J. Vet. Malaysia (1999) 11(1): 41-44

SHORT COMMUNICATION

SEROTYPIC ANALYSIS OF *PASTEURELLA MULTOCIDA* ISOLATED FROM HEALTHY AND DISEASED RABBITS

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SUMMARY

Serotypic characterisation of forty-six *Pasteurella multocida* isolated from healthy and diseased rabbits was conducted. The major serotypes were A:3 (50.0%) and A:1 (28.2%). Other serotypes included D:1 (10.9%), D:3 (8.7%) and A:12 (2.0%). Pneumonia and rhinitis were associated with serotypes A:1 and A:3 whereas serotypes D:1, D:3 and A:3 were isolated from rabbits with septicaemia. Serotypes D:1 and D:3 were also isolated from rabbits with rhinitis and pneumonia, respectively. Serotype A:12 was associated with rhinitis only.

Keywords: Serotypic analysis, *Pasteurella multocida*, rabbits

INTRODUCTION

Pasteurella multocida is a Gram negative bacteria which is known to cause rhinitis and pneumonia in rabbits leading to high morbidity and mortality (Flatt, 1974). Some virulent strains can also cause septicaemia resulting in acute and peracute deaths (Flatt and Dungworth, 1971).

Serotypic characteristics of *P. multocida* have been widely studied especially in the United States (Chengappa *et. al.*, 1982; Lu *et. al.*, 1983; Rimler and Brogden, 1986) Japan (Kawamoto *et. al.*, 1990) and Israel (Mushin and Schoenbaum, 1980). The organism has been serologically characterised based on the cell wall and capsular antigens (Carter and Subronto, 1973; Carter and Rundell, 1975). The known capsular types of *P. multocida* are serotypes A, B, C, D, E and F (Shewen and Rice Conlon, 1993) while the common capsular types isolated from rabbits are types A and D (Carter, 1967; Chengappa *et al.*, 1982; Lu *et. al.*, 1983; Rimler and Brogden, 1986).

Somatic characterisation of *P. multocida* isolated from rabbits revealed serotypes 1, 3, 4, 11 and 12 (Brogden, 1980; Lu *et al.*, 1983). The commonly isolated somatic types were A:3 and A:12 (Brogden, 1980; DiGiacomo *et. al.*, 1983) while serotype 12 is the dominant type for both capsular types A and D (Chengappa *et. al.*, 1982). Little documentation is available on the serotypes of *P. multocida* and the associated lesions in rabbits.

In Malaysia, Al-Haddawi *et al.* (1998) were the first to report the capsular serotypes of *P. multocida* isolated from rabbits. Capsular types A and D were isolated from healthy rabbits as well as from rabbits with rhinitis, pneumonia and septicaemia. Somatic serotypes in diseased and healthy rabbits are, however, still unknown.

This study reports the serotypes of *P. multocida* isolated from healthy and diseased rabbits in the country.

MATERIALS AND METHODS

Bacterial isolation

The method for bacterial isolation was as described by Al-Haddawi *et. al.* (1998). Nasal swabs were collected from rabbits at Universiti Putra Malaysia by inserting sterile swabs into the nasal cavity. Lungs and other organs from dead rabbits were also used for bacterial isolation. Swabs were immediately streaked onto blood agar containing 5% horse blood and MacConkey agar (Oxoid) before incubated at 37°C for 24 hours.

Capsular typing

Type A and D cultures of *P. multocida* were identified based on the methods described previously (Carter and Subronto, 1973; Carter and Rundell, 1975). To identify type A, isolates of *P. multocida*

were transversely streaked across the whole plate of dextrose starch agar (DSA, Difco Laboratories) followed by a heavy streak of hyaluronidase producing strain of *Staphylococcus* at right angle to the *P. multocida* streaked lines. Positive hyaluronidase test was observed as diminution in the size of type A *P. multocida* colonies surrounding the *Staphylococcus* streaks (Carter and Rundell, 1975). Type D cultures were identified by the acriflavine flocculation test. Type D strains flocculated and precipitated when acriflavine was added to broth suspension of the organism (Carter and Subronto, 1973).

Somatic serotyping

Somatic serotyping was was carried out using the gel diffusion and precipitin tests (GDPT) as described by Heddleston *et al.* (1972). Antibodies against all somatic serotypes were raised in 12-16 weeks old chickens by the method of Borgden and Rebers (1978).

RESULTS

A total of 46 isolates of *P. multocida* were examined. The serotypes were A:3 (50.0%), A:1 (28%), D:1 (11%), D:3 (9%) and A:12 (2%). All serotypes, except A:12, were isolated from healthy and diseased rabbits. Serotype A:12 was isolated from only one case of rhinitis (Table 1).

Most isolates (41%) were from healthy rabbits with lowest isolations (24%) from the dead rabbits (Table 1). Serotype A:3 was the most prevalent in diseased rabbits followed by A:1, D:1 and D:3. Serotype A:1 was most commonly isolated from healthy rabbits (Table 1).

Both serotypes A:3 and A:1 were associated with pneumonia and rhinitis but serotypes D:1, D:3 and A:3 were associated with septicaemia. Serotype A:3 was the only serotype isolated from rabbits with rhinitis, pneumonia and septicaemia (Table 1).

Table 1. Number of isolate of *Pasteurella multocida* serotypes and health status of the host

Serotype	Healthy Host	Host with rhinitis	Dead host	Total
A:1	7 (15%)	5 (11%)	1 (2%)	13 (28%)
A:3	10 (22%)	7 (15%)	6 (13%)	23 (50%)
A:12	0	1 (2%)	0	1 (2%)
D:1	1 (2%)	3 (7%)	1 (2%)	5 (11%)
D:3	1 (2%)	0	3 (7%)	4 (9%)
Total	19 (41%)	16 (35%)	11 (24%)	46(100%)

Only one serotypes A:1 was isolated from dead rabbit with pneumonia. Six serotype A:3 were isolated from dead rabbits; 5 were from pneumonic lungs with one from septicaemic case. Serotype A:12 was not isolated from the dead rabbit while only one serotype D:1 was isolated from the dead rabbit with septicaemia. The three serotype D:3 isolated from the dead rabbits involved two septicaemic cases and a pneumonic lung.

DISCUSSION

The results of this study revealed that serotypes A:3 and A: 1 were the common serotypes isolated from healthy and diseased rabbits. These results, however, differed from earlier studies, which reported that serotype A:12 was the predominating serotype and serotype A:3 was isolated only from diseased rabbits (Chengappa *et al.*, 1982; DiGiacomo *et al.*, 1983). The present study revealed that serotype D:1 and D:3 were the prominent while Chengappa *et al.* (1982) revealed that serotype D:12 as the most common serotype.

The isolation of *P. multocida* A:1 and A:3 from rabbits with rhinitis and pneumonia was in agreement with the findings of Lu *et al.* (1983). However, the present study was able to isolate serotype A:3 from cases of septicaemia and serotype A:12 from a case of rhinitis. Our ability to isolate serotype D:3 from a rabbit with pneumonia is consistent with that of Percy *et al.* (1986). Furthermore, our study was able to isolate this serotype from both septicaemic and healthy rabbits.

The results of the present study reveal that although *P. multocida* can be isolated from healthy rabbits, the organism can induce different types of respiratory lesions and septicaemia. The ability to cause disease depends on the ability of the organism to successfully establish and maintain colonisation at different sites in the respiratory tract. The various virulence factors of the organism contribute to the virulence and site colonisation, which influence the isolation.

It was observed in this study that serotypes D:1 and D:3 were successfully isolated from different organs of rabbits that had died of septicaemia as well as from healthy rabbits. This suggests a possible transformation of *P. multocida* from the dormant, non-invasive commensals to pathogenic, invasive strain as observed in *P. haemolytica* infection of cattle (Gonzales and Maheswaran, 1993).

Brogden (1980) observed that different serotypes could be cultured from rabbits in various geographic locations. Differences in the environment and climate in different geographic locations would predispose rabbits to different levels of stress and thus, influencing the severity and possibly types of lesions. Similarly,

pre-treatment with hydrocortisone, a chemical stressor, increased the possibility of colonisation of *P. multocida* in the nasal cavity and lung leading to much severe infection by *P. multocida* (Dillehay *et al.*, 1991).

ACKNOWLEDGMENTS

The authors wish to thank Mr. Basri Kasim and Mr. Hajaraih Salamat for their technical assistance. The financial support from IRPA grant number 51173 is gratefully appreciated.

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RINGKASAN

ANALISIS SEROTIP PASTEURELLA MULTOCIDA *YANG DIPENCILKAN DARIPADA ARNAB SIHAT DAN BERPENYAKIT*

Pencirian serotip empat puluh enam Pasteurella multocida *yang dipencilkan daripada arnab sihat dan berpenyakit telah dijalankan. Serotip utama ialah A:3 (50%) dan A:1 (28.2%). Serotip lain termasuk D:1 (10.9%), D:3 (8.7%) dan A:12 (2.0%). Pneumonia dan rinitis telah dikaitkan dengan serotip A:1 dan A:3 sementara septisemia pula dikaitkan dengan serotip D:1, D:3 dan A:3. Serotip D:1 dan D:3 masing-masing dipencilkan daripada arnab mengidap rinitis dan pneumonia. Serotip A:12 berkait dengan rinitis sahaja.*