

Somatic Serotyping and Pathogenicity Testing of *P. multocida* Strains Isolated from Layer Chickens

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Abstract: During the year 2000-2001, *P. multocida* isolated from natural outbreaks of fowl cholera were subjected to somatic serotyping for the first time in Pakistan. Serotyping was carried out by the gel-diffusion precipitin test. *P. multocida* serotype 1 predominated with the percentage of 47.62, followed by serotype 3(28.57%), 4(14.29%) and 12(09.52%). All the serotypes found highly virulent with 100% mortality after challenge by serotype 1 and 3, 80% by serotype 4 and 60% by serotype 12.

Key words: Avian pasteurellosis, Fowl cholera, *P. multocida*, Somatic serotyping, Gel-diffusion precipitin test, Pathogenicity testing

Introduction

Fowl cholera, caused by *Pasteurella multocida*, remains a disease of major economic importance despite more than 100 years of research (Rhoades and Rimler, 1991). Several systems have been used for serologic or immunologic characterization of *P. multocida* (Rhoades and Rimler, 1990). Those more commonly used have been reviewed and compared (Borgden and Packer, 1979). The system developed by Heddleston *et al.* (1972) based on a gel diffusion precipitin test is currently the system of choice. To date, 16 somatic serotypes (1-16) have been recognized, all of which have been isolated from avian hosts (OIE, 2000). Serological identification of *P. multocida* is useful for determination of the reservoir of infection and the geographical spread of this organism, recognition of the appearance of new serotypes and production of vaccines (Mushin, 1979). The purpose of the present project was to provide information on the prevalence of various somatic serotypes of *P. multocida* and their virulence, isolated from local outbreaks of FC in layer flocks in and around Faisalabad and Rawalpindi.

Materials and Methods

Cultures

A total of 21 *P. multocida* isolants were isolated from natural outbreaks of FC in layer flocks in and around Faisalabad and Rawalpindi. Identification of each isolant was based on the cultural, morphological, sugar fermentation and selected biochemical reactions (Collee *et al.*, 1989).

Serotyping procedure

The test was carried out according to the method described by Heddleston *et al.* (1972). The antigens for serotyping were heat-stable formalinized saline extracts. The specific antisera (Heddleston type 1-16) were obtained through the courtesy of Poultry diagnostic and Research centre, Georgia, USA. Five milliliters of Nobel agar was pipetted onto standard 25 × 75 mm microscope slide. Two patterns consisting of 5 wells (4 peripherally around a center well) were cut on a single slide. For one antigen two slides were used. The 16 antisera were placed in the outer wells and the antigen was placed in the central wells. The slides were incubated for 24 hours at 37°C in covered petridishes having water soaked cotton swab and examined under a magnifying glass with background illumination. A somatic serotype was determined by a precipitin line that occurred between a test antigen well and opposing well of any 16 serotypes specific antisera.

Pathogenicity test

All the identified *P. multocida* serotypes were examined for their pathogenicity in White leghorn layer chickens. Twenty-five, day-old White leghorn layer chicks were raised in isolation in the poultry house of Department of Vet. Microbiology, University of Agriculture, Faisalabad. At 4 weeks of age the chicks were divided into five groups I-V, each having 5 chicks. Chicks of group I, II, III and IV were inoculated with *P. multocida* serotype 1, 3, 4 and 12, respectively through intramuscular route at the dose rate of 10⁶ organisms while group V was kept as uninoculated control. The chicks of each group were reared in isolation and observed daily for upto seven days when survivors were slaughtered and autopsies were performed. Liver and heart blood were cultured for the isolation of *P. multocida*.

Results and Discussion

A total of 21 *P. multocida* isolants were serotyped by an agar gel immuno-diffusion test (Heddleston *et al.*, 1972). This test determines somatic serotypes based on antigenic variation in the bacterial lipopolysaccharide component. It is simpler to conduct, results in fewer untypable strains and provides information of more epizootiologic and immunologic value than other systems (Rimler *et al.*, 1989).

The serotypic distribution of isolants in the present study showed that serotype 1 predominated (47.62%), followed by serotype 3 (28.57%), serotype 4(14.29%) and serotype 12(9.52%). This is the first attempt in Pakistan that *P. multocida* isolated from natural outbreaks of FC were serotyped somatically. These results are in line with the findings of Jurga and Borkowska (2001), who serotyped 123 avian strains of *P. multocida*, and found the predominance of serotype 1 (66.6%), followed by serotype 3 (26%) and rarely occurring serotypes 5, 6, 8 and 10 (6.5%). They reported that the strain of serotype 1 occurred in poultry in the whole country. Rimler (1987) reported that *P. multocida* serotypes 1, 3 and 4 are more commonly isolated from cases of fowl cholera. Aye *et al.* (2001) found somatic serotype 1 most commonly from cases of FC in commercial turkeys.

It has been clear from the previous studies that a number of serotypes of *P. multocida* may cause fowl cholera. The antigenic heterogeneity of *P. multocida* strains accounts in part for the irregular results obtained from the use of currently available imported FC bacterins.

Pathogenicity of all the *P. multocida* serotypes (1, 3, 4 and 12) was confirmed in chickens in the present study and all the serotypes proved highly virulent with 100% mortality after challenge by serotype 1 and 3, 80% by serotype 4 and 60% by serotype 12. No death was recorded in the control group. Some of the more acute cases showed symptoms of classical fowl cholera within 72 hours with mucoïd droppings and viscous nasal and oral discharges, but otherwise general dullness and inappetence were the main signs of ill health in sick birds. When acute deaths occurred postmortem lesions of a septicæmic nature were found. In some of these cases there were pinhead necrotic foci in the liver and pericarditis. An active inflammatory reaction at the injection site was present in these acute cases. In the survivors lesions were mainly in the musculature around the inoculation site. Slight to sever erosive lesions with swelling of adjacent tissues, discolouration of the muscle, patchy hyperæmia, petechiation and the development of a fibrinous exudates on the muscle surface and between muscle groups was observed. Air-sacculites and sternal bursitis were also observed in some survivors. *P. multocida* was isolated from liver and heart blood of all the dead birds. The findings of the present study are in line with the results of Rimler (1987) who reported that *P. multocida* strains varied in virulence and those strains representing serotypes 1, 3 and 4 were the most virulent. The degree of virulence has been reported to vary depending on the host, the bacterial strain and the environmental interaction of the two (Snipes *et al.*, 1987; Hansen and Hirsh, 1989; Christensen and Bisgaard, 2000).

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