MOLECULAR IDENTIFICATION AND EPIDEMIOLOGY

Demonstration that Australian Pasteurella multocida isolates from sporadic outbreaks of porcine pneumonia are non-toxigenic (toxA⁻) and display heterogeneous DNA restriction endonuclease profiles compared with toxigenic isolates from herds with progressive atrophic rhinitis

S. P. DJORDJEVIC, G. J. EAMENS, H. HA*, M. J. WALKER* and J. C. CHIN

NSW Agriculture, Elizabeth Macarthur Agricultural Institute, PMB 8, Camden NSW 2570 and *Department of Biology, University of Wollongong, Wollongong, Australia

Capsular types A and D of Pasteurella multocida cause economic losses in swine because of their association with progressive atrophic rhinitis (PAR) and enzootic pneumonia. There have been no studies comparing whole-cell DNA profiles of isolates associated with these two porcine respiratory diseases. Twenty-two isolates of P. multocida from diseased pigs in different geographic localities within Australia were characterised genotypically by restriction endonuclease analysis (REA) with the enzyme CfoI. Seven of 12 P. multocida isolates from nasal swabs from pigs in herds where PAR was either present or suspected displayed a capsular type D phenotype. These were shown to possess the toxA gene by polymerase chain reaction (PCR) and Southern hybridisation, and further substantiated by production of cytotoxin in vitro. The CfoI profile of one of these seven isolates, which was from the initial outbreak of PAR in Australia (in Western Australia, WA), was identical with profiles of all six other toxigenic isolates from sporadic episodes in New South Wales (NSW). The evidence suggests that the strain involved in the initial outbreak was responsible for the spread of PAR to the eastern states of Australia. Another 10 isolates, representing both capsular types A and D, were isolated exclusively from porcine lung lesions after sporadic outbreaks of enzootic pneumonia in NSW and WA. CfoI restriction endonuclease profiles of these isolates revealed considerable genomic heterogeneity. Furthermore, none of these possessed the toxA gene. This suggests that P. multocida strains with the toxA gene do not have a competitive survival advantage in the lower respiratory tract or that toxin production does not play a role in the pathology of pneumonic lesions, or both. REA with polyacrylamide gel electrophoresis and silver staining was found to be a practical and discriminatory tool for epidemiological tracing of P. multocida outbreaks associated with PAR or pneumonia in pigs.

Introduction

Pasteurella multocida is implicated as an aetiological agent in progressive atrophic rhinitis (PAR) in pigs and is commonly isolated from the lungs of pigs with enzootic pneumonia. The progressive degeneration of nasal turbinate tissue seen in PAR is predominantly associated with toxigenic strains of P. multocida [1–3].

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as diagnostic tools [7, 8]. Furthermore, a recombinant toxin derivative with a deletion of 121 amino acids in the N-terminal region of the native DNT [9] has been shown to be non-toxigenic and protective as a vaccine against PAR in gilts challenged intranasally with Bordetella bronchiseptica and P. multocida [10].

Five capsular types (A, B, C, D and E) are recognised within the genus Pasteurella, with types A and D most frequently isolated from the respiratory tract of pigs [11]. Although both toxigenic and non-toxigenic varieties of capsular types A and D have been described, toxigenic isolates from pigs with PAR in Australia are almost exclusively capsular type D [2] whilst P. multocida isolates from pulmonary lesions are typically non-toxigenic, capsular type A [3, 12–15]. Two field surveys conducted in New South Wales (NSW), Australia, during 1989–1990 revealed that lung isolates of P. multocida are widespread across different herds with a strong association between lesion severity and frequency of isolation of the organism [3]. Non-toxigenic, capsular type A isolates of P. multocida have also been associated with severe pleuritis in two North American herds, where grower and finisher pigs showed severe emaciation and a high mortality rate [13].

Toxigenic P. multocida cannot be reliably differentiated biochemically or by colony morphology from non-toxigenic varieties. Thus, various toxin detection assays including cell culture [2, 16], ELISA [17], immunoblotting [18], dot-blot hybridisations [4] and PCR [18–20] have been developed and evaluated. Restriction endonuclease analysis studies have been applied to compare whole-cell DNA fingerprints of isolates of P. multocida from herds with clinical signs of PAR [21, 22]. However, there are few reports addressing genetic heterogeneity among pulmonary isolates of P. multocida or a comparison of whole-cell DNA restriction endonuclease profiles between strains of P. multocida causing pneumonia and PAR. In this study whole-cell DNA was purified from 22 isolates of P. multocida and digested with CfoI. Restriction endonuclease profiles, resolved by polyacrylamide gel electrophoresis and visualised by silver staining, were compared. Twelve of the 22 isolates were from the nasal passages of pigs with clinical symptoms of PAR whilst the remaining 10 isolates were from pulmonary lung lesions during sporadic outbreaks of pneumonia in geographically diverse Australian herds. The capsular type of these strains was determined and the presence of the toxA gene was confirmed by Southern hybridisation and PCR. In addition, toxin production in each of these isolates was assessed by a cytotoxicity assay.

**Materials and methods**

**Culture of P. multocida**

Strains of P. multocida used in this study are listed in Table 1. The geographic location of all 22 isolates is depicted in Fig. 1. P. multocida isolates 7, 10 and 11 were from pulmonary lung lesions from pigs and were stored as freeze-dried ampoules during 1969–1970. Records identifying the sources of these strains are no longer available. However, these isolates were cultured from lungs by the Regional Veterinary Laboratory at Glenfield which serviced the Hunter, central and far west, southern tablelands and south coast of NSW as well as the metropolitan region of Sydney. All lung isolates were from samples obtained from pigs at necropsy and cultured on blood agar (sterile sheep blood agar 10% v/v; Columbia agar base 3.85% w/v; granulated agar 0.73% w/v). Twelve isolates were from nasal swabs of the turbinates of pigs with clinical signs of PAR cultured on selective blood agar [2]. From lyophilised stocks of these isolates, subcultures were grown on blood agar incubated overnight at 37°C in CO2 5% v/v in air. From these plates, brain heart infusion broth (BHIB) 3.7% was inoculated with a single colony of P. multocida and shaken (200 oscillations/min) for 4–6 h until mid-log phase. P. multocida was harvested by centrifugation (10,000 rpm, 15 min; Sorvall SS34 head) and washed twice with sterile TBS (10 mM Tris-HCl, pH 7.4; NaCl 0.9%).

**Phenotypic characteristics**

All P. multocida isolates were stained by Gram's method [23]. The identity of bacterial isolates was confirmed by standard biochemical tests [24].

**Capsular typing of P. multocida isolates**

P. multocida isolates were classified as capsular type A or D by the acriflavine and hyaluronidase test, respectively [2]. Briefly, one drop of freshly prepared acriflavine solution (diluted 1 in 1000 with milliQ water; Sigma) was mixed with a colony of bacteria taken from an overnight blood-agar culture. Type D bacteria, but not type A, produce a coarse flocculent precipitate within 30 min at room temperature. The hyaluronidase test was performed by first impregnating 5 × 30 mm sterile Whatman filter strips in a sterile solution of ovine testicular hyaluronidase (700 U/ml; Sigma). These strips were then laid across blood agar plates pre-streaked with each P. multocida isolate. After incubation at 37°C in CO2 5%, the continuity of growth was assessed. Growth of type A but not type D cultures was inhibited in the immediate vicinity of the hyaluronidase strip.

**Toxin tests**

A cellular cytotoxicity assay [2] was used to determine the production of toxins by isolates of P. multocida. Briefly, bovine turbinate cells (BTC) were cultured in 0.5 ml of basal medium Eagle (modified) with Hanks's salt (BME/H) containing fetal calf serum (FCS) 10% v/v, penicillin 100 U/ml and streptomycin 100 U/ml. BTC had a passage level of 34. Each well of a 24-well plate (Linbro, Flow Laboratories) was seeded with
1.6 × 10^5 cells, incubated at 37°C with CO2 5% in air for 5 days at which time confluency had been reached. The cells were washed once with phosphate-buffered saline (PBS: pH 7.4, free of calcium and magnesium ions) and the cells in each well were overlaid with 0.5 ml of antibiotic-free BME/H containing FCS 4% v/v and agarose (Seaplaque, Marine Colloids) 2% w/v. The agarose top layer was allowed to solidify at room temperature for 10 min before each P. multocida isolate was inoculated on to half of the agarose surface of each well. A toxigenic strain of P. multocida was always included in each test run. Plates were incubated at 37°C and CO2 5% in air for 72 h before observation. The presence of toxin was confirmed by observing the morphology of BTC through an inverted microscope (25× magnification). Typically, cytotoxin-producing strains induced BTC to appear more granular and rounded compared with non-toxigenic isolates of P. multocida [2]. Isolates were tested in triplicate wells for each treatment and examined for cytotoxicity after incubation for 18, 24, 48 and 72 h.

Extraction of genomic DNA

P. multocida isolates were grown overnight on blood agar (3 × 90 mm plates/isolate) which were flooded with 5 ml of sterile PBS and cells were gently removed by scraping with a glass L-shaped rod. The resuspended bacteria were pelleted by centrifugation and washed three times with PBS. DNA was extracted essentially as described previously [25, 26]. Briefly, the bacterial pellets were resuspended in 4 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) containing sucrose 10%. Lysozyme (50 mg) was added and the mixture was incubated at room temperature (RT) for 10 min before the addition of 50 μl of SDS 10% and 30 μl of RNAase A (10 mg/ml), then incubated at 37°C for 1 h before proteinase K (500 μg) was added. The resultant mixture was incubated at 37°C for 2 h followed by incubation at 56°C overnight. This mixture was then extracted three times with phenol:chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol in serum separator tubes (Becton Dickenson). Finally, the aqueous phase containing the DNA was dialysed overnight with three changes of TES buffer (TES: 10 mM Tris HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl). The concentration and purity of DNA were determined spectrophotometrically. Although large amounts of DNA (3–5 μg) were readily recovered by the above protocol, this study was unable to reliably generate clear CfoI restriction profiles with DNA recovered from toxigenic, capsular type D
isolates. An alternate DNA extraction protocol based on hexadecyltrimethylammonium bromide (CTAB) was used to extract whole-cell DNA from these isolates. Briefly, 200 mg of bacterial pellet were resuspended in 2 ml of TSK (TSK: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, SDS 0.5% and proteinase K 200 μg) and incubated at 37°C for 1 h. A 280-μl volume of 5 M NaCl was added followed by 200 μl of CTAB solution (CTAB: hexadecyltrimethylammonium bromide 10% w/v in 0.3 M NaCl). After mixing by inversion, the DNA lysate was incubated for 10 min at 65°C. DNA was purified by phenol:chloroform:isoamyl alcohol extraction as described above.

Southern hybridisation
Plasmid pPMF3.5 (kindly supplied by Dr E. M. Kamp, Department of Bacteriology, Central Veterinary Institute, Lelystad, The Netherlands) was transformed into E. coli strain DH5α, cultured on LB medium containing carbenicillin 50 μg/ml overnight with shaking and pelleted by centrifugation (5000 rpm, SS34 head). Plasmid DNA was extracted by column chromatography according to the manufacturer’s instructions (Qiagen, Hilden, Germany). pPMF3.5, which contains the 3’ half of the toxA gene was mapped with the restriction endonucleases EcoRI, XbaI, HindIII and combinations thereof to confirm the identity of the plasmid construct [4]. pPMF3.5 and lambda DNA digested with HindIII were labelled with 32P with an Amersham random priming kit (Amersham).

P. multocida genomic DNA digested with CfoI was resolved in agarose 1.0% gels, transferred to nylon membrane, cross-linked with ultraviolet light and hybridised with radiolabelled pPMF3.5 [27]. The nylon membrane was washed three times under stringent temperature and salt conditions (65°C, 2 x SSC) and exposed to film (Fuji RX, Japan) at −80°C for 1–3 days.

Polyacrylamide gel electrophoresis
P. multocida whole-cell DNA (1–2 μg) digested with the restriction endonuclease CfoI was loaded on to polyacrylamide 3.5% gels (12 x 16 cm) and immediately pulsed into the gel matrix at 80 V for 10 min. The gels were electrophoresed (17–19 h, 35 V/gel) in TBE buffer (TBE: 90 mM Tris-HCl, 90 mM borate, 2 mM EDTA) with a Hoefer maxi gel apparatus as described previously [25]. The gels were removed, washed once with milliQ water and stained with silver as described by the manufacturer (Rapid Silver Stain, INC Biomedical). Gels were fixed in acetic acid 0.1% and photographed immediately.

Polymerase chain reaction
Primers which amplify a 1.2-kb fragment of the toxA gene of P. multocida [18] were used to confirm the presence of the toxA gene. Primer sequences were as follows: primer 1, 5′-TACTCAATTGAAAAAGCGC TTAATCTCC-3′ and primer 2, 5′-TCTACTACAGTT GCTGGATTTTTAAATAT-3′. PCR amplification conditions were similar to those described by Nagai et al. [18] and consisted of the following: 1 μM of each oligonucleotide primer, 200 μM of each nucleotide triphosphate, 2.5 U of Taq polymerase (Stoffel enzyme), 1 x reaction buffer (10 mM Tris-HCl, pH 8.3, 10 mM KCl) and sample DNA (100 ng) in a final volume of 50 μl. The reaction mixture was subjected to an amplification regimen consisting of an initial denaturation step (95°C, 2 min) followed by 25 cycles of 92°C (1 min), 55°C (1 min) and 70°C (2 min) with a Corbett Research FTS 960 thermocycler. A portion of the reaction mixture (15 μl) was electrophoresed through agarose 1.0% gel with 0.5 × TBE buffer, 60 V for 1.5 h. The PCR amplification product was visualised with ethidium bromide and photographed with Polaroid 667 film.

Results
Capsular typing of porcine P. multocida isolates
P. multocida isolates 1, 3, 4, 7, 8, 9, 10 and 14–21 produced a type D capsule; all other isolates (2, 5, 6, 11, 12, 13 and 22) were capsular type A as defined by the hyaluronidase and acriflavine tests (Table 1).

Evidence of the presence and expression of the toxA gene
Table 1 shows that all 10 P. multocida isolates from pneumatic lesions did not secrete cytotoxic toxin in an agarose overlay bovine turbinate cell assay. In contrast, P. multocida isolates 1, 15, 16, 17, 18, 19, 20 and 21 (capsular type D) which had been isolated from the nasal turbinates of pigs in a herd with symptoms of PAR, induced cytotoxic changes to bovine turbinate cells (Table 1). A single capsular type A isolate (isolate 22 from Cootamundra, NSW, Australia) also demonstrated cytotoxin activity. Isolate 2, which was co-isolated with isolate 1 from the same nasal swab, did not express toxin. Similarly, isolate 3, from the same farm (Byford, Western Australia, Australia), also failed to express toxin. Isolate 8 (Bendigo, Victoria, Australia), from a nasal swab from a pig with nasal discharge but with no evidence of turbinate atrophy, failed to demonstrate cytotoxin activity.

The presence of the toxA gene amongst the 22 isolates of P. multocida was examined by Southern hybridisation and PCR analyses of purified DNA. Southern blotting of the CfoI-digested whole-cell DNA from P. multocida when probed with 32P-labelled pPMF3.5, confirmed that the 10 pneumatic isolates did not possess the toxA gene. Nasal swab isolates 2, 3 and 8 also did not hybridise with 32P-labelled pPMF3.5. However, CfoI-digested whole-cell DNA from toxicogenic isolates 15–21 showed the presence of three strongly hybridising fragments of c. 3.9, 1.9 and a
fragment of <0.56 kb, thereby confirming the presence of the toxA gene (data not shown). These fragment sizes were in agreement with predicted values based on the presence of CfoI restriction sites identified in the DNA sequence of the toxA gene (accession no. X52478).

PCR primers [18] facilitated the amplification of a 1.2-kb fragment of the toxA gene from whole-cell DNA purified from isolates 1 and 15–22. DNA from isolates of P. multocida which were not toxigenic in the agarose gel overlay assay (isolates 2–14) and which did not hybridise with pPmF3.5 (data not shown) also failed to show any amplification product in the PCR (Table 1).

Genomic characterisation

Isolates from nasal swabs. Isolates 15–21 were obtained during sporadic outbreaks of progressive atrophic rhinitis in pig herds at different geographic localities in NSW (Fig. 2a). DNA from these seven isolates displayed remarkably similar CfoI restrictionendonuclease profiles (Fig. 2a). Apart from a single, darkly stained fragment with a molecular size of c. 4.6 kb (arrowhead, lane 5), the CfoI-generated restrictionendonuclease profiles of these seven isolates were indistinguishable. The CfoI DNA profile of isolate 8 (non-toxigenic, capsular type D) was clearly different from the typical CfoI profile of toxigenic capsular type D isolates (Fig. 2b). Toxigenic isolate 1 (Byford, WA) was amongst the first toxigenic isolates detected in Australia soon after the appearance of PAR [8] and approximately 2 years before the appearance of PAR in herds in NSW (isolates 15–21). The CfoI restrictionendonuclease profile of isolate 1 was remarkably similar to the CfoI profiles of NSW toxigenic isolates 15–21 (Fig. 3).

Table 1. Geographic location, capsular type and toxigenic status of P. multocida isolates

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Origin and identity</th>
<th>Type</th>
<th>Syndrome</th>
<th>Source</th>
<th>Cytotoxicity</th>
<th>toxA PCR</th>
<th>toxA probe</th>
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<tbody>
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<td>1</td>
<td>WA 1096-849</td>
<td>D</td>
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<td>N. swab</td>
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<td>PAR</td>
<td>N. swab</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>WA 1098-855</td>
<td>D</td>
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<td>-</td>
</tr>
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<td>12</td>
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<td>Lung</td>
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<td>-</td>
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<tr>
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<td>-</td>
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<td>-</td>
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<td>Lung</td>
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<td>-</td>
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<td>-</td>
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<td>D</td>
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<td>-</td>
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<td>+</td>
<td>ND</td>
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</table>

PAR, progressive atrophic rhinitis; N. swab, nasal swab; N. discharge, nasal discharge; ND, not done.
Fig. 2. Silver-stained polyacrylamide 3.5% gel showing CfoI restriction endonuclease profiles of whole-cell DNA purified from toxigenic isolates of *P. multocida*. **A:** Lane 1, isolate 19 (Deniliquin, NSW); 2, isolate 15 (Canowindra, NSW); 3, isolate 18 (Galore, NSW); 4, isolate 17 (Henty, NSW); 5, isolate 21 (Tyringham, NSW); 6, isolate 20 (Molong, NSW); 7, isolate 16 (Hillston, NSW); 8, lambda DNA digested with HindIII. **B:** Lane 1, isolate 17; 2, isolate 8 (Bendigo, VIC) from a nasal swab. A and B were derived from the same polyacrylamide 3.5% gel.

Discussion

Capsular types A and D of *P. multocida* are economically significant pathogens of commercial pig industries because of their ability to induce PAR and their involvement in pneumonia as secondary invaders that exacerbate lung pathology initiated by primary colonising agents. There have been no reports comparing the genomic profiles generated by REA of isolates from the nasal mucosa and lung lesions of pigs with clinical symptoms of PAR and pneumonia respectively. Although this study compared restriction endonuclease profiles from only a limited number of isolates of *P. multocida*, their origin from lung lesions in sporadic outbreaks of pneumonia and from nasal swabs of pigs in herds displaying symptoms of PAR from different states across Australia sheds light on the degree of genomic heterogeneity inherent in this species. A comparison of the CfoI profiles of genomic DNA purified from eight toxigenic type D isolates of *P. multocida* from two geographically distant states (WA and NSW) showed remarkable similarities. The enhanced resolution of the CfoI profiles afforded by
polyacrylamide gel electrophoresis and silver staining showed that these eight isolates were clonal. These observations confirm the hypothesis that outbreaks of PAR since 1985 in Australia were initiated from a common infectious source comprising a toxigenic capsular type D strain of P. multocida as suggested by Gardner et al. [22].

Mercy et al. [9] described the first outbreak of PAR in WA after the importation of two sows and it was believed that spread was limited to other states [22]. According to Gardner et al. [22], a second outbreak in South Australia (SA) and the associated sale of breeding pigs from this herd to c. 131 herds across five Australian states suggested that the SA herd was the likely source of the toxigenic type D isolate. Gardner et al. [22] used REA based on profiles generated by the restriction endonuclease Smal, to describe two different Smal restriction endonuclease profiles (type B and C) amongst 37 Australian toxigenic type D isolates of P. multocida (including isolates retyped in this study); all displayed a ribotype 2 profile with the restriction endonuclease EcoRI. However, the only difference between Smal profile types B and C resides in the absence of a single DNA fragment c. 1.9 kb which was likely to have been derived from plasmid DNA because of the relatively high staining intensity compared with genomic DNA fragments. Similarly, the present study noted the absence of a darkly staining fragment of c. 4.4 kb in the CfoI profile of isolate 21 which is likely to have arisen from a restriction fragment length polymorphism (RFLP) in plasmid DNA. Plasmids are known to exist in all seven toxigenic, capsular type D strains in this study (data not shown and [22]). This observation is supported by the inability to detect any other RFLPs in the CfoI profile throughout the entire molecular mass range resolvable with a polyacrylamide 3.5% matrix. The presence of a RFLP in the plasmid profiles amongst these isolates is likely to be responsible for the Smal profiles (types B and C) described previously [22]. The results in Figs. 2 and 3 show that the CfoI restriction profiles of the eight capsular type D toxigenic isolates are derived from a single clonal type which probably emanated from the original outbreak of PAR in WA. These data suggest that the second outbreak in SA was probably caused by a type D toxigenic isolate which first appeared in WA.

A single toxigenic capsular type A isolate 22 of P. multocida (isolate 22) displayed a unique CfoI profile. This observation is supported by the findings of Gardner et al. [22] who demonstrated that this isolate produced a unique Smal profile (type G, ribotype 3) unlike the toxigenic type D isolates.

Two non-toxigenic P. multocida isolates from nasal swabs of pigs in a herd displaying clinical symptoms of PAR (2 and 3) showed distinct and highly variable CfoI profiles, suggesting a limited role in the aetiology of PAR. However, non-toxigenic strains of P. multocida may be ubiquitous in the nasal cavities of pigs [28,29] and their ability to colonise the lower respiratory tract and their contribution to the development of pasteurella pneumonia is unknown. Previous studies with European isolates of P. multocida have reported that the vast majority of non-toxigenic isolates do not possess the toxA gene, although a few isolates do possess highly mutated or incomplete toxA sequences [4]. The toxA gene or parts thereof could not be detected in Southern blots (probed with pPmF3.5) of whole-cell DNA purified from the 10 pneumonic isolates and two nasal swab isolates (isolates 2 and 3) used in this study.
Although *P. multocida* has been recognised as an important pathogen in the development of PAR, its role in the aetiology of swine pneumonia is unclear. It is generally considered that *P. multocida* is not a primary colonising agent of the swine respiratory tract and initial infection with *Mycoplasma hyopneumoniae* [30,31] or one of several viruses including adenovirus [32], hog cholera virus [33] or pseudorabies virus [34] is required for subsequent infection with *P. multocida*. In Australia, with the exception of *M. hyopneumoniae*, none of these agents is present. Pijoan and Fuentes [13] described the isolation of non-toxigenic capsular type A isolates from swine with symptoms of severe pleuritis and abscess formation. In that study, several non-toxigenic, capsular type A isolates, as well as one toxigenic, capsular type D isolate, were used in conjunction with pseudorabies virus to induce experimental pneumonia and pleuritis in pigs. Certain non-toxigenic, capsular type A isolates were more capable of inducing severe pleuritis and abscess formation than other isolates and the toxigenic, capsular type D isolate seemed unable to exacerbate the pathological changes. Studies by Iwamatsu and Sawada [14] described 116 *P. multocida* isolates from lung lesions in Nakasaki, Japan. Capsular type D, toxigenic isolates (18.1%) were isolated only from abscesses, whilst the majority of isolates were capsular type A (81.9%) and were from lungs of pigs with pleuritis and consolidation. Although these studies suggest that capsular type A isolates of *P. multocida* are associated with pneumonic lesions in pigs, there have been no reports comparing the genetic relatedness of such isolates from sporadic outbreaks of pneumonia.

In the present study, DNA was purified from whole-cell lysates of 10 isolates (five each of capsular types A and D) of *P. multocida* from lung lesions. These were from sporadic outbreaks of pneumonia mostly in piggeries located in different areas of NSW and WA. Unlike the *CfoI* profiles of the toxigenic capsular type D isolates, these 10 isolates generally displayed heterogenous *CfoI* profiles both amongst themselves and when compared with 12 isolates from the nasal passages of pigs. None of the 10 lung isolates...
expressed cytotoxin and all failed to show the presence of plasmid DNA (data not shown). These data suggest that opportunistic P. multocida are associated with pulmonary pasteurellosis in swine. The inability of plasmidic isolates to reproduce clinical symptoms of respiratory disease in experimental challenge models in pigs without concomitant infection with M. hypneumoniae or a viral agent [30–34] and the ability to recover simultaneously several different genotypes from the same animal are consistent with this hypothesis. Nevertheless, it is interesting that all plasmidic isolates of P. multocida in the present study did not possess the toxA gene. It is likely that toxin production does not play a role in the pathogenesis of pneumonia or pleuritis. Multilocus enzyme electrophoretic studies with 35 disease isolates of P trehalosi, a pathogen causing systemic infection in the upper alimentary tract and lungs of sheep, identified 16 different electrophoretic types indicative of an opportunistic mechanism of pathogenicity [35]. Further studies are required to determine the degree of genetic heterogeneity amongst a larger number of isolates of P. multocida associated with pneumonia. Finally, polycrylamide gel electrophoresis and silver staining produce highly informative DNA fingerprints which should be useful in studies tracing outbreaks of PAR and pneumonia in swine.

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References


