EVALUATION OF SEROLOGICAL CROSS-REACTIVITY BETWEEN SARCOCYSTIS HIRSUTA (SARCOCYSTIS BOVIFELIS) AND TOXOPLASMA GONDII

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ABSTRACT
Serological cross-reactivity between Sarcozystis hirsuta (Sarcocystis bovifelis) and Toxoplasma gondii was investigated by means of modified agglutination test (MAT) and latex agglutination test (LAT) in human serum positive to T. gondii antibody and hyperimmune rabbit serum against S. hirsuta. The tests conducted in heterologous manner did not show any agglutination. This indicated absence of serological cross-reaction between the two closely related parasite species. The results of the tests were discussed in the light of available literature.

Key Words: Sarcozystis hirsuta, Toxoplasma gondii, MAT, LAT, Serology

INTRODUCTION
Sarcozystis and Toxoplasma are two groups of cyst forming coccidia prevalent worldwide in a variety of animals and man (Dubey, 2010; Chabra and Samantaray, 2013) and have obligatory or facultative prey-predator life cycle with certain epidemiological variabilities. Infection with different species of Sarcozystis are extra ordinarily common but mostly subclinical in domestic animals and a few species of them along with Toxoplasma gondii bear a great significance due to their zoonotic potentiality. Diagnosis of infections in the intermediate host animals is made by microscopic demonstration of parasite stages in tissues, molecular detection of parasite DNA and serological analysis. The former two procedures employed during ante mortem examination may result misdiagnosis due to inhomogeneous distribution of tissue stages in various organs. Serology in this situation offers a valuable diagnostic alternative for conclusive assessment. A good number of serodiagnostic commercial kits have been made available in the market for diagnosis of T. gondii infection in man. Of these, agglutination tests with comparable sensitivities are applicable for diagnosis in man and animals since they have additional advantage of not requiring species specific conjugate (Sucilathangam et al., 2012, 2013). In view of high prevalence of Sarcozystis with T. gondii infection recorded in cattle (Kalita, 2003; Sharma et al., 2008), there was a need to investigate serological cross-reactivity between the two species for possible false diagnosis during epidemiological investigation in animals which are usually infected in nature with multiple closely related and even phylogenetically distinct parasites (El-Moghazy and Abdel-Rahman, 2012). Hence the present study was undertaken to investigate serological cross reactivity between cat-borne T. gondii and S. hirsuta of cattle using modified agglutination test (MAT) and latex agglutination test (LAT).

MATERIALS AND METHODS
Hyperimmune rabbit serum positive to S. hirsuta antibody and human serum positive to T. gondii antibodies were employed in the MAT and LAT to check cross-reactivity between the two parasite species. Formalin killed whole tachyzoites of T. gondii obtained from the Animal Parasitology Institute, U.S. Department of Agriculture, Maryland, USA and formalin killed bradyzoites prepared inhouse from the macrocysts of S. hirsuta recovered from the oesophageal muscles of cattle during slaughter at a local abattoir were used as the test antigens for MAT. The test was performed in round bottomed microplates with 1:25 diluted sera as per the method described by Dubey and Desmonts (1987) in heterologous manner against positive (homologous) and negative controls. The tests were read after incubation of the plate overnight at 37°C for any visible agglutination compared to positive and negative controls. A clear cut button shaped blue deposition at the bottom of the well was interpreted as negative reaction and a complete carpet of agglutinated organisms was considered positive.

Commercial Toxogen Kit [Tulip Diagnostics (P) Ltd., Goa, India] which contained T. gondii antibody positive and antibody negative human serum, T. gondii antigen coated latex reagent was used similarly in homologous and heterologous manner in the test sera diluted 1:16 as per manufacturer’s instruction on a slide supplied with the kit. The test was read after 5 minutes for any visible agglutination against positive (homologous) and negative controls.

RESULTS AND DISCUSSION
The results of serological study are shown in Table 1. T. gondii antibody positive serum did not show agglutination reaction with S. hirsuta antigen in MAT. This conformed to the earlier negative findings observed between S. cruzi and T. gondii antibody in indirect haemagglutination test (Lunde and Fayer, 1977), slide agglutination test (Michael et al., 1979), immunodiffusion test (Saito et al., 1994) and ELISA (Opsteegh et al., 2011) despite contradictory report of cross reaction at low serum dilutions in IFAT and ELISA made by Moon (1987).
Table 1: Results of cross reactivity between sarcocystis and Toxoplasma in MAT and LAT

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Serum</th>
<th>Serological test</th>
<th>Test antigen</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rabbit hyperimmune serum against Sarcocystis</td>
<td>MAT</td>
<td>a) Formalin killed tachyzoites antigen of Toxoplasma</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LAT</td>
<td>b) Toxoplasma Latex antigen</td>
<td>Negative</td>
</tr>
<tr>
<td>2.</td>
<td>Human serum positive to Toxoplasma antibodies</td>
<td>MAT</td>
<td>a) Fomalin killed bradyzoites antigens of S. hirsuta</td>
<td>Negative</td>
</tr>
</tbody>
</table>

MAT and LAT conducted in serum positive to S. hirsuta antibody in the present investigation also showed no agglutination against T. gondii antigen. No report on the use of similar test is available to compare with the present findings, however cross reactivity between S. tenella and S. cruzi antibody positive serum and T. gondii antigen was noted in IFAT and ELISA (Moon, 1987; Uggla and Buxton, 1990; Savini et al., 1994).

Performance of conventional agglutination tests still being used in detection of T. gondii antibody is found to be comparable to modern serological and molecular methods (Opsteegh et al., 2011; Sucilathangam et al., 2012; Kalita and Sarmah, 2015). Advantages of such tests are that they do not require species specific serum or conjugate and are rapid, cost effective and can be performed easily in all laboratories. Similarly several tests have been recommended for detection of anti-sarcocystis antibody (Uggla and Buxton, 1990; Sroka et al., 2008). Recent application of MAT using formalin fixed bradyzoites of Sarcocystis on the line of MAT performed in Toxoplasma screening also revealed satisfactory results (Kalita, 2003). Serological cross reaction has a negative impact on accurate diagnosis in field cases having multiple parasitic infections (El-Moghazy and Abdel-Rahman, 2012) Present study conducted on T. gondii and S. hirsuta may not appear to cause significant disease in cattle but infection with former parasite bears a great significance from epidemiology and zoonotic point of view.

CONCLUSION

MAT and LAT offer valuable diagnostic alternative and can be applied in all laboratories for discriminating cat borne T. gondii and S. hirsuta infection in cattle.

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