Serology based immunological cross-reactivity among various isolates of *streptococcus agalactiae* from mastitic buffaloes

R.K. Dad, A. Shakoor, M. Avais, G. Muhammad, R. Hussain

University of Agriculture, Faisalabad Pakistan

Corresponding author: R. K. Dad. University of Agriculture, Faisalabad Pakistan. raikhudadad@yahoo.com

ABSTRACT: To measure the degree of immunological cross-reactivity among different isolates of *Streptococcus agalactiae* from mastitic cases of buffaloes and cows in four districts (Faisalabad, Jhang, Toba Tek Singh and Sargodha), two tests were used. The isolates of *Streptococcus agalactiae* obtained from mastitic animals from these districts were purified on Edward’s medium. Hyper immune sera were raised by injecting 1.2 x 10⁹ cfu/ml I/V of the isolates separately into rabbits. The sera thus raised were tested for *in vitro* immunological cross-reactivity through counter immuno-electrophoresis and bacterial agglutination test. Eight *Streptococcus agalactiae* isolates were selected and named A, B, C, D, E, F, G and H. In the bacterial agglutination test, all variants were similar antigenically and showed positive reactions, but a few reactions were negative, so the cross-reactivity among the variants was (93.75 %) with respect to the positive reactions. The percentage of negative reactions was (6.25 %). The cross-reactivity among the *Str. agalactiae* isolates was 98.43% by modified counter immuno-electrophoresis assay.

Key words: *Streptococcus agalactiae*, Mastitis, Serology, Immunology.

INTRODUCTION - Mastitis is an inflammatory disease which if not prevented or treated in the early phase, leads to permanent damage to mammary tissue of the infected animals. In Pakistan, field surveys of major livestock diseases have indicated that mastitis is one of the most important health hazards of the dairy industry in the country (Ajmal, 1990). Generally, well-recognized organisms responsible for mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysagalatiae*, *Streptococcus bovis*, *Corynebacterium pyogenes*, *Pseudomonas auroginosa* and *Escherichia coli* etc. (Radostits et al., 2000). *Streptococcus agalactiae* produces hyaluronidase (spreading factor) which can digest connective tissue, so it is responsible for production of more spreading factor than *S. aureus*. The present study has been designed: to see the cross reactivity among various isolates of *Streptococcus agalactiae* collected from Faisalabad, Jhang, Toba Tek Singh and Sargodha districts of Punjab province of Pakistan.

MATERIAL AND METHODS - One hundred milk samples (from buffaloes clinically positive for mastitis), twenty-five from each from Faisalabad, Toba Tek Singh, Jhang and Sargodha districts of Punjab, were collected aseptically for the isolation of *Streptococcus*
agalactiae isolates according to the National Mastitis Council Inc. (1990). Each milk sample was streaked onto blood agar and incubated at 37ºC for 24 hours. Colony characteristics and Gram’s staining did the morphological examination. The colonies that were suspected for Streptococcus agalactiae were streaked onto Edward’s medium to get pure cultures of Streptococcus agalactiae. Streptococcus agalactiae isolates were confirmed by the Catalase test, Esculin test, CAMP test and hemolytic properties on blood agar. These isolates were preserved in Trypticase soy broth (Difco Lab. Detroit, Michigan) containing 20% glycerol and stored at -20ºC for further studies.

Preparation of Streptococcus agalactiae antigen: Eight Streptococcus agalactiae isolates, i.e. A and B from Faisalabad, C and D from Toba Tek Singh, E and F from Jhang, and G and H from Sargodha, were selected and inoculated on to nutrient broth in separate flasks containing 10% sterile bubaline whey prepared by rennet precipitation of fresh defatted bubaline milk to prepare antigens. These were incubated at 37ºC for 48 hours on an orbital shaker at 60 rpm. Inactivation was done with formalin (0.4%) for 24 hours at room temperature. The inactivated Streptococcus agalactiae isolates were then harvested by centrifugation at 6000 x g for 1 hour at 4ºC. Two washings with sterile PBS (pH 7.2) were given. The isolates thus obtained were suspended in PBS. The concentration of Streptococcus agalactiae in antigenic preparation was adjusted to 1.2 x 10⁹ cells/ml by spectrophotometer.

Raising of hyperimmune sera: Eight healthy adult rabbits were selected and injected intravenously with antigens containing Str. agalactiae isolates, i.e. A, B, C, D, E, F, G and H, respectively, as described by Malik, (2001). Blood samples were collected from these rabbits seven days after the last injection to raise hyperimmune sera. Serum samples were stored at –20ºC till further use.

Cross-reactivity among Streptococcus agalactiae isolates: The cross-reactivity among various isolates of Streptococcus agalactiae was determined by bacterial agglutination test (Carmichael and Joubert, 1987) and counter immunoelectrophoresis (Obi and Patrick, 1984).

RESULTS AND CONCLUSIONS - All the eight isolates were cocci in shape, 0.5-1.5µm in size, gram positive, and non-motile. The colony size of the isolates was ranged between 0.5 and1.0mm on Edward’s medium. This medium is highly selective for Streptococcus agalactiae as it inhibits the growth of all gram negative bacteria and most gram positive bacteria due to the presence of the crystal violet. All the isolates of Streptococcus agalactiae were negative for catalase and Esculin but positive for the CAMP test (arrow head formation). These finding are congruent with those of Merchant and Packer (1983). Isolates A, B, C, E, G and H gave complete (ß) haemolysis while D and F gave partial (α) haemolysis on blood agar.

In the bacterial agglutination test, isolate A showed positive reactivity against sera of rabbits A, B, C, D, E, G and H. Isolates B, C, D, E and H gave positive reactions against sera of rabbits A, B, C, D, E, F, G and H. Isolate F gave a positive reactions against sera of rabbits A, B, C, D, E, F and G. (Table 1)
So, in this study the cross-reactivity among the *Str. agalactiae* isolates was reported to be 93.75% with respect to the positive bacterial agglutination reactions. The percentage of negative reactions was (6.25%). These findings corresponds to the findings of Margaretha et al. (2000).

In modified counter immuno-electrophoresis assay, isolates A, B, C, D, E, F and H gave positive reactions against sera of rabbits A, B, C, D, E, F, G and H. (Table 2).

### Table 1. Results of bacterial agglutination test in rabbits.

| Isolate | Hyperimmune sera of rabbit | *PC | **NC |
|---------|-----------------------------|-----|------|
| A       | + + + + + + + + + +       | +   | -    |
| B       | + + + + + + + + + +       | +   | -    |
| C       | + + + + + + + + + +       | +   | -    |
| D       | + + + + + + + + + +       | +   | -    |
| E       | + + + + + + + + + +       | +   | -    |
| F       | + + + + + + + + + +       | +   | -    |
| G       | + + + + + + + + + +       | +   | -    |
| H       | + + + + + + + + + +       | +   | -    |

+ Indicates agglutination - indicates no agglutination

*Positive control** Negative control

### Table 2. Results of Modified Counter Immunoelectrophoresis Assay in rabbits.

| Isolate | Hyperimmune sera of rabbit | *PC | **NC |
|---------|-----------------------------|-----|------|
| A       | + + + + + + + + + +       | +   | -    |
| B       | + + + + + + + + + +       | +   | -    |
| C       | + + + + + + + + + +       | +   | -    |
| D       | + + + + + + + + + +       | +   | -    |
| E       | + + + + + + + + + +       | +   | -    |
| F       | + + + + + + + + + +       | +   | -    |
| G       | + + + + + + + + + +       | +   | -    |
| H       | + + + + + + + + + +       | +   | -    |

+ formation of precipitation band - no precipitation band formation

*Positive control** Negative control

The cross-reactivity among the *Str. agalactiae* isolates was 98.43% by modified counter immuno-electrophoresis assay. The difference in percent cross reactivity of the two tests
may due to the increased sensitivity of modified counter immuno-electrophoresis assay. These findings are in complete agreement with the findings of Tahir (1998), who reported that modified counter immuno-electrophoresis is more sensitive and specific.

REFERENCES - Ajmal, M. (1990). Livestock wealth of Pakistan. In Proceedings of the 3rd Internation Congress. Pakistan Vet. Med. Assoc. Pakistan. Carmichael, L. E. and J. C. Joubert. (1987). A rapid slide agglutination test for the serodiagnosis of Brucella canis infection that employs a variant (M-) organism as antigen. Cornell Vet., 77: 3-12 Malik, B. S. (2001). A laboratory manual of veterinary microbiology II. Immunology and serology. CBS Publishers & Distributers. New Delhi, India. Merchant, I. A. and R. A. Packer (1983). Veterinary Bacteriology and Virology. 7th ed. CBS Publishers &Distributers. Delhi, India. pp: 211-222. National Mastitis Council, Inc., (1990). Microbiological Procedures for the Diagnosis of Bovine Udder Infection. National Mastitis Council, Inc., Arlington V.A., USA. Obi, T. U. and D. Patrick. (1984). The detection of peste des petits ruminatns (PPR) virus antigen by agar gel precipitation test and counter immuno-electrophoresis. J. Hygiene, 93: 579-586. Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff. (2000). Veterinary Medicine. 9th ed. W.B. Saunders Company Ltd., London, UK. Tahir, M. T. (1998). Serological studies on the pest of small ruminants using counter immunoelectrophoresis. M. Sc. Thesis. Dept. of Vet. Microbiology, University of Agriculture, Fsd. Pakistan. Margaretha S. C., A. Thomas, L. Charlotte and L. Gunnar (2000). Cross-Protection between Group A and Group B Streptococci Due to Cross-Reacting Surface Proteins. The Journal of Infectious Diseases, 182: 142-149.