Studies on \textit{Pasteurella multocida}: Indirect Haemagglutination Test for the Identification of Serological Types

Raja Farrukh Imran Kayani, Zahid Hussain Naqvi, Tariq Mahmood Chaudhry and Munazza Shauket
Department of Zoology, Government College Faisalabad (RFIK, ZHN)
Nuclear Institute for Agriculture and Biology, Jhang Road, Faisalabad (TMC, MS), Pakistan

Abstract: Haemorrhagic septicaemia (HS) is an acute, infectious disease of cattle and buffaloes, caused by a bacterium \textit{Pasteurella multocida}. All of the isolates of \textit{P. multocida} gave consistent results for nitrate reduction test, methyl red test, catalase test, indole production test and gelatin liquification test. Variable results were obtained for H2S production test. Sugar fermentation tests were uniform for sucrose, glucose, mannose, fructose and salicin but were variable for lactose and maltose. The results of IHA showed that all of the thirteen isolates agglutinated with the anti-serum raised against Roberts Type I in rabbits. It has been concluded that prevalent serotype of \textit{P. multocida} causing haemorrhagic septicemia in cattle and buffalo in Faisalabad, Pakistan is Roberts type I, which is equivalent to Carter’s type B.

Key words: Haemorrhagisepticemia, Indirect haemagglutination test, \textit{Pasteurella multocida}

Introduction
Haemorrhagic septicaemia (HS) commonly effects cattle and buffalo in Southern Europe, North, Central and East Africa, the Southern and South East Asia, including Pakistan. Morbidity due to this disease is more than 45% and mortality is 92% in diseased animals. In Pakistan HS is causing a loss of Rs. 1.8 billion rupees per annum. The prevalence of disease varies from region to region. In tropical countries, the greatest incidence is in the rainy season, although isolated cases may occur at any time during the year. It is suggested that some extraneous factor is necessary to precipitate the outbreak of disease. The causative bacterium is known as \textit{Pasteurella multocida}. It has been reported to occur normally in the respiratory tract of healthy animals. Disease appears when the resistance of the animal is lowered. The strains of \textit{P. multocida} are classified on the basis of capsular and somatic antigens. An indirect haemagglutination test (IHA) identifies five capsular groups A, B, D, E and F (Carter, 1955; Rimler and Rhoades, 1987), a gel diffusion precipitin test identifies 16 somatic types 1 to 12 (Namioka and Murata, 1961; Namioka, 1978) and less commonly used agglutination test identifies 12 somatic types 1 to 12 (Heddleston et al., 1972; Brogden et al., 1978) and less commonly used agglutination test identifies 12 somatic types 1 to 12 (Heddleston et al., 1972; Brogden et al., 1978). The serotypin of biochemically confirmed isolates are recognized as the cause of a specific disease. Haemorrhagic septicemia. Serogroup B is widely distributed but serogroup E and somatic serotype 2 (Namioka serotype 6) \textit{Pasteurella multocida} are recognized as the cause of a specific disease, Haemorrhagic septicaemia. Serogroup B is widely distributed but serogroup E and somatic serotype 2 (Namioka serotype 6) \textit{Pasteurella multocida} are recognized as the cause of a specific disease, Haemorrhagic septicaemia.

Serotyping: The serotyping of biochemically confirmed isolates was done by indirect haemagglutination test.

Preparation of hyperimmune rabbits antiserum: A 6-8 hour broth culture of the- reference strain was seeded onto casein sucrose yeast extract agar and incubated at 37°C overnight. Growth on each plate was checked for its purity by the rapid slide agglutination test. The growth was harvested by washing the plates using 2-3 ml per plate of a 0.3 percent formalinized buffered saline. The turbidity was adjusted by spectrophotometer at 640 nm corresponding to approximately 10^8 organism/ml. Two mature rabbits were inoculated by the intravenous route (I/V) with the suspension of already prepared antigen. The inoculation schedule consisted of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml volumes respectively at 4-day intervals. Seven days after the last inoculation 0.5 ml of a live 6-hour broth culture of the reference strain was injected intravenously. Rabbits were bled from the ear vein 10 days after this injection. The serum was then separated and stored at -20°C.

Preparation of antigen for IHA: To separate the capsular antigen, the bacterial suspension was given heat treatment at 60°C for 30 minutes. After heat treatment, suspension was centrifuged at 2000 rpm for 30 minutes and supernatant was collected. The optimal dilution of the antigen for the sensitization of human ‘0’ erythrocytes was determined by carrying out an antigen titration against hyper immune rabbit antiserum. The antigen dilution used...
in the IHA test was fixed as 2 haemagglutination units. Indirect haemagglutination test was performed by the procedure described by Carter (1955).

Results and Discussion
On account of multi host nature, organism is heterogeneous in its characteristics with considerable difference in host predilection, pathogenicity, biochemical activities, colonial morphology and antigenic structure. Therefore, the effective control of HS in cattle and buffalo by the use of type specific vaccine requires a comprehensive knowledge about the various serotypes of *P. multocida* prevailing in particular area.

Out of 28 blood samples *P. multocida* could be isolated from 13 samples. *P. multocida* from blood samples was difficult to grow on solid media directly as compared to broth media. Colonies on CSY agar were round sticky and were of mucoid consistency, slightly raised in the center, on blood agar non hemolytic, on MacConkey agar and citrate agar no growth could be observed. Microscopic examination revealed that the organisms were bipolar coccobacilli. With repeated sub culturing organism tend to diminish in size and became some what rounded and sometimes even lost its bipolar character. All of these observations were in agreement with those studied by Bain et al. (1982) and Wilson et al. (1984).

All the isolates fermented glucose. This has also been reported by Aslam et al. (1988). In case of sucrose and mannose all the isolates were able to ferment while salicin was not fermented by any of the isolate. These results were similar to Mohan et al. (1994). Fermentation of maltose and lactose was variable in our study, similar to the studies of Kozarev and Mamadudian (1988). All the isolates were positive for indole production test and also for catalase test but were negative for methyl red test, urease test, and gelatin liquefaction test. The similar results were obtained by Aslam et al. (1988).

Serotyping was done on the basis of capsular antigens employing ever reliable and simple method of indirect Hemagglutination test. All of the isolates showed agglutination reaction with the serum raised against Robert’s type I which showed that all of the isolates collected from Faisalabad district belonged to Robert’s type I which was thought to be equivalent to Carter’s type B. These results are also in agreement with those obtained by Ahmad and Anjum (1972) and Ajmal et al. (1985) who conducted investigation on serotypes prevalent in Pakistan. Aslam et al. (1988) proved that only Robert’s type I is prevalent in Pakistan. This study confirmed the previous findings and proved that there is only one serotype of *P. multocida* responsible for Haemorrhagic septicaemia in and around Faisalabad. Screening of prevalent bacterial strains in any particular area would be regular procedure to detect the bacterial mutation, if any in the area, because it is necessary for successful vaccination programmes for control and eradication of disease from livestock.

References


