

Standardization of Indirect Haemagglutination Test for Monitoring *Mycoplasma mycoides* Subspecies *capri* Antibodies Raised in Rabbits and Goats

RAHMAN, S.U., M. SIDDIQUE, I. HUSSAIN, K. MUHAMMAD† AND M.H. RASOOL

Department of Veterinary Microbiology, University of Agriculture, Faisalabad–38040, Pakistan

†University of Veterinary and Animal Sciences, Lahore–Pakistan

ABSTRACT

Indirect haemagglutination (IHA) test was standardized with certain modifications for measuring the antibodies against *Mycoplasma mycoides* subspecies *capri*. Four different concentrations of antigen were maintained at each of the protein contents of 5, 10, 15 and 20 mg mL⁻¹ for the sensitization of sheep erythrocytes. Two different concentrations of sensitized sheep red blood cells (RBCs) (1 and 2%) were also prepared to perform IHA test. Constant results of IHA antibody titers were found at 15 and 20 mg mL⁻¹ protein concentration, while variable results were recorded in lower dose of antigen. Similarly, 1% concentration of sensitized sheep RBCs showed fast and better resolved antibody titers in contrast to 2% sensitized sheep RBCs (SSRBCs), where more time was required for better resolution of weak and particularly last dilution of high antibody titer.

Key Words: Indirect haemagglutination test; Standardization; *Mycoplasma mycoides ssp. capri*; Antibodies

INTRODUCTION

Goat farming in the country provides milk, meat, skin, mohair and manure at small scale. Existing goat population of 47.4 million head (Govt. of Pakistan, 2000), still providing about 28% of the total meat demand in Pakistan. For the last many years slow progress of goat and sheep industry in the country may be due to continuous threats of respiratory problems confronting to goat and sheep population. Despite regular vaccination programme, there are still many factors, which lag behind the continuous growth and health of the goat population. One of the major factors includes the lack of proper serological test for monitoring antibody response against *Mycoplasma mycoides* subspecies *capri*.

A variety of serological tests were used to demonstrate the presence of *Mycoplasma* antibodies or to identify antigens. Most commonly employed tests include agglutination, precipitation, complement fixation, fluorescent antibody and enzyme immunoassay. Each test has its own merits and demerits, however, agglutination tests are mostly practiced. Agglutination test requires that the antigen be particulate such as bacterial cells, red blood cells and latex particles coated with antigens. Similarly, in the light of this a passive haemagglutination test was developed for the diagnosis of *Mycoplasma pneumoniae* infections in human by Ghyka *et al.* (1974). This technique led to rapid and highly specific results.

Now-a-days for routine diagnosis, a variety of serodiagnostic tests are in use. Indirect haemagglutination (IHA) and complement fixation tests (CFT) are used to assess the antibody response of goat to F-38 strain of

Mycoplasma capri. Though IHA has positive correlation with CFT, yet it is more reliable and sensitive test (Muthomi & Rurangirwa, 1983).

Keeping in view the efficacy and simplicity of this test, present study was conducted to standardize IHA test conditions for measuring antibodies against *Mycoplasma mycoides* subspecies *capri* by using four different antigen concentrations at two different SSRBCs concentrations.

MATERIALS AND METHODS

Following the method described by Hayat *et al.* (1991) IHA test was standardized with the following modifications.

Source of erythrocytes. Defibrinated sheep blood was collected and washed thrice in phosphate buffered glucose (PBG). Finally 20% suspension of washed erythrocytes was prepared in PBG and stored at 4°C for further use.

Preparation of antigen. *Mycoplasma capri* (PG3) strain was grown in PPLO broth over an incubation of 48 h at 37°C. The harvested growth was washed thrice and finally the pellet, obtained at 22,000Xg was resuspended in normal saline. The bacterial suspension was sonicated for 3 min at 200 watt (Ultrasonics Ltd, USA). The protein contents of sonicated suspension were determined by proteinometer. Four different antigen concentrations were maintained at each of the protein concentration of 5, 10, 15 and 20 mg mL⁻¹ for sensitization of erythrocytes.

Sensitization of erythrocytes. i) Equal volume of sheep RBCs were mixed with 0.2% glutaraldehyde solution (Cho *et al.*, 1976) and incubated at 37°C for 15 min.

ii) Glutaraldehyde fixed sheep RBCs (GA-SRBC) were washed thrice in sterilized N.S. containing 0.1% sodium

azide and finally resuspended in 0.01M PBS to maintain 20% suspension.

iii) Five parts of GA-SRBC were mixed with two parts of *Mycoplasma capri* (PG3) sonicated antigen (V/V) in separate glass beakers to maintain four different concentrations of antigen.

iv) Suspensions were incubated at 37°C for overnight period with occasional agitation. Later, the suspensions were washed thrice in PBS. Finally each of the suspension was separately maintained into two independent working suspensions of 1 and 2% (SSRBC). These were kept at 4°C for further use.

Indirect haemagglutination test. Hyperimmune sera were separately prepared in five rabbits and five goats according to the method described by Rahman (2001). All the serum samples were heat inactivated at 56°C for 30 min in water bath. Two fold serial dilutions of known positive serum of rabbits and goats (five each) and two known negative serum (one from each animal) were prepared in normal saline solution (Hayat *et al.*, 1991). Fixed amount of SSRBC (1 and 2%) was mixed separately with each diluted serum samples into the microtitration plates (96 wells titertekplates).

The data regarding IHA antibody titer was recorded for comparison among different test conditions.

Reproducibility of results. Hyperimmune serum of rabbits and goats were stored at -20°C in separate aliquots and IHA antibody titers were recorded for verification of reproducibility after 48 h.

RESULTS AND DISCUSSION

Antigens play an important role in the performance of IHA test. In different methods described for the preparation of antigens, Cho *et al.* (1976) first time reported a technique for preparation of *Mycoplasma* antigen. Washed cell suspension was sensitized at a protein concentration of

10 mg mL⁻¹. Later, Kirchhoff *et al.* (1982) adopted the technique in equines with the modification of using sonicated antigen for IHA and CFT. Hayat *et al.* (1991) have suggested sonicated antigen of *Mycoplasma* at protein concentration of 10 mg mL⁻¹.

It was evident from the results of present study that sonicated *Mycoplasma* antigen at the protein concentration of 5 and 10 mg mL⁻¹ showed some variable results of antibody titers in rabbit and goat sera, however, the concentration of 15 mg mL⁻¹ and above (20 mg mL⁻¹) showed consistently non-variable results of antibody titers. Results also indicated that optimum protein concentration of *Mycoplasma* was 15 mg mL⁻¹ whereas, the protein concentration below 15 mg mL⁻¹ may not be sufficient for complete coating of erythrocytes and it was further evident from the protein concentration above 15 mg mL⁻¹ where there was non significant variation in the antibody titers in IHA test as detailed in the Table I.

The final concentration of sensitized sheep RBC in the IHA test also affected its reproducibility. In the present study, it was evident that 1% concentration of SSRBC showed response within 20 min with better readability but it also showed consistently low antibody titer as detailed in Table I. This might be due to the suboptimum sensitization dose of antigen (5 and 10 mg mL⁻¹). These findings also suggested that the optimum sonicated antigen concentration of 15mg mL⁻¹ for sensitization and 1% concentration of SSRBC were the most suitable conditions for optimum reproducibility with enhanced readability of IHA antibody titers against *Mycoplasma mycoides* subspecies *capri*. Serum of rabbits and goats behaved equally good with similar optimum conditions of indirect haemagglutination test. Such optimum test conditions of IHA test may be suggested for better evaluation of antibody titres among the vaccinated rabbits and goats.

Table I. Results of IHA antibody titration in rabbit and goat known positive serum using different concentrations of *Mycoplasma mycoides* subspecies *capri* (PG3) antigen on sensitization rate and percentage of sensitized (sheep) erythrocytes

Animal Serum	Antigen concentration (mg mL ⁻¹)							
	5 mg mL ⁻¹		10 mg mL ⁻¹		15 mg mL ⁻¹		20 mg mL ⁻¹	
	Sensitized RBC concentration (percentage)							
	1%	2%	1%	2%	1%	2%	1%	2%
R1	512	512-1024	1024	1024	1024	1024	1024	1024
R2	128-512	256-512	512	256-512	512	512	512	512
R3	128-256	256	256	256	256	256	256	256
R4	16-32	32-64	64-128	128	128	128	128	128
R5	4-8	8-16	16-32	16-32	64	64	64	64
G1	512-1024	512-1024	512-1024	1024	1024	1024	1024	1024
G2	256	256-512	256	512	512	512	512	512
G3	128-256	64-128	128-256	256	256	256	256	256
G4	64-128	32-64	128-256	64-128	128	128	128	128
G5	8-16	16-32	32-64	32-64	64	64	64	64

R = Rabbit serum; G = Goat serum

ACKNOWLEDGEMENT

Authors are thankful to the Vice Chancellor for providing full funding under the University Research Promotion Fund (URPF) in the completion of the present study.

REFERENCES

- Cho, H.J., H.L. Ruhnke and E.V. Langford, 1976. The indirect haemagglutination test for the detection of antibodies in cattle naturally infected with mycoplasmas. *Canadian J. Comp. Med.*, 40: 20-9
- Ghyka, G., G. Sorodoc, N. Stoian and P. Periulescu, 1974. Serologic diagnosis of *Mycoplasma* infections by passive haemagglutination using sheep red blood cells sensitized by gluteraldehyde. *Rev. Roum. Virol.*, 25: 93-4
- Govt. of Pakistan, 2000. Economics Survey (1999-2000). Govt. of Pakistan. pp: 12-4. Fin. Div. Eco. Adv. Wing. Islamabad, Pakistan
- Hayat, O., M. Siddique and M.A. Awan, 1991. Evaluation of indirect haemagglutination and gel immuno-diffusion tests for detecting antibodies against *Mycoplasma mycoides* subspecies *capri*. *Pakistan Vet. J.*, 11: 163-6
- Kirchhoff, H., A.M. Ammar, J. Heitmann, H. Dubenkropp and R. Schmidt, 1982. Serological investigation of horse sera for antibodies against *Mycoplasma* and *Acholeplasmas*. *Vet. Microbiol.*, 7: 147-56
- Muthomi, E.K. and F.R. Rurangirwa, 1983. Passive haemagglutination and complement fixation as diagnostic test for contagious caprine pleuropneumonia caused by the F-38 strain of mycoplasma. *Res. Vet. Sci.*, 35: 1-4
- Rahman, S.U., 2001. Studies on immunoprophylaxis of contagious caprine pleuropneumonia. *Ph.D. Thesis*, Dept. Vet. Microbiology, Univ. of Agri., Faisalabad, Pakistan

(Received 11 April 2003; Accepted 25 May 2003)