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# IDENTIFICATION OF IMMUNOGENIC PROTEIN OF *ASCARIS SUUM*

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## ABSTRACT

Drug resistance by many naturally occurring nematodes amongst animals has intensified the efforts of scientists for exploring the antigenic protein necessary for survival of parasite. Therefore the present study was undertaken to explore the excretory-secretory (ES) product of adult *Ascaris suum* for identifying the protein with immunogenic activity associated this fraction of worms. For the purpose, antigenic protein was isolated and purified from the culture medium of adult worms by gel permeation chromatography on Sephadex G-200 and individual elutes were assessed for their antigen city against hyper immune sera, raised in rabbits. The elutes depicting the positive results were pooled and concentrated with PEG-6000 and characterized by SDS-PAGE. The most immunodominant protein was further assessed by immunoblotting. The protein 36 kD depicted immunogenicity and therefore, may be an ideal candidate for the development of vaccine against the helminth infection. In addition, this antigenic protein may also be explored for serodiagnosis of ascariasis in animals under field conditions.

**KEYWORDS:** *Ascaris Suum*, Double Immuno-Diffusion Test, SDS-PAGE, Western Blot

## INTRODUCTION

*Ascaris suum*, the largest among nematodes, was originally identified as ubiquitous and pathogenic parasite (Stankiewicz and Jeska, 1990) that lives in micro-aerobic environment of pig intestine (Roepstorff and Nansen, 1994). Despite the high prevalence of *Ascaris* infection, no good method for diagnosing ascariasis in the context of an epidemiological investigation has yet been devised except for parasitological screening for the presence of eggs in feces. In addition, anthelmintic resistance among nematode population and concerns about the effect of drug residues on animal health and the environment have focused an attention on the prospect of developing an effective anti-nematode vaccine (Knox and Smith, 2001). Therefore, development of reliable and efficient measures for control of this parasitic infection especially on the prospect of developing effective anti-nematode vaccine (Smith and Zarlenga, 2006), is the major element in ensuring the sustainability of animal production. Many of the materials released during *in-vitro* are receiving an increasing attention as potential sources of diagnostic and protective antigens. These products are collectively termed as excretory/secretory (ES) and are the subject of high antibodies responses in both infected and experimental animals (Ogilvie and de Savigny, 1982).Hence, the present study was under taken describes the purification and characterization of released excretory-secretory (ES) proteins of *A. suum* which may be useful markers for diagnosis of *Ascaris* infection in an epidemiological study.

## MATERIALS AND METHODS

### Parasites

Adult *Ascaris suum* were collected from naturally infected pigs, slaughtered at local abattoirs at Jabalpur, Madhya Pradesh.

### Preparation of Antigen

The worms were brought alive to the laboratory within a short span of time and washed thoroughly in 0.15M physiological saline. These were maintained in RPMI-1640 (0.5ml per worm) with 25mM HEPES containing 2% glucose and antibiotics as specified earlier by Kushwah *et al*., 2004. After 48 hr, the medium was carefully withdrawn (Smith and Zarlenga, 2006) and centrifuged at 5000 rpm for 5-10 minutes. The resultant supernatant was passed through 0.22µm filter membrane and was designated as crude ES products and stored at -20$^0$C after addition of 1mM EDTA as chelating agent and 1mM PMSF (Andrade *et al*., 2005) until further use. Protein in the extract was estimated using diagnostic kit (Bangalore Genei).

### Hyper Immune Sera

New Zealand white strain rabbits of age 12-18 months old each weighing about 0.5-1.0 kg were procured from D.R.D.O., Gwalior (Madhya Pradesh) and maintained under strict animal husbandry conditions. The animals were specific pathogen free and seronegative for *A. suum* antibodies. These were allowed to be fed on diet prepared under hygienic conditions and water *ad-libitum*. The animals were immunized against E/S products of *A. suum* by intramuscular route (Hay and Westwood, 2002) and later by intravenous route (Malick, 1996). On 34$^{th}$ day, the rabbits were bled, serum separated and stored in aliquots at -40$^0$C. The presence of antibodies were tested upon DID assay (Rodero *et al*., 2002).

### Separation of Proteins by Gel Permeation Column Chromatography

The dialyzed crude ES products were subjected to Sephedex G-200 gel permeation column chromatography (Kushwah and Sharma, 2005) subsequently the presence of proteins in each elute was estimated spectrophotometrically at 280nm.

### Identification of Antigenic Protein

Elutes, depicting the presence of protein, were tested for their antigenicity against hyper-immune sera, as obtained above. Upon DID assay, all the fractions depicting the positive results were pooled, subjected to ammonium sulfate precipitation and dialyzed against sucrose and later against PBS (pH 7.2). Finally, the dialyzed fractions were concentrated using polyethylene glycol (PEG) 6000 (Merek, India).

### Immunoblot

The dialyzed antigenic protein was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (Laemmali, 1970) using 4.5% stacking and 12.5% separating gel. Electrophoresis was carried out at a constant current of 20 mA. Molecular weight markers were incorporated and the gel was stained with Coomassie brilliant blue dye.

Following SDS-PAGE, the protein bands in unstained gel were transferred onto nitrocellulose membrane (Hybond C, Amersham, Sweden) in semi dry blotting apparatus (ATTO, Japan). The transblot was carried out at a constant current of 0.8 to 2.0 mA/cm$^2$ for 90 min. The membrane after transfer was incubated overnight at 4$^0$C in blocking buffer.

After blocking, the membrane was washed thrice with PBS-T and incubated with 1:400 diluted rabbit anti protein serum at $37^0$C for 1hr.Thereafter, the membrane was washed thrice with PBS-T as described above and incubated for 1hr at $37^0$C with 1:2500 goat anti rabbit HRPO conjugate (Sigma, USA). After washing thrice with PBS-T, the antigen-antibody reaction was detected by incubating the membrane with substrate. The color of the reaction was terminated by exhaustive washing with distilled water.

## RESULTS AND DISCUSSIONS

Nematode surface coat and ES products are considered to play an important role in the host-parasite interaction of the host animal (Lopez *et al*., 1999). In the present study, the protein in the crude ES products were resolved into two distinct broad peaks with almost equal distribution (Figure 3) and with more than 70% protein recovery. The presence of antigenic protein was assessed by DID assay (Figure 2) since the technique is simple, economical and needs no special equipment and has also been successfully used by earlier workers for immunodiagnostic purpose (Saini *et al*., 1997). Whilst the presence of antigenic protein was located in the second peak indicating it to be of low molecular weight Earlier workers indicated fractionation of ES antigenic protein of *A. lumbricoides* into ten fractions by gel chromatography on Sepharose-12 column in FPLC. Of these, the third fraction showing binding activity with IgG and IgE antibodies of A. lumbricoides infected sera was further resolved into two fractions when passed through mono Q column (Chatterje *et al*., 1996).

Upon SDS-PAGE, the dialyzed antigenic protein of ES was resolved into 36kDa band (Figure 4) that correlated with the observations of the earlier workers also (Andrade *et al*., 2005). Upon subjecting it to Immunoblotting, good reactivity was also observed with sera from rabbits, earlier immunized with ES antigenic protein from *A. suum* (Figure 5). This indicated infections with parasitic nematode initiates both allergic and immunomodulatory response (Peterson *et al*., 2002). Therefore ES products could conveniently be explored for further experimentation.

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## APPENDICES

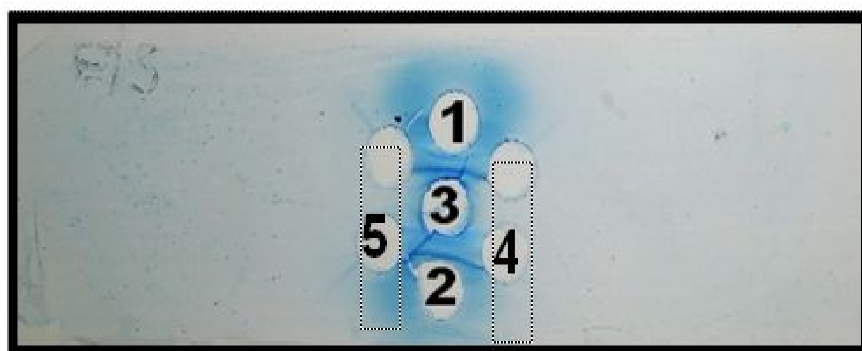


**Figure 1: DID Assay of Hyper Immune Sera of Rabbits**

- Sera of Rabbit-I immunized with ES protein
- Sera of Rabbit-II immunized with ES protein
- ES Protein
- Sera of controlled Rabbits

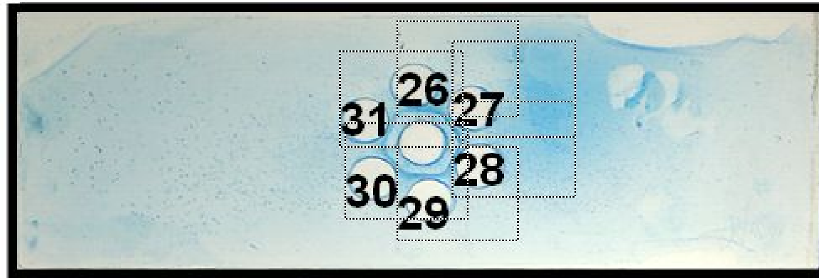


**Figure 2: DID Assay of Eluted ES Protein**

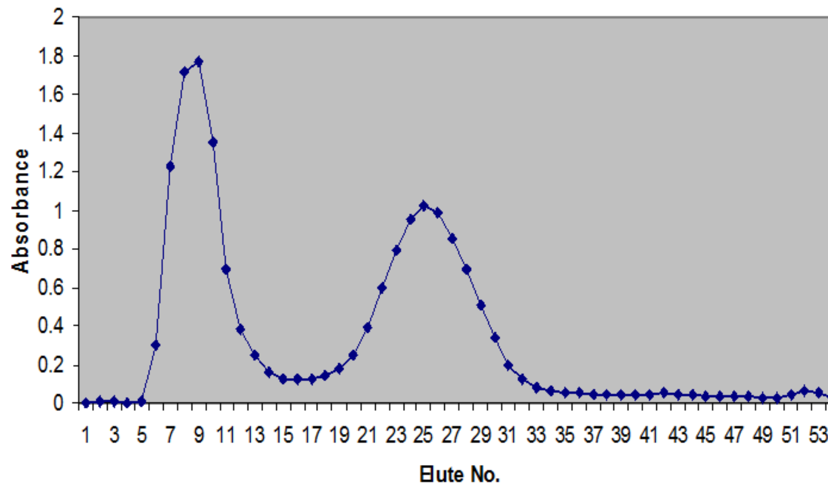


**Figure 3: Sephadex G-200 Chromatogram of ES Protein of *A. suum***

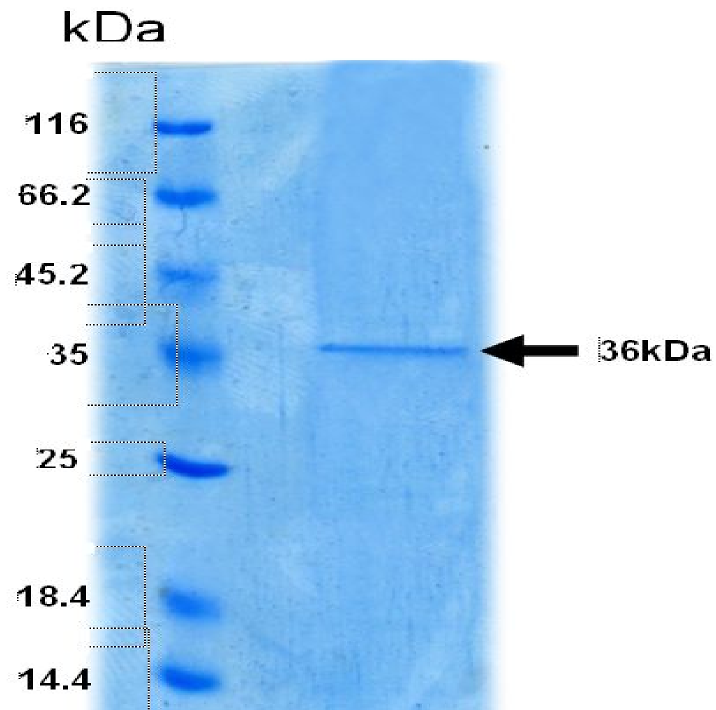


**Figure 4: Fractionation of Purified E/S Antigenic Protein by SDS-PAGE**

**Figure 5: Identification of Specific Immunogenic Protein**

**Table 1: Absorbance of Eluted E/S Protein at 280 nm Upon Chromatographic Separation**

| S. NO. | Absorbance | S. NO. | Absorbance | S. NO. | Absorbance | S. NO. | Absorbance |
|---|---|---|---|---|---|---|---|
| 1 | 0.004 | 15 | 0.123 | 29 | 0.505 | 43 | 0.041 |
| 2 | 0.005 | 16 | 0.121 | 30 | 0.341 | 44 | 0.042 |
| 3 | 0.127 | 17 | 0.125 | 31 | 0.195 | 45 | 0.037 |
| 4 | 0.000 | 18 | 0.143 | 32 | 0.121 | 46 | 0.034 |
| 5 | 0.006 | 19 | 0.174 | 33 | 0.084 | 47 | 0.035 |
| 6 | 0.301 | 20 | 0.249 | 34 | 0.059 | 48 | 0.033 |
| 7 | 1.226 | 21 | 0.395 | 35 | 0.052 | 49 | 0.027 |
| 8 | 1.717 | 22 | 0.597 | 36 | 0.051 | 50 | 0.029 |
| 9 | 1.767 | 23 | 0.793 | 37 | 0.047 | 51 | 0.045 |
| 10 | 1.351 | 24 | 0.948 | 38 | 0.047 | 52 | 0.063 |
| 11 | 0.692 | 25 | 1.022 | 39 | 0.043 | 53 | 0.055 |
| 12 | 0.382 | 26 | 0.987 | 40 | 0.041 | 54 | 0.031 |
| 13 | 0.245 | 27 | 0.854 | 41 | 0.045 | | |
| 14 | 0.163 | 28 | 0.696 | 42 | 0.049 | | |