai., 1993; Cuquerella et ai., 1991; Knox et ai., 1999; Pena et al., 2004) who recognized several polypeptides/proteins from *H. contortus* antigen by using immunoblotting (Jasmer and McGuire, 1991), Thiol-Sepharose chromatography (Knox et ai., 1999) techniques.

Based upon the results of present study, it can be concluded that MCIE is a simple, rapid and inexpensive technique that does not require any sophisticated equipments and reagents. It can be used as a preliminary screening tool in sheep/goat flocks to detect the *H. contortus* infection.

References

Almazan C, G Avila, H Quiroz, F Ibarra and P Ochoa, 2001. Effect of parasite burden on the detection of *Fasciola hepatica* antigens in sera and feces of experimentally infected sheep. Veterinary Parasitology, 97: 101–112.

Barrett JT, 1988. TextBook of Immunology: An introduction to immunochemistry and immunobiology. 5th ed. The C.V. Mosby Company, Washigton, DC, pp. 226-227.

Bradford MM, 1976. A dye binding assay for proteins. Analytical Biochemistry, 72: 248-254.

Charley J, C Bourdieu, G Laffau and P Prey, 1981. Immune response of sheep to *H. contortus*, serum antibodies against cross reacting antigens between parasites. Annales-de-Recherches-Veterinaries, 12: 123-128.

Cuquerella M, MT Gomez-Munoz and JM Alunda, 1991. Serum IgG response of Manchego lambs to infections with *Haemonchus contortus* and preliminary characterization of adult antigens. Veterinary Parasitology, 38: 131-143.

Eysker M, HW Ploger, RJ Martin, HD Schalling and LH Chapperl, 2000. Value of present diagnostic methods for gastrointestinal nematode infections of ruminants. Veterinary Parasitology, 120: 109-119.

Githigia SM, SM Thamsborg, WK Munyua and N Maingi, 2001. Impact of gastrointestinal helminths on production in goats in Kenya. Small Ruminant Research, 42: 21–29.

Gomez-Munoz MT, IA Domínguez, LA Gomez-0Iglesias, FJ Fernandez-Perez, S Mendez, C de la Fuente, JM Alunda and M Cuquerella, 2000. Serodiagnosis of haemonchosis with a somatic antigen (Hc26) in several breeds of sheep. Journal of Veterinary Diagnostic Investigations, 12: 354-360.

Iqbal Z, M Akhtar and MN Khan, 1993. Prevalence and economic significance of haemonchosis in sheep and goat slaughtered at Faisalabad abattoir. Pakistan Journal of Agricultural Sciences, 30: 51-53.

Jan SU, Z Iqbal, CS Hayat, MN Khan and M Akhtar, 1993. Distribution of serum immunoglobulins against *Haemonchus contortus* in experimentally induced sheep. Pakistan Veterinary Journal, 13: 171-174.

Jasmer DP and TC McGuire, 1991. Protective immunity to a blood-feeding nematode (*Haemonchus contortus*) induced by parasite gut antigens. Infection and Immunity, 59: 4412-4417.

Knox DP, SK Smith and WD Smith, 1999. Immunization with an affinity purified protein extract from the adult parasite protects lambs against infection with *Haemonchus contortus*. Parasite Immunology, 21: 201-210.

Koski KG and ME Scott, 2001. Gastointestinal nematodes, nutrition and immunity: Breaking the negative spiral. Annual Reviews of Nutrition, 21: 297-321.

Li X, A Du, W Cai, Y Hou, L Pang and X Gao, 2007. Evaluation of a recombinant excretory secretory *Haemonchus contortus* protein for use in a diagnostic enzyme-linked immunosorbent assay. Experimental Parasitology, 115: 242-246.

Mortensen LL, LH Williamson, TH Terrill, R Kircher, M Larsen and RM Kaplan, 2003. Evaluation of prevalence and clinical implications of anthelmintic resistance in gastrointestinal nematodes of goats. Journal of the American Veterinary Medical Association, 23: 495–500.

Munn EA, TS Smith, M Graham, CA Greenwood, AS Tavernor and G Coetzee, 1993. Vaccination of merino lambs against haemonchosis with membrane-associated proteins from the adult parasite. Parasitology, 106: 63-66.

Ndao M, 2009. Diagnosis of Parasitic Diseases: Old and New Approaches Interdisciplinary

Perspectives on Infectious Diseases. doi:10.1155/2009/278246.

Obi TU and D Patrick, 1984. The detection of peste des petits ruminantis virus antigen by agar gel precipitation test and counter immunoelectrophoresis. Journal of Hygiene, 93: 579-586.

Pena MT, JE Miller and DW Horohov, 2004. Effect of dexamethasone treatment on the immune response of Gulf Coast Native lambs to *Haemonchus contortus* infection. Veterinary Parasitology, 119: 223–235.

Tahir MT, MA Anjum, Z Ahmad, I Hussain and M Ashfaq, 1998. A modified counter immunoelectrophoresis technique. International Seminar on Microbial diseases, College of Veterinary Sciences Lahore, March 21, pp: 20.

Vatta AF, BA Letty, MJ van der Linde, EF vanWijk, JW Hansen and RC Krecek, 2001. Testing for clinical anaemia caused by *Haemonchus* spp. in goats farmed under resource-poor conditions in South Africa using an eye color chart developed for sheep. Veterinary Parasitology, 99: 1–14.

Wakelin D, 1996. Immunity to Parasites. 2nd ed. Cambridge University Press, London, UK.