



Determination of immune reactive proteins in cystic membrane of *Cysticercus tenuicollis* of goat origin*

S NATH¹, S PAL², P K SANYAL³ and S C MANDAL⁴

Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattishgarh 491 001 India

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Commonly affected animals due to *Cysticercus tenuicollis*, the metacestode of *Taenia hydatigena*, are sheep and goat where the prevalence of cysticerci may reach up to 30% even more (Pathak and Gaur 1982). The cysticercosis causes considerable economic losses due to high degree of morbidity and mortality in livestock (Abidi *et al.* 1989). Diagnosis of *T. hydatigena* cysticercosis is usually made by post mortem examination. For ante-mortem diagnosis, specific serological tests are essential. Cross reaction is usually common especially in helminthic infections due to sharing of antigens. Looking to the importance of the diagnosis of infection, a study on the determination of immune reactive proteins in *Cysticercus tenuicollis* cystic membrane of goat origin was conducted.

Cysticercus tenuicollis were collected from goat slaughterhouse at Supela, Bhilai, Chhattisgarh. The cysts were cleaned and the membranes were collected aseptically after aspirating the fluid.

Preparation of antigens: The membranes of *C. tenuicollis* were collected and mixed with phosphate buffer saline (PBS, pH 7.4) containing phenyl methanesulphonyl fluoride (PMSF) @ 10 µg/ml and homogenized with the help of tissue homogenizer till a homogenous suspension was obtained. The homogenate was sonicated in batches at 10µ amplitude for 8 min, each cycle for 2 min with 1 min interval between two successive cycles. The sonicated antigen was centrifuged at 16 800 g for 1 h at 4° C, the supernatant was collected and designated as *C. tenuicollis* membrane antigen (CTMAg). Following protein estimation, CTMAg was stored in 2 ml aliquots at –20° C till further use.

Naturally infected goat sera: Blood samples from both *C. tenuicollis* positive and negative cases were collected from

the slaughterhouse of small ruminants, Supela, Durg (C.G.). Sera were separated and stored in sterilized vials at –20° C for further use.

Raising of hyper immune sera: Hyper immune sera against membrane antigen were raised both in parasite free rabbits and kids. For this, 2 New Zealand white rabbits and 3 healthy Jamunapari desi cross kids were selected. Sera were collected in the sterilized storage vials and stored at –20° C.

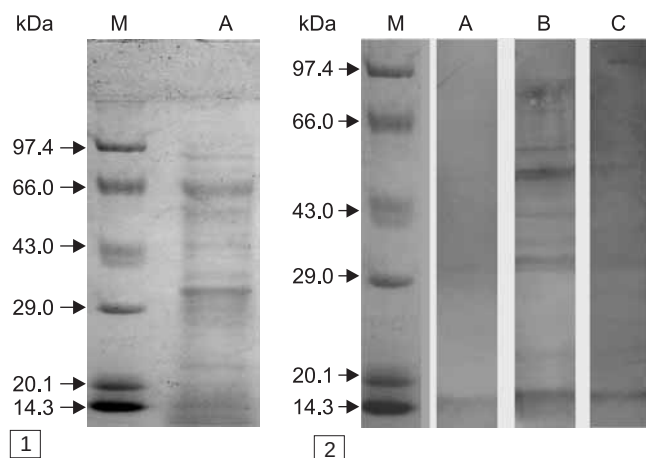
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE): The cystic membrane antigen was characterized by 12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis using a vertical mini slab gel electrophoresis system as per Laemmli (1970) with slight modification. The protein of 50 µg was charged in 4 lanes along with the molecular weight markers ranging from 97.4 to 14.3 kDa, in a single lane. Electrophoresis was run at a constant potential difference of 50 volts. After completion of running, the gel was removed carefully and a portion containing marker and 1 antigen lane was separated off and stained with Coomassie Brilliant blue R-250 while other portion was left for western blotting. The molecular weight (MW) of different polypeptides of membrane antigen was determined according to Shapiro *et al.* (1967) by plotting the MWs of standard markers on a semi logarithmic paper against their relative mobility (R_f).

Western blotting: The electrophoretic transfer of membrane proteins on nitrocellulose paper (NCP) was performed at 0.05 ampere for 3 h. The NCP was removed after electrophoretic transfer and was cut according to each lane after washing with PBS/Tween-20 (0.05%). Thereafter, all strips were immersed in blocking solution containing 3% bovine serum albumin (BSA) in PBS/T and incubated at 37° C for 2 h. The strips were then washed 4 times of 5 min each with washing solution. Then the strips were immersed in serum samples diluted at 1: 50 in PBS/T with BSA (3%) and incubated for 2 h at 37° C. The strips were given 4 washings and again incubated for 1 h at 37° C with anti-species HRPO conjugate at a 1: 1000 dilution in PBS/T. Finally, all strips

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Present address: ¹Teaching Associate (e mail: sanyalpk@rediffmail.com), College of Veterinary Sciences and Animal Husbandary, Jabalpur, Madhya Pradesh 482 001.

^{2,4}Associate Professor, ³Professor and Head, Department of Parasitology, College of Veterinary Sciences and Animal Husbandary.



Figs 1-2. 1. SDS-PAGE of *C. tenuicollis* cystic membrane (A) on coomassie brilliant blue staining. Lane 'M' indicating the molecular weight marker. 2. Western blotting of *C. tenuicollis* membrane antigen with the experimentally and naturally infected sera. A, Naturally infected goat serum; B, rabbit anti-membrane serum; C, goat anti-membrane serum.

were washed as before and immersed in substrate solution till the bands appeared. Immediately thereafter, the strips were immersed in distilled water to avoid excessive background color and dried. The immunodominant bands were identified and calibrated based on standard molecular weight marker.

The SDS-PAGE profiles of membrane antigens of *C. tenuicollis* cyst in the 12.5% gel are shown in Fig. 1. The cystic membrane antigen revealed 15 polypeptides bands having MW of 10–131 kDa (lane A). Out of 15 polypeptides, 10 peptides were showing major bands with the molecular weights (MW) of 90, 66, 58, 44, 38, 34, 31, 22, 14 and 10 kDa. The result was closer to the findings of Mathur *et al.* (2003) who reported 22 bands in cystic membrane. The slight difference may be due to the host variation as they used the antigens of *C. tenuicollis* of sheep origin.

As *C. tenuicollis* cyst membrane antigen was immunoblotted by using sera from naturally and experimentally infected goat and rabbit. The bands of 34 and 14 kDa were observed in the nitrocellulose membrane in the naturally infected goat serum (Fig.2; lane A), while the bands of 90, 66, 58, 44, 38, 34, 31 and 14 kDa were noticed in the anti-membrane rabbit serum (lane B) and 66, 58, 44, 34 and 14 kDa in the anti-membrane goat serum (lane C). Above observation indicated that the bands of 34 and 14 kDa were common and would be considered as parasitic protein components. The 66 and 44 kDa bands were found in the experimentally infected goat serum but absent in the naturally infected goat serum may be due to their lack of exposure to immune system of the host. The components of 90, 38 and 31 kDa observed in the rabbit membrane serum and absent in the goat sera, might be of host origin.

Although, the immunoreactive components of membrane antigen of *C. tenuicollis* cyst of goat origin were investigated, using Western blotting but Kara and Doganay (2005) reported 5 protein bands of 26, 38, 42.5, 66 and 116 kDa in the *C. tenuicollis* cyst fluid antigen of sheep origin when immunoblotted with dog sera monospecifically infected with *Taenia hydatigena*. They found the proteins of 38 and 42.5 kDa as specific for the diagnosis of *T. hydatigena*. Burgu *et al.* (2000) reported 5 protein bands in the sheep hydatid cyst fluid out of which 58 and 38 kDa were common with our findings. It may indicate that these proteins are common to other helminth parasites, nonspecific and cannot be used for the diagnosis hepatitis cysticercosis. Therefore, it is concluded that the proteins of 34 and 14 kDa may be specific for their use in diagnosis of *Taenia hydatigena* cysticercosis. However, it requires further studies to establish the above findings.

SUMMARY

The SDS-PAGE profiles of membrane antigens of *C. tenuicollis* revealed 15 polypeptides bands having MW of 10–131 kDa. Immunoblotting results revealed bands of 66, 58, 44, 34 and 14 kDa on the nitrocellulose membrane in the anti-membrane goat hyperimmune serum, while the bands of 90, 66, 58, 44, 38, 34, 31 and 14 kDa were noticed in the rabbit anti-membrane hyperimmune serum and 34 and 14 kDa were found in the naturally infected goat serum. Thus bands of 34 and 14 kDa being common could be explored for the diagnosis of *Taenia hydatigena* cysticercosis in goat.

REFERENCES

- Abidi S M A, Nazami W A, Khan P, Ahmad M and Irshadullah M. 1989. Biochemical characterization of *Taenia hydatigena* cysticerci from goats and pigs. *Journal of Helminthology* **63**: 333–37.
- Burgu A, Doganay A, Gonenc B, Sarimehetoglu H O and Kalinbacak F 2000. *Turkey Journal of Animal Science* **24**: 493–500.
- Kara M and Doganay A. 2005. Investigation of antigenic specificity against *Cysticercus tenuicollis* cyst fluid antigen in dogs experimentally infected with *Taenia hydatigena*. *Turk Veterinerlik ve Hayvanlık Dergisi* **29** (3): 835–40.
- Laemmli U K. 1970. Cleavage of structural proteins during the assembly of the head Bacteriophage T4. *Nature* **227**: 680–85.
- Mathur V C, Aiyasami S S, Latha B R, Lalitha J and Jhon L 2003. Western bolt analysis of *Cysticercus tenuicollis* antigens. *Indian Journal of Animal Sciences* **73** (8): 837–39.
- Pathak K M and Gaur S N. 1982. The incidence of adult and larval stage *Taenia hydatigena* in Uttar Pradesh, India. *Veterinary Parasitology* **10**: 91–95.
- Shapiro A L, Vinuela E and Maizel J V. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS polyacrylamide gels. *Biophysics and Biochemical Research Communication* **28**: 8–15.