

## Serodiagnosis of *Taenia solium* cysticercosis in pigs by indirect haemagglutination test

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**SELVAM, P., P. E. D'SOUZA, M. S. JAGANNATH: Serodiagnosis of *Taenia solium* cysticercosis in pigs by indirect haemagglutination test. Vet. arhiv 74, 453-458, 2004.**

### ABSTRACT

The indirect haemagglutination test (IHAT) was evaluated for the detection of circulating antibodies in serum samples obtained from pigs naturally infected with *Taenia solium* cysticercosis. A sensitivity of 85.71 per cent and 80.95 per cent, respectively, was found with the whole cyst antigen, and antigen B and 19.75 and 15.43 per cent of pigs which were not detected during meat inspection but which were probably lightly infected or previously exposed to the disease, were also detected by this test with the two antigens viz., WCA and Ag-B, respectively.

**Key words:** *Taenia solium*, cysticercosis, pigs, indirect haemagglutination test, antigens

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### Introduction

*Taenia solium* cysticercosis is one of the most common infections in pigs, with a significant zoonotic and economic impact. *Cysticercus cellulosae* is commonly found in skeletal muscles, tongue, diaphragm, heart and other organs, including brain and eye. This infection cannot be detected easily in live animals and the conventional meat inspection technique is also known to fail in the detection of mildly infected slaughtered pigs (D'SOUZA and HAFEEZ, 1999). Diagnosis of this occult infection is therefore largely dependent on serological tests. MAHAJAN et

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al. (1995) reported that IHAT was useful in the detection of neurocysticercosis in man. Hence, this study was undertaken to evaluate IHAT in the diagnosis of *Taenia solium* cysticercosis in pigs. The indirect haemagglutination test (IHAT) was evaluated in the diagnosis of *T. solium* cysticercosis in pigs with two different antigens, viz., whole cyst antigen (WCA) and antigen - B (Ag-B), a partially purified antigen in the present study.

### Material and methods

Blood samples were collected from 507 pigs at the KMPMCL slaughterhouse, Bangalore, of which 21 samples were from pigs positive for *T. solium* cysticercosis on meat inspection (Group I), and was considered as known positive. Group II comprised 19 serum samples from pigs reared in an organised farm and which were negative during meat inspection. Group III included the remaining serum samples, which were from pigs negative on meat inspection. Animals in Groups I and III consisted of pigs reared employing the free range system of rearing.

*Antigens.* The antigens were prepared from cysticerci obtained from the muscles and organs of naturally infected pigs. The cysts were collected in phosphate buffered saline (PBS) (pH 7.2) containing 40 mg each of kanamycin, nalidixic acid and ampicillin, along with the protease inhibitor, phenylmethylsulphonyl fluoride (PMSF).

*Whole cyst antigen (WCA)* was prepared with 10 g of the cysticerci disrupted in 20 ml volume of PBS containing 0.1 mM PMSF and 0.01 per cent sodium azide and then homogenized manually with 100 strokes in a tight fitting glass homogeniser at 4 °C. The homogenate was further subjected to sonication four times for a period of four min at 20 KHZ 1 mA, with a 30 sec cooling interval on an ice bath. The homogenate was spun at 14000 ×g for one hour at 4 °C in a refrigerated centrifuge. The supernatant was used as WCA and its protein content was estimated as per the method of LOWRY et al. (1951). It was found to be 8.59 mg ml<sup>-1</sup>.

*Antigen B.* The antigen B was prepared as per the procedure of BERTHA et al. (1982). The WCA without vesicular fluid was dialyzed against 0.5 M acetic acid (pH 2.3) for 12 hours at 4 °C. The dialysed material was centrifuged at 14000g for one hour. After estimation of the protein content of the supernatant it was adjusted to 0.5 mg ml<sup>-1</sup>. It was then precipitated with 0.86 M sodium chloride and resuspended in 0.5 M acetic acid.

*Control sera.* Hyperimmune serum was raised against WCA and antigen B in normal healthy rabbits by injecting 500 µg of protein mixed with Freund's complete adjuvant. After 8 -10 days a booster dose was given using the same antigen, with Freund's incomplete adjuvant. After three such inoculations at intervals of 8 - 10 days, the final bleeding was carried out 10 days after the final injection. The hyperimmune serum was used for standardization of the test, which was performed as per the procedure of VARMA et al. 1986.

Tanned sheep erythrocytes were sensitized with an equal volume of antigen and 3 ml of buffered saline for 15 min, then washed thrice with PBS, resuspended in 10 ml of PBS containing 0.1% BSA. A two-fold dilution of serum from 1:2 to 1:512 and 100 µl of 0.5% sensitized RBC was added and incubated at 37 °C for one h. Lattice formation was an indication of positivity, whereas a button formation was considered as negative. Antigen concentration of 5 mg/ml was found to be optimum for both antigens and was used in this test.

## Results

The undiluted WCA resulted in lattice formation in 18 out of 21 known positive serum samples up to 1:32 serum dilution with a sensitivity of 85.71 percent, whereas undiluted Ag-B produced a reaction in 17 out of 21 known positive serum samples up to 1:8 to 1:16 serum dilutions with a sensitivity of 80.95 per cent. A serum dilution of 1:32 and 1:16 with WCA and Ag-B, respectively, was considered to be optimal for the diagnosis of *T. solium* cysticercosis in pigs by IHAT.

Results of IHAT in the three different groups of sera test are presented in Table 1.

In the present study, no reaction could be observed in any of the 19 known negative samples by the IHAT with both WCA and Antigen B with a specificity of 100 per cent. Positivity of 19.75 and 15.43 per cent with WCA and Ag-B was observed respectively in serum samples collected from pigs apparently negative on meat inspection, indicating either low grade infection or previous exposure to infection. A statistically significant difference was recorded at five per cent level of significance between the two different antigens in the IHAT.

Table 1. Indirect haemagglutination test to detect *Taenia solium* cysticercosis in pigs

Group	Number tested	Number positive	
		Whole cyst antigen	Antigen - B
Group I - Known Positive (free range)	21	18 (85.71)	17 (80.95)
Group II - Known negative (farm reared)	19	0 (0.00)	0 (0.00)
Group III - Serum found negative on meat inspection (free range)	486	96 (19.75)	75 (15.43)

Figures in parentheses indicates percentage values; Chi-square value = 0.644; \*\*  $P \leq 0.05$

## Discussion

Agglutination tests are more sensitive than other simple tests, such as the precipitation test, for the detection of antibodies. By absorbing soluble antigens to the surface of carrier particles it is possible to convert precipitation into agglutination reaction, which is more convenient and sensitive and is also known as the passive agglutination test (GOLDSBY et al., 2000). In generalized human cysticercosis, the haemagglutination test was considered to be more sensitive than either the precipitation test or complement fixation test (CFT) by BIAGI and TAY (1958). Also, the cysticercal antigen was found to be superior to adult worm antigen with regard to sensitivity.

The sensitivity of IHAT in the detection of porcine cysticercosis in the present study of 85.71 per cent with WCA was in close agreement to the observation of MAHAJAN et al. (1975) in human cysticercosis. Similarly, in the study of VARMA et al. (1986) in porcine cysticercosis, the WCA resulted in sensitivity of 86.36 per cent, which indicated that the WCA gives consistent results in IHAT, which was noticed in the present study also. SHINDE et al. (1991) reported that IHAT with scolex

antigen was 84 per cent effective in detecting cysticercosis in naturally infected pigs, but sensitivity in the present study was slightly better with WCA. Further, the preparation of this antigen was simpler.

In IHAT, the WCA, although crude in nature, gave better sensitivity than Ag-B in the present study in the diagnosis of naturally infected pigs. The lowered sensitivity of Ag-B in IHAT could be due to the elimination of vesicular fluid in its preparation, and also to a probable loss of some components in the chemical purification procedure. Despite the disadvantages, such as poor specificity and batch variability, crude antigen was considered by CRAIG et al. (1996) to be useful in immunodiagnostic screening for larval taeniid infections. However, Ag-B, a partially purified antigen, was found to be valuable and gave better results in other immunodiagnostic tests such as CIEP and ELISA (D'SOUZA, 1998) compared to the WCA.

It was concluded that the IHAT is a moderately sensitive method of serodiagnosis of *T. solium* cysticercosis in pigs and that WCA is useful in this test compared to the partially purified Ag-B.

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#### Acknowledgements

The first author is grateful for the award of Junior Fellowship by the ICAR, New Delhi. The authors thank Indian Council of Agricultural Research, New Delhi, for the research grant and the facilities made available through the Centre of Advanced Studies in Veterinary Parasitology

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Received: 11 November 2003

Accepted: 8 November 2004

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**SELVAM, P., P. E. D'SOUZA, M. S. JAGANNATH: Serološka dijagnostika cisticerkoze svinja uzrokovane vrstom *Taenia solium* testom neizravne hemaglutinacije. Vet. arhiv 74, 453-458, 2004.**

**SAŽETAK**

Istraživana je primjena testa neizravne hemaglutinacije za dokaz specifičnih protutijela u uzorcima seruma svinja prirodno invadiranih cisticerkom *Taenia solium*. Osjetljivost testa iznosila je 85,71% primjenom antigena iz cijele ciste, a 80,95% antigenom B. U svinja u kojih inspekcijom mesa nije bila otkrivena cisticerkoza, jer su vjerojatno bile slabo invadirane ili su ranije bile izložene bolesti, osjetljivost je iznosila 19,75% uporabom antigena iz cijelih cista i 15,43% s antigenom B.

**Ključne riječi:** *Taenia solium*, cisticerkoza, svinje, neizravna hemaglutinacija, antigeni

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