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### Project Update: Effect of enrofloxacin on Haemophilus parasuis infection, disease and immune response

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#### Background

- Haemophilus parasuis colonizes the upper respiratory tract of pigs, invades the bloodstream and causes polyserositis (pericarditis, peritonitis, meningitis, arthritis) resulting in mortality losses in nursery pigs.
- Antimicrobials limit bacterial infections by decreasing bacterial load, permitting the host to activate immune defenses, and eliminating the pathogen without excessive inflammation. However, in certain occasions, antimicrobials can have unintended consequences by preventing the development of immunity (i.e if the pathogen is removed very efficiently).
- Because certain antimicrobials can be highly effective against H.parasuis, we hypothesized that they could have a detrimental effect on the establishment of an immune response if given at the time of colonization.

#### Objective

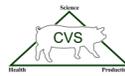
To study the relationship between H. parasuis infection, immunity, protection, and use of antibiotics. More specifically to characterize the clinical outcome and immune responses to H. parasuis in pigs treated with enrofloxacin before or after low dose inoculation with a pathogenic H. parasuis strain.

#### Material and methods

- An animal model using conventional pigs was used. Pigs were inoculated with a low dose of a pathogenic H. parasuis strain at weaning and were treated with enrofloxacin, followed by homologous challenge with a high dose of H. parasuis. This model attempted to mimic field conditions of H. parasuis colonization, infection, and antibiotics use.
- There were 6 experimental groups: Three groups of pigs (EXP, EXP/ABT, ABT/EXP) were inoculated with a low dose of pathogenic H. parasuis (exposure) on day 0. Group ABT/EXP was also treated with enrofloxacin before inoculation, on day -3 of the study. Group EXP/ABT was treated with enrofloxacin 3 days post inoculation (DPI). Group ABT was treated with enrofloxacin 3 DPI but was not inoculated with low dose of pathogenic H. parasuis, and served as a control for the effect of the antibiotic treatment alone. Pigs in the group CHA were only inoculated with a high dose of H. parasuis on day 21 (challenge) and served as positive controls. Groups EXP, EXP/ABT, ABT/EXP and ABT were also challenged at 21 DPI. Pigs in the negative control group (NEG) were untreated, non-exposed, and non-challenged. At the termination of the study, all pigs were euthanized at either 25 or 35 DPI.
- Pigs were clinically evaluated throughout the study, samples collected for bacterial culture and seroconversion.



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## Results:

- After *H. parasuis* infection (low dose inoculation), clinical signs were observed in 6/10 pigs from the EXP group and 5/10 pigs from the ABT/EXP group (Table below). Pigs in the other four groups did not exhibit clinical signs. The proportion of pigs with clinical signs in groups EXP and ABT/EXP were not statistically different from each other, but were significantly different from those observed in the EXP/ABT and the non-exposed groups (ABT, CHA and NEG) ( $p < 0.05$ ).

Clinical outcome after low dose *Haemophilus parasuis* Nagasaki strain inoculation.

Groups	Number of inoculated pigs	Number of clinically affected pigs <sup>1</sup>	Days from inoculation to clinical signs
EXP	10	6 <sup>a</sup>	4,4,4,4,8,8
ABT/EXP	10	5 <sup>a</sup>	7,7,8,8,8
EXP/ABT	10	0 <sup>b</sup>	–
ABT	0	0 <sup>b</sup>	–
CHA	0	0 <sup>b</sup>	–
NEG	0	0 <sup>b</sup>	–

<sup>1</sup>Differences in superscripts indicate statistical differences at  $p < 0.05$ .

<sup>1</sup> Affected pigs included pigs that had clinical signs of fever  $>40^{\circ}\text{C}$ , prostration, swollen joints, respiratory distress, and lateral recumbency.

- Results of clinical outcomes after high dose challenge are presented in the next table. Pigs from the NEG, EXP and ABT/EXP groups did not exhibit any signs of *H. parasuis* disease. In contrast, 4 out of 10 pigs in the EXP/ABT and CHA groups and 8 out of 10 pigs in the ABT group had clinical signs of disease. The proportion of affected pigs in groups EXP/ABT, ABT and CHA was statistically higher when compared to non-affected pigs in EXP, ABT/EXP and NEG groups ( $p < 0.05$ ).

Clinical outcome after challenge by inoculating pigs with a high dose of *Haemophilus parasuis* Nagasaki strain.

Groups	Number of clinically affected pigs <sup>1</sup>	Days from challenge to clinical signs	Presence of lesions at necropsy
EXP	0/8 <sup>a</sup>	–	0/8 <sup>a</sup>
ABT/EXP	0/8 <sup>a</sup>	–	0/8 <sup>a</sup>
EXP/ABT	4/10 <sup>a</sup>	3,3,3,3	4/10 <sup>b</sup>
ABT	8/10 <sup>b</sup>	2,2,3,3,3,3,3,4	6/10 <sup>b</sup>
CHA	4/10 <sup>a</sup>	2,2,3,3,	4/10 <sup>b</sup>
NEG	0/10 <sup>a</sup>	–	0/10 <sup>a</sup>

<sup>a,b</sup>Distinct superscripts indicate differences among groups in each column ( $p < 0.05$ ).

<sup>1</sup> Affected pigs included pigs that had clinical signs of fever  $>40^{\circ}\text{C}$ , prostration, swollen joints, respiratory distress, and lateral recumbency.

- After *H. parasuis* infection (low dose inoculation), pigs from group EXP and ABT/EXP developed an IgG serological response and this response remained high after challenge. There was not a significant increase in IgG antibodies in pigs from EXP/ABT and control groups after low dose infection. However, at 14 days post challenge, IgG antibodies significantly increased in the group that was treated after *H. parasuis* infection. Levels of antigen specific IgG remained low in the NEG group throughout the study. The poor induction of antibodies in *H. parasuis* exposed animals treated with enrofloxacin correlated with decreased protection observed in this group. Serological results not shown.

## Conclusions and Implications:

- Our results indicated that enrofloxacin given 3 days after pigs were inoculated with a low dose exposure of *H. parasuis* controlled infection with pathogen, but these pigs were neither able to activate an immune response nor protected against subsequent high dose challenge. In contrast, enrofloxacin given 3 days before inoculation did not interfere with the development of an immune response and pigs being protected against challenge.
- In summary, we demonstrated that antibiotic treatment can alter the development of protective immune responses to *H. parasuis* and in this study, pigs only treated before *H. parasuis* infection seroconverted and were protected against subsequent challenge.
- Results from this study can help determine timing of antimicrobial use and contribute to our current understanding of judicious antibiotic use.