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Study of the molecular profile in strains of *Pasteurella multocida* serotype A from lung lesions in swine

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Introduction

Pasteurella multocida A (*P. multocida*) is a common bacteria isolated from swine respiratory tract. Despite of been considered an opportunistic [5], this pathogen has been associated with lung lesions, pleurisy and pericarditis observed in field outbreaks and experimental challenges [3]. The objective of this study was to evaluate the molecular profile of different strains of *P. multocida* from eight Brazilian States. Studies included information on species-specific genes, capsular typing and virulence genes.

Materials and Methods

A total of 157 *P. multocida* strains, previously characterized, by standard biochemical procedures, like *P. multocida* serotype A were analyzed. The strains were recovered from pneumonic lungs of pigs from farms and slaughterhouses in the eight major pork producer states in Brazil. DNA samples were analysed by multiplex PCR to detect capsular genes type A, D, F and the species-specific *kmt1* gene [6]. Virulence genes were detected by PCR specific for *toxA* (dermonecrotic toxin), *tbpA* (binding protein hemoglobin), *hgbB* (mechanisms of iron acquisition) and *pfhA* (filamentous hemagglutinin-bacterial adhesion factor) [1]. One amplified fragment from each gene was sequenced, and the specificity was confirmed by the GenBank database using the BLAST tool.

Results

All studied 157 strains were positive for the *P. multocida* species-specific gene *kmt1*. In addition, 94.9% (149/157) were positive for capsule type A and 5.1% (8/157) for the capsule type D. None strains were identified as type F. Investigation of virulence factors, showed no positive results for *toxA* and *tbpA* genes. However, 31.8% (50/157) and 82.8% (130/157) of the strains harbored the *pfhA* and *hgbB* genes, respectively. Only 14% (10/157) of the samples harbored both *pfhA* and *hgbB* genes, therefore, 85.4% (134/157) of strains were positive to a single one of these genes. *P. multocida* positive for *pfhA* and *hgbB* genes, important virulence factors, were identified in all investigated Brazilian states.

Conclusions and Discussion

All strains of this study were verified as *P. multocida* and the capsular serotype A was widely predominant. The high prevalence of hemoglobin binding protein coding by *hgbB* gene suggests that an additional advantage to increase the pathogenicity [7]. The *hgbB* gene was detected in the majority of studied strains, even though *P. multocida* does not show visible and classical hemolysis [7]. Possibly, this gene's presence is related to occurrence of lesions. The genetic arrangement of *tbpA* support a possible transpositional recombination event of

this genetic locus from non or low pathogenic to highly virulent *P. multocida* strains [2]. Both the *toxA* gene coding the dermonecrotic toxin and the *tbpA* gene, which was previously described factor involved in hemorrhagic septicemia in cattle [2] were not detected. The *toxA*, *tbpA* and *pfhA* as well as the capsule biosynthesis genes are important marker genes to define the pathogenic potential of *P. multocida* strains [2]. The *pfhA* gene codes a protein associated to a bacterial adhesion factor in respiratory tract. This gene was present in 31.8% of strains, in agreement with Ewers [2]. Experimental studies in pigs using different virulence genes profiles are needed to understand their effect in the pathogenicity. In conclusion, the *P. multocida* A was the most prevalent serotype and it is widely distributed in pneumonic lesions in pigs from farms and slaughterhouses in Brazil. In addition, *P. multocida* A strains harbor different virulence related genes.

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