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Serodiagnosis of nasal and visceral schistosomosis in cattle by counter current immuno electrophoresis

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ABSTRACT

Counter current immunoelectrophoresis (CIEP) was evaluated for serodiagnosis of nasal and visceral schistosomosis in cattle. The whole worm antigens of *Schistosoma nasale* and *S. spindale* with protein content of 3.5mg/ml were prepared and the CIEP test was conducted. A clear cut positive band indicating positivity was observed in hyperimmune sera raised against both species. This technique was 98.62 and 99.50% sensitive in *S. nasale* and *S. spindale* infections in known positive cattle, respectively. CIEP could also detect infections in 85.36% and 92.70% of the cattle in which neither eggs nor worms could be recovered on post-mortem indicating previous exposure to infection.

Key words: *Schistosoma nasale*, *S. spindale*, serodiagnosis, counter current immuno electrophoresis, cattle

Introduction

S. nasale and *S. spindale* cause clinical and pathological manifestations even in moderate infections in cattle. These blood flukes adversely affect the health and productivity of cattle and subclinical incidence is high. Infections as high as 79.50 and 79.16% of *S. nasale* and *S. spindale*, respectively were observed in an abattoir survey in Bangalore (SUMANTH et al., 2002).

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A typical snoring sound and dyspnoea in affected animals aids in the diagnosis of nasal schistosomosis. Although clinical symptoms in cattle may be of diagnostic value for *S. nasale* infection, in the case of carrier animals such as buffaloes and sheep a specific test is required for confirmation. No attempts have been made to develop immunodiagnostic tests for nasal schistosomosis in order to confirm carrier animals or those suffering from early stages of the disease.

Natural *S. spindale* infection is not diagnosed frequently by faecal examination since direct smear and salt flotation faecal methods have poor sensitivity and eggs are often missed. Sensitivity of all faecal examination methods is found to be poor and immunodiagnosis is considered essential for correct diagnosis (AGRAWAL 2004). Certain methods, such as acid-ether, sieving and hatching methods, rectal biopsy and liver biopsy, have been evaluated but have produced varying results. Limited studies have been conducted on the immunodiagnosis of *S. spindale.* The development of a rapid, inexpensive, easy and reliable immunodiagnostic method for detection of schistosomosis is therefore highly desirable. A study was undertaken to evaluate Counter Current Immuno Electrophoresis (CIEP) in the detection of nasal and visceral schistosomosis, since encouraging results were obtained with this test in the serodiagnosis of other parasitic diseases (D'SOUZA and HAFEEZ, 1999).

Materials and methods

Antigens. Two antigens were prepared from whole worms of *S. nasale* and *S. spindale* as per the procedure of PRESTON and DUFFUS (1975) for *S. bovis* with modifications. The worms were collected from nasal cutting and mesenteries of slaughtered cattle in phosphate buffered saline containing 40 mg each of Kanamycin, Nalidixic acid and Ampicillin with protease inhibitor Phenylmethylsulphonyl fluoride (PMSF). The whole worm antigen (WWA) was prepared with ten g. of *S. nasale* or *S. spindale* worms taken in a 20 ml volume of PBS containing 0.1mM PMSF and 0.01% sodium azide, and then homogenised manually in a tight fitting glass homogeniser at 4 ^{0}C. The homogenate was subjected to sonication 4 times at an interval of 2-3 seconds at 20 KHz 1mA for 90 seconds on an ice bath. The homogenate was spun at 13000 g for 60 minutes at 4 ^{0}C in a refrigerated centrifuge. The supernatent was used as WWA and the protein content was estimated by the method of LOWRY et al. (1951) and adjusted to 3.5 mg/ml for both species.

A total of 640 serum samples were collected from cattle slaughtered at the Karnataka Meat and Poultry Marketing Corporation Limited, Slaughterhouse, Bangalore. Nasal scrapings and cuttings, intestinal scrapings and mesenteric cuttings, were examined to ensure that they were positive by worm and egg detection. Eighty-two and 96 of 320 serum samples belonged to cattle negative by worm and egg detection for *S. nasale* and *S. spindale*, respectively. Forty serum samples collected from twenty cattle kept in confinement in organised farms with no history of schistosomosis or exposure to snails, and negative for eggs on microscopic examination of nasal discharges and faecal samples, served as negative control for each species (Table 1).

Table 1. Particulars of cattle serum samples used in serodiagnosis of schistosomosis

Animal group	Status of infection	*S. nasale*	*S. spindale*
I	Positive by worm and egg detection in slaughtered cattle	218	204
II	Negative by egg and worm detection in slaughtered cattle	82	96
III	Healthy control sera from farm animals	20	20
Total		320	320

Hyperimmune serum against whole worm antigen was raised in 2 healthy adult New Zealand White rabbits for *S. nasale* and *S. spindale* antigens, respectively, by injecting subcutaneously1.5 ml of antigen (500 μg of protein) mixed with an equal volume of Freund's complete adjuvant. Four further weekly injections were given with antigen, mixed with Freund's incomplete adjuvant.

The procedure for the CIEP test was as per the method of D'SOUZA and HAFEEZ (1999) using whole worm antigen of either *S. nasale* and *S. spindale* with corresponding hyper immune serum for evaluation of the test, and later with serum from known positive or negative animals.

Results

The CIEP test performed with hyperimmune sera of both species gave clear cut bands. In Group I, which comprised cattle confirmed as positive based on egg and worm detection, the test showed positivity in 212 (97.24%) and 200 (98.03%) of

218 and 204 animals infected with *S. nasale and S. spindale*, respectively (Table 2). The serum samples of 173 cattle infected with *S. nasale* of 215, and 151 out of 203, for *S. spindale* revealed one precipitin band, and 42 of 215 for *S. nasale*, and 52 of 203 for *S. spindale* revealed two to three bands respectively, indicating an overall sensitivity of 98.62% and 99.50%, respectively.

Table 2. Detection of *S. nasale* and *S. spindale* infections in cattle by CIEP

Group Nº	Nº of serum samples tested		Nº and % positive		Nº and % negative	
	S. nasale	*S. spindale*	*S. nasale*	*S. spindale*	*S. nasale*	*S. spindale*
I	218	204	212 (97.24)	200 (98.03)	6 (2.75)	4 (1.96)
II	82	96	70 (85.36)	89 (92.70)	12 (14.64)	7 (7.3)
III	20	20	-	-	20 (100)	20 (100)
Total	320	320	282 (88.12)	289 (90.31)	38 (11.87)	31 (9.68)

S. nasale $\chi^2 = 166.30$, $P \leq 0.01$; *S. spindale* $\chi^2 = 201$, $P \leq 0.01$

In the serum samples of cattle in which neither eggs nor worms were observed (Group II) 70 (85.36%) and 89 (92.70%) were positive by CIEP, respectively, for *S. nasale* and *S. spindale* in which 51 and 62 showed a single band and 19 and 27 showed two to three bands. All the sera (Group III) from cattle reared in a confined farm were negative. Chi-square values indicated a significant difference at $P \leq 0.01$ levels. Sensitivity was 97.24% and 98.03% in Group I, and 85.36% and 92.70% in Group II, respectively.

In some cases, when the band showing positive reaction was feeble, the visibility of the precipitin band was clearer after the slide was kept at 4 ^{0}C for three hours. However, only when a clear semi-lunar precipitin band was visible was it considered as positive.

Discussion

CIEP was first designed to detect hepatitis-associated antigen in man and was used for diagnosis of a variety of infectious diseases for antibody detection, such as amoebiosis trichinosis, filariasis, cysticercosis and fascioliosis. This test had also been evaluated in human schistosomosis (AKHIANI et al., 1988; FERNANDES et al., 1988; MADWAR et al., 1988; MALKED et al., 1988) but no attempt was made to detect animal schistosomosis using this technique.

CIEP detected 285 and 292 cases for *S. nasale* and *S. spindale* in the present study out of 300 samples each. None of the 20 control serum samples revealed any bands indicating that CIEP is sensitive, and a specific test in detecting animal schistosomosis similar to human schistosomosis. CIEP with whole WWA with some samples showed one band, whereas a certain percentage showed 2-3 bands. This could be due to the fact that WWA was a crude antigen and that it is more complex in nature. It is also speculated as to whether it was due to relationship in severity or stage of infection. However, MADWAR et al. (1988) compared CIEP, ELISA and immuno diffusion test for antibody detection. Of 34 cases passing *S. mansoni* eggs and 26 controls the test detected antibodies in 33.23 and 15 cases and antigens in 15, nine and six cases, respectively. They concluded that ELISA was more sensitive than CIEP and ID test for antigen and antibody detection.

HUEY-RV HWU et al. (1978) conducted CIEP to detect antibodies to *S. japonicum* with a soluble egg antigen in 118 sera from people living in an endemic area in the Philippines. The sera were also tested for antibodies by circum oral precipitation test (COPT). Fifty three percent were found positive by CIEP and 48% by COPT. No significant differences were found between the tests. Correlation with the presence of eggs in the stools was considered good, despite the small number of stool samples that were positive. ASSMAR et al. (1991), HILLTER (1976) and YOUSSEF et al. (1991) conducted CIEP for human fasciolosis using crude *Fasciola hepatica* antigen and copro antigens and concluded that the assay is simple, rapid, sensitive and specific for diagnosis. In the present study whole worm *S. nasale* and crude *S. spindale* antigens were used to detect antibodies in CIEP and they gave good results, which is in agreement with the above workers in being simple, rapid, sensitive and specific for the diagnosis of early as well as established infection.

D'SOUZA and HAFEEZ (1999) reported that CIEP was 86-96 and 80-100 percent sensitive and specific with three different antigens in detection of *C. cellulosae* infection in pigs. CIEP also detected 26-36% infections in free-range reared pigs which could not be diagnosed on routine meat inspection. In the present study the whole worm antigen detected *S. nasale* and *S. spindale* infection with a sensitivity of 97.24 and 98.03%, respectively. CIEP also detected 85.36% and 92.70% infection of *S. nasale* and *S. spindale*, respectively, which could not be diagnosed by routine observations during carcass dressing but indicating previous exposure to infection.

From the above observations it can be concluded that CIEP is advantageous since very small amounts of antigen with rapid electrophoretic mobility are required. It also detected 85.36 and 92.70% infection of *S. nasale* and *S. spindale* of infected cattle that were considered negative on dressing of carcass by detection of worms and eggs. The test could also be considered for ante-mortem diagnosis of schistosomal infection with a high degree of reliability.

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References

AGRAWAL, M. C. (2004): Problems of human schistosomosis in India - status and strategy. Abstracts and Programme, 3rd Global meeting on Parasitic Diseases, Bangalore, India, PL 1, 2-3.

AKHIANI, A. A., L. A. NILSSON, ONCHTERLONGO (1988): Comparative antigen analysis of different life stages of *S.mansoni* by crossed immuno electrophoresis. Int. Arch. Aller. Appl. Immunol. 95, 266-272.

ASSMAR, A., AMIR-KHAINI, D. YADEGARI, K. FORGHAN PARAST, H. NAHRAVANIAN, N. PIAZAK, A. ESMAYLL, Z. HOVASVESIANI (1991): Sero-epidemiological investigations of fascioliasis in Northern Iran. Med. J.Islamic Rep. Iran 5, 23-27.

D'SOUZA, P. E., M. D., HAFEEZ (1999): Detection of *C. cellulose* infection in pigs by counter current immuno electrophoresis. Int. J. Anim. Sci. 14, 215-219.

FERNANDES, M., I. IRUIEGUL, D. M. C. LIMA, C. P. ABRANTES, M. S. CRESSONI, C. H. CHRISTO, L. SILVA, C. TAKIGUTIC (1988): Detection of circulating polysaccharide antigen of *S. mansoni* in hamster sera by counter current immuno electrophoresis. Revista do instituto de medicina tropical de Sao Paulo 30, 72-78.

HILLTER, G. V. (1976): Immuno diagnosis of human fascioliasis by counter current immunoelectrophoresis. J. Parasitol. 62, 1011-1013.

HUEY-RV HWU, T. C. BANZON, J. H. CROSS (1978): Counter current immuno electrophoresis in detection of antibodies to *S. japonicum.* Amer. J. Trop. Med. Hyg. 27, 276-280.

LOWRY, O. H., N. J., ROSENBROUGH, A. C., FARR, R. J. RANDALL (1951): Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265-275.

MADWAR, M. A., EL-TAHAWAI, A. M. FARGHALT (1988): Immuno diffusion, Counter current immuno electrophoresis and ELISA for detection of circulating schistosomal antigen and antibodies. J. Egyptian Soc. Parasitol. 8, 411-418.

MALKED, M. K., N. H. MOHAMMED, S.A. SOFFAR, M. M. EL-SIBAE, K. KHALED (1988): Double counter current immuno electrophoresis in detecting circulating parasitic antigens and antibodies in Schistosomiasis. J. Egyptian Soc. Parasitol 18, 361- 66.

PRESTON, J. M., W. P. H. DUFFUS (1975): Diagnosis of *S. bovis* infection in cattle by an Indirect haemagglutination test. J. Helm. 49, 9-17.

SUMANTH, S., P. E. D'SOUZA, M. S. JAGANNATH (2002): The status of nasal and visceral schistosomosis in cattle based on an abbatoir study in Bangalore, South India. Sci. Tech. Rev. Off. Int. Epi. In press.

YOUSSEF, F. G., N. S. MANSOUR, A. G. AZIZ (1991): Early diagnosis of human fascioliasis by detection of copro antigens using CIEP. Trans. Royal Soc. Trop. Med. Hyg. 85**,** 383-384.

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SAŽETAK

Istraživana je primjena imunoelektroforeze (CIEP) u serološkoj dijagnostici nazalne i visceralne shistosomoze u goveda. Pripremljeni su antigeni cijelih parazita vrsta *Schistosoma nasale* i *S. spindale* s koncentracijom proteina od 3,5 mg/ml i proveden je CIEP test. Ustanovljena je jasna pozitivna vrpca s hiperimunim serumima za obje vrste ukazujući na pozitivan nalaz. Osjetljivost testa iznosila je 98,62% za vrstu *S. nasale* i 99,50% za vrstu *S. spindale* u pozitivnih goveda. Testom CIEP dobiven je također pozitivan nalaz u 85,36% i 92,70% goveda u kojih obdukcijom nisu nađena jaja ni paraziti što ukazuje na prijašnju izloženost invaziji.

Ključne riječi: *Schistosoma nasale*, *S. spindale*, serološka dijagnostika, CIEP, govedo