

## Protein profile and serodiagnosis of *Taenia solium* bladder worm infections in pigs

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### ABSTRACT

A study on the protein profile of the bladder worms of *Taenia solium* was conducted. Three antigens, including whole cyst antigen (WCA), scolex antigen (SA) and excretory secretory antigen (ESA), were evaluated and the presence of 18, 15 and 12 polypeptides was observed, respectively. The WCA, SA and ESA had 11, 10 and 9 polypeptides respectively as immunodominant when these were allowed to react with homologous hyper immune sera. When infected pig sera were allowed to react, 4 bands in WCA, 2 in SA and 4 in ESA were found to be highly reactive.

**Key words:** *Taenia solium*, bladder worm, protein profile

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### Introduction

*Taenia solium* cysticercosis is a public health problem in most developing countries where pigs are raised and pork is consumed and where poverty, illiteracy and deficient sanitary infrastructures are common (DORNY et al., 2003).

Infection with bladder worms of *Taenia solium* results in a specific antibody response, mainly of the IgG class. Some patients have IgM, IgA and IgE antibodies (GOODMAN et al., 1997). It is possible that most infected hosts produce multiple antibodies of different specificities, which may appear at different intervals after infection, apparently in response

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to the qualitative and quantitative changes in excretory-secretory antigens and somatic antigens released during various phases of parasitic development (SCHANTZ, 1996).

Research on the antigenic properties and improved protein purification techniques have resulted in much more reliable serological tools (GOTTSTEIN et al., 1986; PARKHOUSE and HARRISON, 1987; TSANG et al., 1989; ITO et al., 1998). The most specific test developed is the enzyme-linked immunoelectro transfer blot (EITB).

Purification of glycoproteins by isoelectric focussing was recently shown to produce very specific antigens which are applicable both in immunoblot and ELISA (ITO et al., 1998). The specificity and sensitivity of ELISA was reported to match those of the immunoblot (ITO et al., 2002). The enzyme-linked immunosorbent assay (KUMAR and GAUR, 1987; 1989; DEKA and GAUR, 1993; PATHAK et al., 1994; D'SOUZA and HAFEEZ, 1999) and enzyme immuno transfer blot (GOTTSTEIN et al., 1986; GONZALEZ et al., 1990; SREENIVASAMURTHY et al., 1999) have been conducted and have shown varying sensitivity and specificity.

MURALIDHAR et al. (1990) characterized whole cyst antigen, cyst fluid antigen, scolex antigen and normal pork muscle antigen using crossed immunoelectrophoresis. Characterization of whole cyst antigen using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) has been attempted by GOTTSTEIN et al. (1986), LACLETTE et al. (1987), and SREENIVASAMURTHY et al. (1999). KO and NG (1998) characterized the whole cyst antigen, cyst fluid antigen and excretory-secretory antigens, and LARRALDE et al. (1986) analyzed the vesicular fluid antigen.

To identify the most specifically active component a systematic approach is needed. Every component from the whole parasite must be examined for its specific antigenic activity. Hence, a study was designed to characterize the whole cyst antigen (WCA), scolex antigen (SA) and excretory-secretory antigen (ESA) on a comparative basis to analyse the protein profile and immunodominant epitopes using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and enzyme immuno transfer blot (EITB).

### **Materials and methods**

*Whole cyst antigen (WCA)*. WCA was prepared as per the method of MAHAJAN et al. (1974) with a few modifications. The cysts were collected from naturally infected pigs slaughtered at the Corporation slaughterhouse in Bangalore and washed in phosphate buffered saline (PBS) pH 7.2-7.4, containing antibiotics, viz. penicillin G (500 IU/ml) and streptomycin (500 µg/ml). Ten g of cysticerci were taken into 20 ml of PBS containing 0.1 mM phenyl methyl sulphonyl fluoride (PMSF) (Merck) as preservative. The cysticerci were disrupted in a glass tissue homogeniser at 4 °C. The homogenate was sonicated four times in a sonicator at 20 kHz, 1 mA for 60 seconds in an ice bath. The homogenate

was then centrifuged at 14000 g for 60 minutes at 4 °C in refrigerated centrifuge and the supernatant was used as the antigen.

*Scolex antigen (SA)*. The scolices (10 g) were dissected out, immediately placed in five volumes (50 ml) of sucrose / [2-(4- {2-hydroxyl ethyl} 1-piperaziny) ethane sulphonic acid] (HEPES) / PMSF buffer (0.05 M HEPES/ sodium hydroxide (NaOH), 0.25 M sucrose, 2.0 mM ethylene diamine tetracetic acid (EDTA), 5 mM PMSF, pH 7.2) at 4 °C, drained, blotted dry and 10 g of the scolices were used to prepare SA as per the procedure for WCA.

*Excretory secretory antigen (ESA)*. Cysticerci were dissected out from the skeletal muscles of infected pigs, carefully avoiding host tissue, and only the intact cysts were isolated. Then the cysts were washed for 30 minutes in 3 changes of sterilized saline with penicillin G (500 IU/ml) and streptomycin (500 µg/ml). Twenty cysts were transferred to milk dilution bottles containing Roswell Park Memorial Institute (RPMI) 1640 medium (Himedia) with 1000 mg/L penicillin, 2 mg/L gentamicin, 5 mg/L fungizone and 20 mg/L cefotaxime. The culturing procedure and preparation of ESA was as per the method of D'SOUZA and HAFEEZ (1999).

*Host antigen*. The skeletal tissues of uninfected pigs from shoulders, thigh and heart of normal pigs after their slaughter were collected from the Corporation slaughterhouse, homogenated in PBS with antibiotics, centrifuged at 14000 g in a refrigerated centrifuge and the supernatant was then used as antigen.

*Raising of hyperimmune serum (HIS)*. Hyperimmune serum was prepared by injecting of the three antigens to three separate rabbits. Three hundred micrograms of antigen were injected at weekly intervals after mixing with equal volumes of Freund's complete adjuvant (FCA), and for subsequent injections Freund's incomplete adjuvant (FIA) was used. The animals were bled 14 days after last injection; sera were separated and kept in aliquots at -20 °C until use.

*Sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE)*. SDS-PAGE was performed according to LAEMMLI (1970) in 10% separating gel and 4.5% stacking gel. The gels were stained with Coomassie Brilliant Blue and molecular weight was determined using standard molecular weight markers (Genei, Bangalore).

*Enzyme immuno transfer blot (EITB)*. EITB was carried out on Nitrocellulose membrane in a mini-Transblot cell (Biorad) as per the procedure of TOWBIN et al. (1979), and HARLOW and LANE (1988). The free binding sites were saturated with 5% skimmed milk powder. Protein A conjugate with horseradish peroxidase was diluted at 1:1000 (v/v) with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) / ortho dianisidine dihydrochloride (ODD) substrate system.

## Results

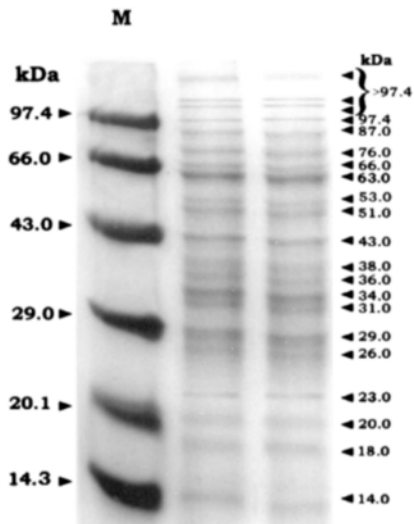


Fig. 1a. SDS-PAGE profile of whole cyst antigen (WCA)

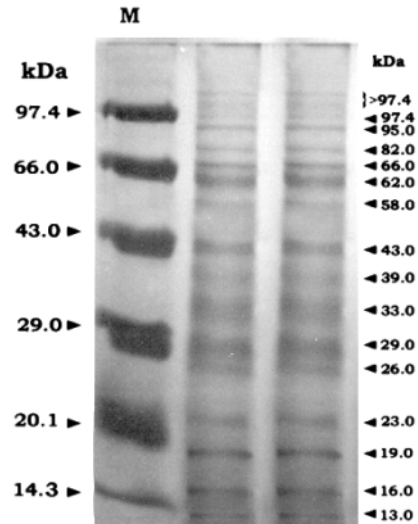


Fig. 1b. SDS-PAGE profile of scolex antigen (SA)

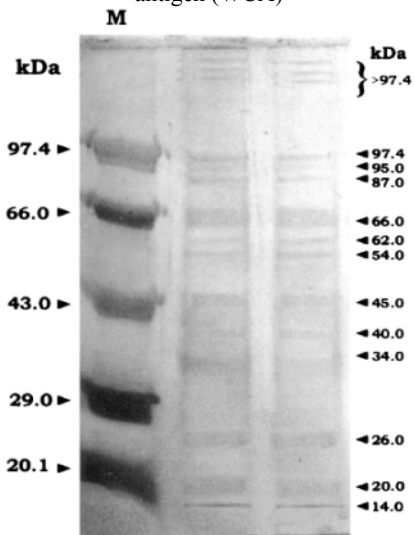


Fig. 1c. SDS-PAGE profile of excretory-secretory antigen (ESA)

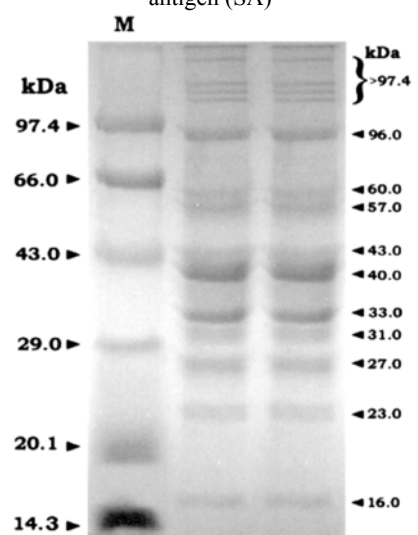


Fig. 1d. SDS-PAGE profile of host antigen (HA)

**SDS-PAGE.** The Whole cyst antigen (WCA), Scolex antigen (SA), and excretory secretory antigen (ESA) when analyzed by SDS-PAGE, revealed 18, 15 and 12 bands respectively, which ranged from 13 kDa to 97.4 kDa. Also, all three antigens had three bands of more than 97.4 kDa. Host antigen had a total of 10 polypeptides, ranging from 96.0 to 16.0 kDa (Fig 1. a, b, c, d).

**Immunoreactive polypeptides of WCA, SA, ESA and HA as detected by EITB.** When rabbit positive serum was used as developing serum, 11, 10 and 9 highly reactive antigenic components were demonstrated in WCA, SA and ESA, respectively. WCA and SA when reacted with heterologous HIS 43 kDa and 26 kDa were found to be reactive. When the same heterologous HIS was allowed to react with ESA no protein was found to react. This indicates that ESA does not have any band in common with others. This indicated that 43kDa and 26kDa in WCA might be from the scolex region. To check the presence of host antigens, the HIS raised against WCA, SA and ESA were allowed to react with the host antigen and no antigen antibody reaction in the form of band could be seen.

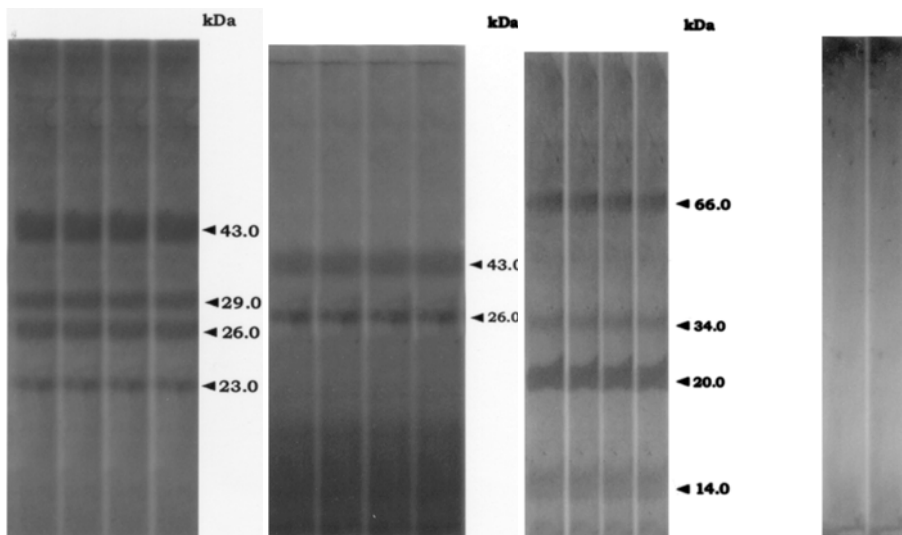


Fig. 2a, 2b, 2c. EITB profiles of serum samples of pigs positive for *Taenia solium* bladder worms using WCA, SA and ESA.

Fig. 2d. EITB profiles of serum samples of pigs negative for *Taenia solium* bladder worms.

Using the pig positive serum as the developing serum, antigenic bands of 23.0, 26.0, 29.0 and 43.0 kDa were highly reactive in WCA. Bands of 26.0 and 43.0 kDa were highly reactive in SA. In ESA the bands of 14.0, 20.0, 34.0 and 66.0 kDa were highly reactive (Figs 2a, b, c). The number of antigenic bands decreased when the antigens were probed

with pig positive serum, which may be due to the fact that the non-specific epitopes, which are reactive with rabbit antiserum, are not reactive with the pig positive serum. When the serum from control animals was allowed to react with WCA, SA and ESA there was no antibody reactivity to any of these antigens (Fig. 2d).

### Discussion

In the present study whole cyst antigen was selected in view of its high sensitivity (KUMAR and GAUR, 1987). The SA was very significant as it has been said to be the most antigenic component of the cysticerci (NASCIMENTO et al., 1987). The selection of ESA was of special interest. Multicellular parasites such as *Cysticercus cellulosae*, unlike unicellular parasites, are not amenable for phagocytosis due to which a significant number of the antigens of the helminth parasite might remain unknown to the host's immune system, while the metabolic product of these live parasites was likely to sensitize the host immune system (LIGHTOWLERS and RICKARD, 1988).

SDS-PAGE and EITB techniques were used to separate and identify antigenic parasite molecules binding to immuno globulin of sera. In the present study the three antigens with respective immunodominant epitopes represent possible potential immunodiagnostic antigens.

The most commonly recognized antigen bands by EITB from infected pig serum were located at 23.0, 26.0, 29.0 and 43.0 kDa of WCA, which is similar to reports by PATHAK et al. (1994), ITO et al. (1999), and SREENIVASAMURTHY et al. (1999). The 26.0 and 43.0 kDa bands of SA appear to be an ideal antigenic source for further fractionation and purification of specific immunodominant antigen. In ESA 14.0, 20.0, 34.0 and 66.0 kDa bands are immunodominant. However, 48.0 and 66.0 kDa bands are said to be immunodominant (KO and NG, (1998), who used anti porcine conjugate and medium 199 (contains Earle's salts in extra when compared to RPMI 1640 medium) for culturing of the cyst as against protein A conjugate and RPMI 1640 medium. Similarly, Centricon 30 (Amicon) was employed in purification of the antigen, which was not used in the present study.

In conclusion, WCA, SA and ESA appear to be an ideal antigenic source for further fractionation and purification of specific immunodominant antigen. In addition, EITB can be made suitable for routine application by storing the nitrocellulose membranes blotting with specific immunodominant antigen at 4 °C after fixing.

### References

- DEKA, D., S. N. S. GAUR (1993): Serodiagnosis of *Taenia solium* cysticercosis in naturally infected pigs by ELISA. J. Vet. Parasitol. 7, 86-92.

- DORNY, P., J. BRANDT, A. ZOLI, S. GEERTS (2003): Immunodiagnostic tools for human and porcine cysticercosis. *Acta Tropica*. 87, 79-86.
- D'SOUZA, P. E., Md. HAFEEZ (1999): Detection of *Taenia solium* cysticercosis in pigs by ELISA with Excretory Secretory antigen. *Vet. Res. Commun.* 23, 293-298.
- GONZALEZ, A. E., V. CAMA, R. H. GILMAN, V. C. W. TSANG, J. B. PILCHER, A. CHAVERA, M. CASTRO, T. MONTENEGRO, M. VERASTEGUI, E. MIRANDA, H. BAZALAR (1990): Prevalence and comparison of serologic assays, necropsy and tongue examination for the diagnosis of porcine cysticercosis in Peru. *Am. J. Trop. Med. Hyg.* 43, 194-199.
- GOODMAN, K., A. CARPIO, S. A. BALLAGH (1997): Case-control study of seropositivity for cysticercosis in Cuenca, Ecuador. *J. Neurol. Sci.* 150, S326.
- GOTTSTEIN, B., V. C. W. TSANG, P. M. SCHANTZ (1986): Demonstration of species specific and cross reactive components of *Taenia solium* metacestode antigens. *Am. J. Trop. Med. Hyg.* 35, 308-313.
- HARLOW, E. D., D. LANE (1988): *Antibodies: A Laboratory Manual*. Cold spring Harbor Laboratory, U.K.
- ITO, A., A. PLANKARTE, Y. MA, L. KONG, A. FLISSER, S. Y. CHO, Y. H. LIU, S. KAMHAWI, M. W. LIGHTOWLERS, P. M. SCHANTZ (1998): Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. *Am. J. Trop. Med. Hyg.* 59, 291-294.
- ITO, A., A. PLANCARTE, M. NAKAO, K. NAYAKA, T. IKEJIMA, Z. X. PIAO, T. KANAZAWA, S. S. MARGONO (1999): ELISA and immunoblot using purified glycoproteins for serodiagnosis of cysticercosis in pigs naturally infected with *Taenia solium*. *J. Helminthol.* 73, 363-365.
- ITO, A., Y. SAKO, M. NAKAO, K. NAKAYA (2002). Neurocysticercosis in Asia: serology /sero-epidemiology in humans and pigs. In: *Cestode Zoonoses: Echinococcosis and Cysticercosis* (Craig, P., Pawlowski, Z., Eds.), IOS Press, pp. 25-31.
- KO, R. C., T. F. NG (1998): Evaluation of excretory/secretory products of larval *Taenia solium* as diagnostic antigens for porcine and human cysticercosis. *J. Helminthol.* 72, 147-154.
- KUMAR, D., S. N. S. GAUR (1987): Serodiagnosis of porcine cysticercosis by enzyme linked immunosorbent assay (ELISA) using fractionated antigens. *Vet. Parasitol.* 24, 195-202.
- KUMAR, D., S. N. S. GAUR (1989): Comparative evaluation of various immunodiagnostic tests for the diagnosis of *Taenia solium* cysticercosis in pigs, using fractionated antigens. *J. Helminthol.* 63, 13-17.
- LACLETTE, J. P., M. T. MERCHANT, K. WILLMS (1987): Histological and ultrastructural localization of antigen B in the metacestode of *Taenia solium*. *J. Parasitol.* 73, 121-129.
- LAEMMLI, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685.
- LARRALDE, C., J. P. LACLETTE, C. S. OWEN, I. MADRAZO, M. SANDOVAL, R. BOJALIL, E. SCIUTTO, L. CONTRERAS, J. ARZATE, M. L. DIAZ, T. GOVEZENSKY, R. M. MONTOYA, F. GOODSID (1986): Reliable serology of *Taenia solium* cysticercosis with antigens from cyst vesicular fluid: ELISA and haemagglutination tests. *Am. J. Trop. Med. Hyg.* 35, 965-973.

- LIGHTOWLERS, M. W., M. D. RICKARD (1988): Excretory secretory products of helminth parasites - effects on host immune responses. *Parasitol.* 96, 123-166.
- MAHAJAN, R. C., N. L. CHITKARA, J. S. CHOPRA (1974): Evaluation of cysticercosis and adult worm antigens in serodiagnosis of cysticercosis. *Indian J. Med. Res.* 62, 1310-1313.
- MURALIDHAR, K. K., C. JAGANNATH, G. N. GOKUL, A. CHANDRAMUKHI, S. SEHGAL (1990): Antigenic analysis of *Cysticercus cellulosae* by crossed immunoelectrophoresis and its role in immunodiagnosis of Neurocysticercosis. *Indian J. Med. Res.* 91, 39-43.
- NASCIMENTO, E., P. M. P. NOGUEIRA, C. A. P. TAVARES (1987): Improved immunodiagnosis of human cysticercosis with scolex protein antigens. *Parasitol. Res.* 73, 446-450.
- PARKHOUSE, R. M. E., L. J. S. HARRISON (1987): Cyst fluid and surface associated glycoprotein antigens of *Taenia* spp. metacestodes. *Parasite Immunol.* 9, 263-268.
- PATHAK, K. M. L., J. C. ALLAN, K. ERSFELD, P. S. CRAIG (1994): A western blot and ELISA assay for the diagnosis of *Taenia solium* infection in pigs. *Vet. Parasitol.* 53, 209-217.
- SCHANTZ, P. M. (1996): Cysticercosis in non-endemic countries: the example of the United States. In: *Taeniasis: cysticercosis pork T. solium* (Garcia, H. H., Martinex, S., Eds.), Editorial Universo S.A, Lima, pp. 277-286.
- SREENIVASAMURTHY, G. S., P. E. D'SOUZA, M. S. JAGANNATH (1999): Enzyme linked immunotransfer blot in the diagnosis of *Taenia solium* cysticercosis in pigs. *J. Parasitic Dis.* 23, 85-88.
- TSANG, V. C., J. A. BRAND, A. E. BOYER (1989): An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *J. Infect. Dis.* 59, 50-59.
- TOWBIN, H., T. STACHELIN, J. GORDON (1979): Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and cone applications. *Proc. Natl. Acad. Sci., USA*, 76, 4350-4354.

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**DHANALAKSHMI, H., M. S. JAGANNATH, P. E. D'SOUZA: Proteinski profil i serološka dijagnostika invazije cisticercima *Taenia solium* u svinja. *Vet. arhiv* 75, 505-512, 2005.**

**SAŽETAK**

Provedeno je istraživanje proteinskog profila cisticerka *Taenia solium*. Istražena su tri antigena, uključujući antigen cijele ciste (WCA) s utvrđenih 18 polipeptida, antigen skoleksa (SA) s 15 polipeptidai ekskretorno sekretorni antigen (ESA) s utvrđenih 12 polipeptida. Kod antigena WCA, SA i ESA kao imunodominantni su se pokazali polipeptidi 11, 10 i 9 u reakciji sa homolognim hiperimunim serumom. U reakciji sa serumima invadiranih svinja utvrđene su kao visoko reaktivne četiri trake kod WCA, dvije kod SA i četiri kod ESA antigena.

**Ključne riječi:** *Taenia solium*, cisticerk, proteinski profil

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