NOVEL IDENTIFICATION OF *PASTEURELLA MULTOCIDA* SEROGROUP F IN MALAYSIA

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ABSTRACT. This study will highlight the novel identification and confirmation of *Pasteurella multocida* serogroup F in Malaysia. The organism was isolated from a two-year-old Large White crossed Landrace female pig which presented with a history of emaciation. Necropsy revealed multiple abscess in its lungs. Upon isolation and identification, the grey and non-haemolytic bacteria colonies were identified as *Pasteurella multocida* by Sarawak State Veterinary Diagnostic Laboratory (SVDL) at Kuching, Malaysia using a series of biochemical tests and were subjected to serogroup identification in the Veterinary Research Institute (VRI) at Ipoh, Malaysia. The DNA of the bacteria was amplified by multiplex polymerase chain reaction (PCR) which produced an amplicon of 851 bp. Further partial DNA sequence of the *fcbD* gene analysis showed it to be 100% identical to *P. multocida* serogroup F from the National Center for Biotechnology Information (NCBI) database and was closely related to *P. multocida* serogroup A. In the VRI diagnostic laboratory, there were reports of a few sporadic cases due to *P. multocida* serogroup F infection in various animal species such as chicken, goat and deer. This revealed that the *P. multocida* serogroup F is circulating in Malaysia and this affects livestock clinically. Therefore, further research is needed to understand the characteristics and pathogenicity by targeting the virulence factor (VFs) encoding of outer membrane proteins, iron acquisition factors, bacterial adhesions and colonisation factors, extracellular enzymes and dermonecrotoxin of the isolate and the virulence of the serogroup F strain.

Keywords: *Pasteurella multocida* identification, phylogenetic analysis, serogroup F, livestock, Malaysia.

INTRODUCTION

Pasteurella multocida is a gram-negative coccobacillus found as a normal flora in the upper respiratory tract of healthy animals. It can act as a primary pathogen causing haemorrhagic septicaemia (HS) in cattle, buffalo, and fowl cholera in avian. In secondary infections, it may multiply and colonise in the lungs, causing pneumonic pasteurellosis in stressed animals. The serogroup of *P. multocida* are identified based on differences in capsular polysaccharides and are designated as A, B, D, E and F by Carter (1955). VRI adopted capsular typing using molecular PCR as suggested by the World Organisation for Animal Health (OIE). The presence of the capsular type is one of the important

virulence factors that influence the ability of *P. multocida* to invade and multiply in the hosts. *P. multocida* serogroup F was first isolated from turkeys in the USA (Rimler and Rhoades, 1987). Over the years, it has been isolated from a few other mammalian species worldwide. In the Czech Republic, serogroup F have been isolated from rabbits with clinical signs of rhinitis and pneumonia (Jaglic *et al*., 2005). Recently, there was a reported case of isolation from pigs in China (Peng *et al*., 2017). In Malaysia, the study on prevalence of *P. multocida* and predominant serogroups are also available (Khoo *et al*., 2017; Nafizah *et al*., 2014; Arumugam *et al*., 2011). However, *P. multocida* serogroup F are just briefly mentioned in those reports without any details of the history and further description. This study will highlight a novel finding on the identification and confirmation of *Pasteurella multocida* serogroup F in Malaysia.

MATERIALS AND METHOD

Bacterial strains

P. multocida isolate (VRI-2507-2015) was cultured on a nutrient agar slant isolated from lungs of a pig at VRI for *P. multocida* capsular identification. Necropsy and bacterial isolation were performed at SVDL using standard culture protocol. In VRI, the isolate was cultured on 5% ox blood agar and the *Pasteurella multocida* colonies were tested using a series of biochemical tests prior to molecular identification.

Molecular analysis

The bacterial DNA was extracted from an overnight culture on blood agar using Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol. Multiplex PCR was performed as described by Townsend *et al*. (2001) with a slight modification in the total reaction volume of 50 µl using MyTaq Mix

Table 1. The capsule serogroup, gene, primer sequences and amplicon sizes (Townsend *et al*., 2001).

Capsule serogroup	Gene	Primer sequence (5′ – 3′)	Amplicon (bp)
CapA	*hyaD-hyaC*	TGC-CAA-AAT-CGC-AGT-CAG TTG-CCA-TCA-TTG-TCA-GTG	1044 bp
CapB	*bcbD*	CAT-TTA-TCC-AAG-CTC-CAC-C GCC-CGA-GAG-TTT-CAA-TCC	760 bp
CapD	*dcbF*	TTA-CAA-AAG-AAA-GAC-TAG-GAG-CCC CAT-CTA-CCC-ACT-CAA-CCA-TAT-CAG	657 bp
CapE	*ecbJ*	TCC-GCA-GAA-AAT-TAT-TGA-CTC GCT-TGC-TGC-TTG-ATT-TTG-TC	511 bp
CapF	*fcbD*	AAT-CGG-AGA-ACG-CAG-AAA-TCA-G TTC-CGC-CGT-CAA-TTA-CTC-TG	851 bp

(Bioline UK), 5 sets of primers (CapA, CapB, CapD, CapE and CapF) and 10.0 µl of DNA template. Amplified DNA was analysed by electrophoresis on 1.5% agarose gel stained with GelRed stain (Biotium, USA) and visualised using gel documentation system (Major Science, USA).

The 851 bp PCR product from the amplified *fcbD* gene was cut from agarose gel. Purification and sequencing were performed by First Base Laboratories (First Base, Malaysia). The primers used for sequence analysis were the same as those used for PCR amplification. The sequences were assembled using Seqman (DNAStar Lasergene, USA). Nucleotide sequences were then analysed using BioEdit program version 7.1.9 and multiple sequence alignment was performed with Clustal W (Hall, 1999). Phylogenetic analysis was carried out by including other *P. multocida* sequences obtained from NCBI for comparison. Phylogenetic tree was constructed with MEGA7 using neighbour-joining Kimura 2 parameter model and 1,000 bootstrapped replications (Tamura *et al*., 2011). In this study, the phylogenetic analysis of the *P. multocida* 2507-2015 isolate was generated based on the region of 828 bp long of *fcbD* gene.

RESULTS AND DISCUSSION

P. multocida VRI-2507-2015 which was isolated from the lungs of an infected pig showed grey, non-mucoid and non-haemolytic colonies on 5% ox blood agar (Figure 1) after 24 hours of incubation at 37 °C. It did not grow on MacConkey agar. The DNA was amplified by multiplex PCR. An amplicon of 851 bp was identified with the presumption that *P. multocida* serogroup F (Figure 2) as only positive controls for *P. multocida* serogroup A, B and D were available. Thus, amplification of the repeated analysis by using single specific primers of

Figure 1. *P. multocida* serogroup F on 5% ox blood agar.

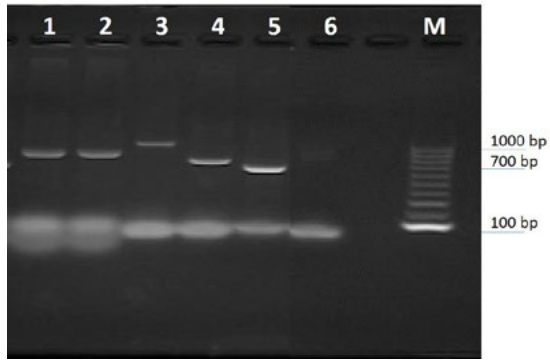

Figure 2. Agarose gel electrophoresis of multiplex PCR product. Lane 1-2, duplicates of *P. multocida* 2507-2015 with amplicon 851 bp (*fcbD* gene); lane 3, *P. multocida* serogroup A; lane 4, *P. multocida* serogroup B; lane 5, *P. multocida* serogroup D; lane 6, negative control; lane M, marker, 100 bp DNA ladder.

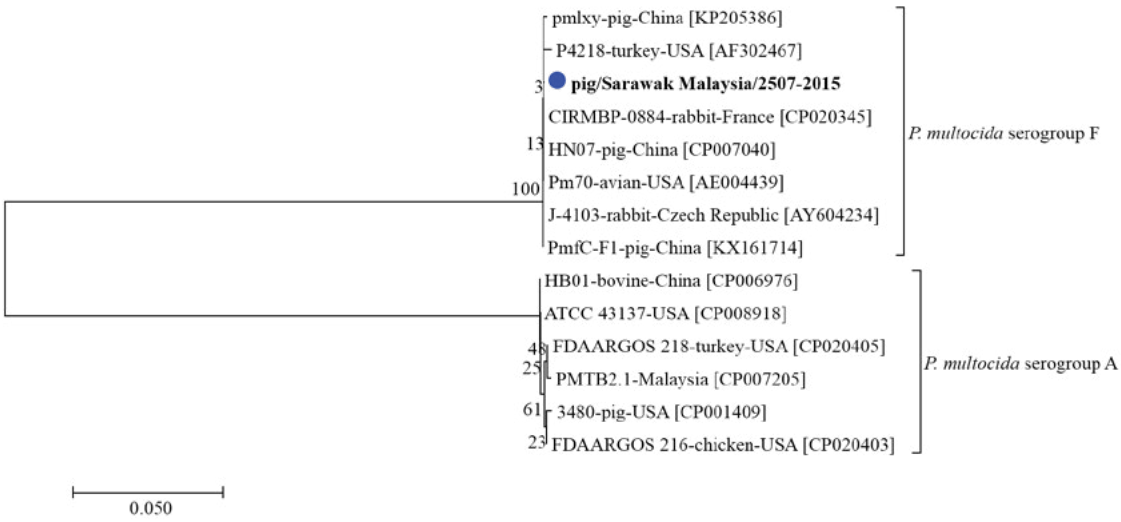

Figure 3. Phylogenetic analysis of the *fcbD* gene based on 828 bp long nucleotide sequences. *P. multocida* 2507-2015 isolated from Malaysia and other isolates were included for analysis using the Neighbour Joining Method. The isolate obtained in the present study are in bold. Accession numbers of the sequences from NCBI are shown in parenthesis. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

CapF targeting *fcbD* gene was successful and was amplified at the expected size of 851 bp. The DNA sequence analysis showed it to be 100% identical to the *P. multocida* serogroup F available in the NCBI database.

P. multocida often causes diseases in various domestic and wild animals. In pigs, infection by this pathogen can cause respiratory symptoms such as cough, pneumonia, atopic rhinitis, and severe breathing. There are five different *P. multocida* serogroup (A, B, D, E and F) based on their capsule (Carter, 1955). Porcine pneumonia is often caused by non-toxigenic *P. multocida* serogroup A, while atrophic rhinitis is often associated with toxigenic strain of *P. multocida* serogroup D (Davies *et al*., 2003). In this particular case, the two-year-old Large White crossed Landrace female pig was reported to be emaciated and had multiple abscess lesions on the lungs. The presumptive diagnosis of that particular of time was tuberculosis or melioidosis infection. To date, there has been only one recent publication on the pathogenicity of *P. multocida* serogroup F in pigs in China indicating high pathogenicity and distinct pneumonic lesions in the lungs of 8-week-old pigs (Peng *et al*., 2017). The reported clinical signs and pathological changes in the pigs were also similar to the experimental rabbits that were challenged with a rabbit-sourced *P. multocida* serogroup F isolate (Jaglic *et al*., 2008). Pasteurellosis in pigs rarely cause severe mortality, but it is economically important because it

significantly reduces the growth rate of an infected herd (Harper *et al*., 2006). Most of the previous reports on *P. multocida* serogroup F are very limited and are only found in avian species such as turkey (Rimler and Rhoades, 1987).

CONCLUSION

This is the first report on the identification and confirmation of *P. multocida* serogroup F isolated from pig in Malaysia by using partial gene sequencing and phylogenetic analysis. Phylogenetic analysis showed that the *P. multocida* serogroup F was most closely related to serogroup A, compared to serogroup D (Harper *et al*., 2006). Since then, there have been a few sporadic cases of *P. multocida* serogroup F detected from various animals such as chicken, goat and deer in West Malaysia in the recent years (source: VRI unpublished diagnostic data). This indicates that *P. multocida* serogroup F is not host specific in terms of its pathogenesis. Continuous research work is essential to understand the characteristics or pathogenicity of the organism by targeting the gene encoding virulence of outer membrane proteins (*ompH*) or dermonecrotoxin (*toxA*) of *P. multocida*. This may contribute to the further understanding of its virulence for the serogroup F strain and add knowledge to this field of research.

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