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RESEARCH ARTICLE

EVALUATION OF MODIFIED AGGLUTINATION TEST IN DETECTION OF ANTIBODIES AGAINST *SARCOCYSTIS CRUZI* AND *SARCOCYSTIS HIRSUTA* OF CATTLE

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ABSTRACT

Performance of Modified Agglutination Test (MAT) in serodiagnosis of *Sarcocystis cruzi* (*Sarcocystis bovicanis*) and *Sarcocystis hirsuta* (*Sarcocystis bovifelis*) in cattle was evaluated with reference to agar gel precipitation test (AGPT), counter immunoelectrophoresis (CIEP) and indirect haemagglutination test (IHAT). MAT performed in rabbit sera hyperimmune to crude antigens of *S. cruzi* and *S. hirsuta* showed positive result against homologous and heterologous antigens similar to those observed in AGPT, CIEP and IHAT. Formalin killed whole bradyzoites of *S. hirsuta* used as antigen also detected antibodies in sera of 88.33% open grazing cattle by MAT while similar *S. cruzi* antigen could detect antibodies in 81.25% of tested sera samples. Crude soluble antigens of *S. hirsuta* coated on tanned sheep red blood cells detected antibodies to *Sarcocystis* in 85% of tested sera of cattle against 82.14% detection with *S. cruzi* antigen in IHAT. However, the AGPT and CIEP results were negative when employed in field sera of cattle. The results of MAT and IHAT in hyperimmune rabbit sera and cattle sera were comparable with evidence of cross reactivity between the two parasite species. MAT thus proved reliable and as effective as IHAT for seroprevalence study of *Sarcocystis* infection.

INTRODUCTION

Three species of *Sarcocystis* namely, *Sarcocystis cruzi* (*S. bovicanis*), *Sarcocystis hirsuta* (*S. bovifelis*) and *Sarcocystis hominis* (*S. bovihominis*) during their life cycle involve cattle as the intermediate host and dog, cat and man respectively as their definitive hosts. These parasites although considered generally nonpathogenic, *S. cruzi* in heavy infection can result abortion, reduced milk production, loss of weight and even death in cattle and *S. hominis* appears to bear zoonotic importance. The sources of infection for cattle are grazing land, feed and water contaminated with infective faeces of the definitive hosts. Previous studies on *Sarcocystis* in India point to a high prevalence in cattle (Dafedar *et al*., 2011; Chabra and Samantaray, 2013) which are frequently exposed to infections with *S. cruzi* and *S. hirsuta* of dog, cat and even wild carnivores. Diagnostic methods employed to detect these infections in cattle are light microscopy, molecular testing and serology. However parasite stages may escape microscopic and PCR diagnosis which are even cumbersome and are not an option in ante mortem diagnosis. Thus serology offers a valuable alternative in which a good number of methods are being used during clinical and epidemiological investigations (Pandit *et al*., 1996). Modern serological tests like indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbant assay (ELISA) are generally preferred (Dubey *et al*., 1989) to the conventional ones for being more sensitive and specific. However, literature on the use of tests like modified agglutination test (MAT) and latex agglutination test (LAT) for diagnosis of *Sarcocystis* infection are very scanty. Therefore the present investigation was undertaken to know whether the MAT could be used in serodiagnosis of *Sarcocystis* infection in cattle.

MATERIALS AND METHODS

For detection of serum antibodies against *S. cruzi* and *S. hirsuta*, Modified agglutination test (Dubey and Desmonts, 1987) was performed with reference to Agar gel precipitation (AGPT), Counter Immunoelectrophoresis (CIEP) and Indirect haemagglutination test (IHAT). The tests were first standardized in hyperimmune sera prepared in rabbits against crude soluble antigens of *S. cruzi* and *S. hirsuta* recovered from the heart and oesophagus of cattle during slaughter at a local abattoir. Subsequently the tests were extended to sera samples from blood obtained during slaughter from 60 open grazing indigenous cattle at the abattoir. AGPT and CIEP were performed according to the methods described elsewhere (Hudson and Hay, 1989; Talwar and Gupta, 1992) using the two antigens against hyperimmune rabbit sera and cattle sera in

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homologous and heterologous manner. IHAT was performed using tanned sheep erythrocytes coated with *S. cruzi* and *S. hirsuta* antigens similarly against hyperimmune rabbit sera and cattle sera as per Lunde and Fayer (1977). For evaluation of MAT, formalin killed whole bradyzoites of *S. cruzi* and *S. hirsuta* at a concentration of $2x10^4$ parasites/µl were prepared as per the method described by Dubey and Desmonts (1987) and used as antigens. The test was performed in homologous and heterologous manners in U bottomed well microtitre plates using 25 µl of antigen and equal amount of serum (rabbit/cattle) in 2-fold dilutions starting from 1:25. The plates were covered with sealing tape and incubated at 37° C overnight. The tests were read against negative controls. A clear-cut button shaped blue deposition at the bottom of the well was interpreted as negative reaction while presence of a complete carpet of agglutinated organisms was considered as positive. The results of MAT were compared with those of other tests performed.

RESULTS AND DISCUSSION

Results of the serological tests conducted in the present study are summarized in Table 1. Hyperimmune rabbit sera collected on 21st day of immunization showed a faint precipitation line in AGPT and CIEP conducted in homologous and heterologous manners. The precipitation line observed was more sharp and distinct with the sera collected on 31st day of immunization. However, the tests were found negative when conducted with sera of open grazing cattle similar to the negative control sera of rabbit collected before immunization. Presence of precipitating antibody in hyperimmune rabbit sera as observed in the present study is in conformity with the reports made by Reddy *et al*. (1990) and Juyal *et al*. (1990). However, results of open grazing cattle sera are not in agreement with Saleque (1990) who observed precipitating reaction in field sera obtained from buffaloes.

Table 1 Homologous and heterologous antibody response to *Sarcocystis hirsuta* and *Sarcocystis cruzi* in hyperimmune rabbit sera (HIS) and test sera of cattle

Serum	Antigen	Result / Antibody titre in			
		AGPT	CIEP	IHAT	MAT
HIS against *S. hirsuta* (rabbit)	*S. hirsuta*	+	+	1:12800	1:3200
	S. cruzi	+	+	1:3200	1:1600
HIS against *S. cruzi* (rabbit)	*S. hirsuta*	+	+	1:12800	1:3200
	S. cruzi	+	+	1:12800	1:3200
Open grazing cattle sera	*S. hirsuta*	-	-	1:100 – 1:12800 (85.00%)	1:100 – 1:3200 (88.33%)
	S. cruzi	-	-	1:100 – 1:12800 (82.14%)	1:100 – 1:13200 (81.25%)

Total no. of open grazing cattle sera examined for *S. hirsuta* antibody = 60.
Total no. of open grazing cattle sera examined for *S. cruzi* antibody = 28.
Figures in parentheses indicate percentage of antibody positive.

The IHAT performed in hyperimmune rabbit sera showed 1:12800 antibody titre against homologous antigens. Heterologously, rabbit sera hyperimmune to *S. cruzi* also showed 1:12800 titre with *S. hirsuta* antigen, but the *S. cruzi* antigen showed 1:3200 titre in the serum of rabbit hyperimmune to *S. hirsuta*. The test when employed with sera of open grazing cattle showed 85% positivity against *S. hirsuta* antigen and 82.14% positive against *S. cruzi* antigen. Reddy *et al*. (1990) using conventional diagnostic tools reported IHAT as the most sensitive, reliable, economical and less time consuming test that detected 92.5% buffaloes positive for antibodies to *Sarcocystis*. The present findings are also in conformity with the reports of Lunde and Fayer (1977) and Dubey *et al*. (1989) who recommended IHAT useful for diagnosis of bovine sarcocystosis.

In MAT, *S. hirsuta* whole bradyzoite antigen showed 1:3200 antibody titre in homologous and heterologous hyperimmune rabbit sera. *S. cruzi* antigen also showed similar titre in homologous sera but a lower antibody titre of 1:1600 in sera of rabbit hyperimmune to *S. hirsuta*. Sera of 88.33% open grazing cattle tested antibody positive against *S. hirsuta* antigen and 81.25% positive against *S. cruzi* antigen. Though no studies regarding the use of MAT in diagnosis of *Sarcocystis* infection have been previously carried out, the conventional agglutination test conducted by several workers (Achuthan,1986) were unsuccessful except Michael *et al*. (1979) who found slide agglutination test highly sensitive when employed using lyophilized antigen in sera of buffaloes positive to macroscopic sarcocysts of *S. hirsuta*. The MAT has been proved to be most sensitive and reliable in serodiagnosis of *Toxoplasma* infection (Devada *et al*., 1998, Sucilathangam *et. al*., 2012). In the present study this test employed in sero-diagnosis of *Sarcocystis* infection in cattle also gave encouraging result as evidenced by 92% and 12.30% prevalence of *S. cruzi* and *S. hirsuta* respectively observed in a parasitological survey conducted in cattle of Assam (Kalita, 2003). Antibody titres observed in all the tests conducted in heterologous manner might suggest presence of cross reactivity between the two species as was reported earlier (Moon, 1987; Savini *et al*., 1994). This further proved that *Sarcocystis* antigens are not species specific. On the other hand sero-prevalence record of *S. cruzi* (85-88%) slightly lower than the parasitological prevalence might suggest waning of antibodies due to chronicity of infection. The antibody titres in MAT observed in the present study were comparatively lower than that of IHAT and this might be due to the use of crude soluble antigen in IHAT and whole bradyzoite antigen in MAT. However, the seropositive results of MAT were found comparable to those of IHAT. It is therefore concluded that MAT can be used in seroprevalence study of *Sarcocystis* infection in cattle similar to its use in detection of *Toxoplasma gondii* antibodies (Kalita and Sarmah, 2015).

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