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DEVELOPMENT OF A QPCR-SEROTYPING SYSTEM FOR ACTINOBACILLUS PLEUROPNEUMONIAE

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Introduction

A total of 16 serotypes of *Actinobacillus pleuropneumoniae* (APP) have been reported so far; nevertheless, data indicating which serotypes are more virulent is still lacking for most countries. Thus, updated information related to a particular geographical area might be precious.

Current techniques of APP serotyping present certain methodological limitations; cross reactions when using serological tests and inaccurate identification through Apx genes. The aim of this work was to develop a complete set of APP typing qPCR reactions to conduct a preliminary survey regarding the current situation of APP in Spain.

Material & Methods

One real time PCR for detecting APP (all serotypes) and 14 qPCR multiplex assays were designed to unequivocally detect serotypes 1,2,3,4,5,6,7,8,9/11,10,12,14,15 and 16. Due to the lack of available

sequence ser13 assay was not developed.

A collection of reference strains containing every single serotype was gently provided by University of Montreal (Canada) to conduct the validation. After that, respective isolations from 40 pleuropneumonia compatible cases collected in Spain from 2015 to date were analyzed.

Results

The proposed qPCR assays detected the required serotype only confirming the specificity of the tests.

Microbiological isolations from clinical cases resulted: ser8(n=4, 10%), ser2(n=5, 12%), ser 9/11(n=5, 12%), ser4(n=11, 28%) and ser13(n=15; 38%). Ser13 strains had to be serotyped by serological techniques after obtaining negative reaction with all herein developed qPCR-typing assays.

Discussion & Conclusion

These results agree partially with a former Spanish report to the extent of high prevalence of ser2, ser4 and ser9/11. However, contrary to what was previously described, we found the ser13 as the most prevalent. Considering the advantages of qPCR technique, further studies are planned to validate the simultaneous detection of every serotype when analyzing directly the biological sample.

We conclude that these sets of qPCR assays are a valuable tool to serotype APP strains.