CRC for High Integrity Australian Pork

2A-107: *Antibiotic sensitivity of Haemophilus parasuis plus Actinobacillus pleuropneumoniae and other respiratory pathogens*

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Aims and Objectives

In the last decade, the emergence of antimicrobial resistant forms of bacterial pathogens has become a problem in the treatment of bacterial diseases of livestock. Despite this problem, no data was available on the resistance status of respiratory bacterial pathogens in Australia. For one of the main pathogens, *Haemophilus parasuis*, there was not even an accepted, validated, standardized methodology for testing for antimicrobial resistance. Therefore, the first aim of this study was to develop a method to test for *H. parasuis* antimicrobial sensitivity. Once validated methods for the testing of *H. parasuis* were developed, the project set out to look at antimicrobial resistance in key respiratory pathogens.

The next objective of the project was to find the underlying genetic cause for the phenotypic resistance observed. A wide variety of genes that have been reported in the literature to cause the kind of resistance observed for the Australian isolates were screened.

The last part of this project looked at the resistance of respiratory bacteria within a pig, across a batch of pigs and between pig batches (all from the same farm) to determine whether resistance is evenly distributed in a bacterial population on a farm. For this study, isolates of *A. pleuropneumoniae* were collected from pigs (from a single farm) at slaughter at three different occasions (ten isolates from the primary isolation plate of each lung). The three antimicrobial agents examined were tilmicosin, tetracycline and amoxicillin.

Key Findings

A new validated method for sensitivity testing for *H. parasuis* has been established and the method has been published. We have also sent the method to different laboratories so it can be implemented for the pig industry This method is not yet accepted at the international level, but we are currently collaborating with a laboratory in Germany and one in the USA to do the testing required for the uptake of the method at the international level.

We have looked at antimicrobial resistance in *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica, H. parasuis* and *Pasteurella multocida*. The resistance to some of the older antibiotics, such as erythromycin and tetracycline, was marked in all species, for example 89% and 75% of *A. pleuropneumoniae* isolates showing resistance to these two agents, respectively. A worrying outcome was the resistance to the newer antimicrobial agents, such as tilmicosin where resistance was found in 25% of the *A. pleuropneumoniae* isolates. As well, an elevated minimal inhibitory concentration for tilmicosin was found in 22% of the *H. parasuis* isolates tested. There were also isolates observed with multiple drug resistance. This work has been published in two peer- reviewed articles in an international journal. Overall, while antimicrobial resistance is less of a problem in Australia than in many other countries, resistance is present in key pathogens and the industry and associated health professionals need to address the issue.

The next objective of the project was to find the underlying genetic cause for the phenotypic resistance observed. A wide variety of genes that have been reported in the literature to cause the kind of resistance observed for the Australian isolates were screened. Isolate resistance to beta-lactams and tetracyclines could be explained to a large extent by resistance genes detected, bla_{ROB-1} and *tet*B, respectively. However, the screening for eight genes reported to be associated with marolide (ie tilmicosin, erythromycin) resistance gave only negative results. So at this point, the resistance to macrolides cannot be explained by any of resistance genes examined in this study. Further studies are needed to explain the resistance mechanism of the Australian isolates resistant to macrolides.

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pleuropneumoniae were collected from pigs (from a single farm) at slaughter at three different occasions (ten isolates from the primary isolation plate of each lung). The three antimicrobial agents examined were tilmicosin, tetracycline and amoxicillin. Genetic fingerprinting analysis established that the isolates collected were clonal. Among the 367 clonal isolates from three different batches of pigs, isolates that were either resistant or susceptible to tilmicosin were found. In some isolates, there was also statistically significant evidence of two populations in the second sampling for tilmicosin and amoxicillin, with one population showing a lower zone diameter (that is tending towards resistance) and the other showing a higher zone diameter (tending towards more susceptible). The results suggest that a single clone of a porcine respiratory pathogen on a farm can consist of both susceptible and resistant types, with variation in zone diameters of multiple isolates varying across time.

Application to Industry

This project has the following outcomes:

- Validated disk diffusion and MIC antibiotic sensitivity method for testing *H. parasuis* isolates
- Knowledge of the current resistance patterns in the key pathogens - *A. pleuropneumoniae*, *B. bronchiseptica*, *H. parasuis* and *P. multocida*.
- Knowledge of the genes responsible for the phenotypic resistance patterns observed for Australian isolates to beta-lactams and tetracyclines.
- Establishment of occurrence of variation in antimicrobial sensitivity of one clonal bacterial population on a farm and thus the need for diagnostic laboratories to consider testing multiple animals and multiple isolates within an animal to determine antimicrobial sensitivity patterns and provide sound advice to clinicians seeking effective antimicrobial treatment strategies on a farm.

This knowledge is essential to establish effective, sustainable antimicrobial treatment and prevention programs.