# Capsular serogroups of *Pasteurella multocida* isolated from animals in Zimbabwe

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**ABSTRACT**

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*Pasteurella multocida* is isolated from a variety of disease conditions from different animal species in our diagnostic laboratory. In order to determine serogroup distribution among the isolates, an indirect haemagglutination test using glutaraldehyde-fixed sheep red blood cells was employed. A serological examination of 79 isolates revealed that 47/79 were of capsular serogroup A, 11/79 capsular serogroup D, 4/79 capsular serogroup B and 17/79 were untypable strains. None of the isolates belonged to either serogroup E or F. All those from cases of classical pasteurellosis could be grouped, but a significantly high proportion of those which originated from companion animals were untypable. The significance of these results is discussed. This report appears to be the first detailed information on the prevalence of various serogroups of *P. multocida* in animals in southern Africa.

**Keywords:** Animal disease, capsular serogroups, *Pasteurella multocida*, prevalence

## INTRODUCTION

*Pasteurella multocida* (*sensu stricto*) represents a heterogeneous taxon comprising a cluster of a single species and subspecies, which are often associated with a wide range of disease manifestations in a variety of hosts. Efforts to assign the species and/or its subspecies to a particular taxon to determine disease significance have been undertaken by phenotypic and genotypic methods. Among the phenotypic methods used are biotyping (Mutters, Ihm, Pohl, Frederiksen & Mannheim 1985; Holst, Rollof, Larsson & Nielsen 1992; Mohan, Sadza, Madsen, Hill & Pawandiwa 1994; Fegan, Blackall & Pahoff 1995) and capsular and somatic typing (Rhoades & Rimler 1987; 1990). Molecular typing has involved DNA hybridization studies (Mutters *et al.*, 1985; Piechulla, Bisgaard, Gerlach & Mannheim 1985), DNA fingerprinting (Wilson, Morgan & Barger 1993), ribotyping (Blackall, Fegan, Chew & Hampson 1998) and random amplification of polymorphic DNA (Chaslus-Dancla, Lesage-Descauses, Leroy-Setrin, Martel, Coudert & Lafont 1996). Other typing methods, which include antibiogram typing, plasmid analysis and phage typing have been reviewed by Fussing (1998). However, capsular serogrouping has been relied upon more frequently than the others. Based on differences in the antigenic capsular polysaccharide, five capsular serogroups designated A, B, D, E and F (Carter 1955; Rimler & Rhoades 1987), have been described. We report in this paper results of capsular groups of *P. multocida* isolates and their prevalence in various animal hosts and respective disease manifestations.

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## MATERIALS AND METHODS

### Isolation and characterization of bacterial isolates

Specimens (from both sick and dead animals) were received from our veterinary teaching hospital and

from private veterinarians for bacteriological examination. Techniques for isolation and characterization of *P. multocida* (*sensu stricto*) have been described by Mohan *et al.* (1994). The isolates were stored either frozen at –70 °C or in a lyophilized state until required.

## Serotyping

Reference serotyping strains (A1113, B925, D42, E978 and F4679) and antisera were obtained from The Royal Veterinary College, London. These were kept at –20 °C until required.

Serogrouping of the isolates was performed by an adaptation of the procedure described by Sawada, Rimler & Rhoades (1982). Briefly, bacteria were streaked onto dextrose starch agar (Difco) and incubated overnight at 37 °C. Cells were harvested into 1 ml of 0,02M PBS (pH 7,2) per agar plate and suspended by vortexing. The suspensions were treated with equal volumes of PBS containing 200 U of hyaluronidase (bovine testis, Sigma) for 2 h at 37 °C. A crude capsular extract was collected following boiling the suspension for 1 h, and centrifuging at 13,000 x *g* for 20 min. The antigenic capsular polysaccharide (contained in the crude capsular extract) was immobilized onto 1 % glutaraldehyde-fixed sheep red blood cells by incubating at 4 °C for 2 h with agitation and a final 0,5 % (v/v) suspension of red blood cells was prepared in PBS containing 0,25 % bovine serum albumin (PBS-A). Two fold serial dilutions of reference antisera were individually prepared in PBS-A in V-shaped microtitre plates. Capsular antigen-coated red blood cells of each isolate were reacted with separate dilutions of reference typing antisera at room temperature. Plates were examined for haemagglutination after 1 to 3 h.

## RESULTS

The isolates included in the study originated from a variety of animal species and were mainly from cases

TABLE 1 Distribution of capsular serogroups of *Pasteurella multocida* from various disease conditions

| Disease | No. of isolates | Capsular serogroups | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | D | E | F | Untypable |
| Pneumonia | 20 | 18 | – | 1 | – | – | 1 |
| Bite wounds | 15 | 10 | – | – | – | – | 5 |
| Rhinitis/bronchitis | 10 | 3 | – | 5 | – | – | 2 |
| Septicaemia | 10 | 7 | 3 | – | – | – | – |
| Tonsillitis/colonisation | | | | | | | |
| Arthritis | 9 | 3 | – | 4 | – | – | 2 |
| Other | 4 | 2 | – | 1 | – | – | 1 |
| Meningitis | | | | | | | |
| Urogenital | 2 | – | 1 | – | – | – | 1 |
| Stomatitis | 2 | 1 | – | – | – | – | 1 |
| Abscesses | 5 | 2 | – | – | – | – | 3 |
| | 2 | 1 | – | – | – | – | 1 |
| Total | 79 | 47 | 4 | 11 | 0 | 0 | 17 |

TABLE 2 Hosts and capsular serogroups of *Pasteurella multocida*

| Disease | No. of isolates | Capsular serogroups | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | D | E | F | Untypable |
| Dogs | 22 | 13 | – | – | – | – | 9 |
| Cats | 14 | 7 | – | 1 | – | – | 6 |
| Cattle | 13 | 9 | 3 | – | – | – | 1 |
| Pigs | 16 | 8 | 1 | 7 | – | – | – |
| Poultry | 4 | 4 | – | – | – | – | – |
| Rabbits | 5 | 3 | – | 2 | – | – | – |
| Other | | | | | | | |
| Sheep | 3 | 2 | – | 1 | – | – | – |
| Goats | 1 | 1 | – | – | – | – | – |
| Horses | 1 | – | – | – | – | – | 1 |
| Total | 79 | 47 | 4 | 11 | 0 | 0 | 17 |

of pneumonia, bite wounds, septicaemia rhinitis and bronchitis (Table 1). Those from bite wounds, stomatitis, rhinitis and bronchitis contributed largely to the untypable proportion. All isolates from classical cases of pasteurellosis, which included bovine haemorrhagic septicaemia, fowl cholera and rabbit septicaemia were found to be groupable.

Of the 79 isolates serotyped, 47/79 were found to be capsular serogroup A, 11/79 serogroup D and 4/79 serogroup B, but 17/79 turned out to be untypable. The distribution of the serogroups among different disease conditions and host species are summarized in Tables 1 and 2, respectively. Serogroup A was predominant among all the hosts and was from a wide range of disease conditions. Serogroup D was mainly of porcine origin, with an exception of two isolates from rabbits and one each from a sheep and a cat. While serogroup B was mainly from cattle, there was a single isolate from a porcine case of meningitis. None of the isolates belonged to either serogroup E or F, and there was a relatively high incidence of untypable isolates.

The host spectrum from which *P. multocida* was isolated is presented in Table 2. Dogs, pigs, cattle and cats represented major host sources of the organism. Isolates from dogs and cats represented a relatively large proportion of the untypable category, while those from pigs, rabbits and poultry were all found to be groupable.

## DISCUSSION

Capsular serogrouping offers a quick taxonomic identification tag to *P. multocida*. However, the procedure has its own shortfalls, the main being the inability in clearly defining an association between a serogroup and disease syndrome or host species. There is a tendency for one serogroup to infect more than one animal species and to cause different disease manifestations. For example, serogroup A has been isolated from cases of calf pneumonia, fowl cholera, pneumonia in pigs and upper respiratory tract infections in dogs and cats. In this study, serogroup A was the most prevalent group, and was isolated from a variety of disease manifestations (Table 2). The isolates from bovine haemorrhagic septicaemia (HS) were found to be serogroup B. A recent report also implicates serogroup B as the causative agent of HS in Namibia (Voigts, Ngaisuiue, Henton & Hubschle 1997). Martrenchar (1993) reported an association of serogroup B with an outbreak of HS in Cameroon. However, HS has previously been associated with serogroup E in Africa (Francis, Schels & Carter 1980; Bastianello & Jonker 1981; Rhoades & Rimler 1992). It is speculated whether this serogroup still causes significant disease in Africa. The association of serogroup B with HS in Africa apparently has implications in vaccine production, mainly because there is no cross-protection between the two serogroups. Serogroup F was not detected among our isolates. Serogroup F is often associated with septicaemic conditions in turkeys (Rimler & Rhoades 1987). Few specimens were received from turkeys, and of those that we examined, none yielded *P. multocida*. Consequently, the prevalence of serogroup F in Zimbabwe is uncertain.

There was a relatively high incidence of untypable isolates, mostly of dog and cat origin. Untypable isolates of *P. multocida* are sometimes encountered, particularly when the organism is in the acapsular state (Rhoades & Rimler, 1987). Acapsular forms can result from loss of capsule which readily occurs during passage on laboratory media (Elberg & Cheng-Lee 1950; Maheswaran & Thies 1979). We also observed that most of our dog and cat isolates were not as comparatively mucoid (suggestive of lack of capsular material) as those from cattle, poultry and pigs.

Although this study did not include a large collection of isolates, there appeared to be some correlation between the capsular group, disease and host, particularly in the typable isolates. The problem of untypable isolates needs further investigation, and there may be need for the development of a molecular-based typing system.

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