

# Comparative Genomics

## Manipulation of commensal gut microbiota as a tool to decrease respiratory disease in swine

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### Introduction:

Respiratory diseases continue to have an important welfare and economic impact for the swine industry worldwide. Enzootic pneumonia, the infection caused by *M. hyopneumoniae*, is regarded as highly prevalent and it is associated with major economic losses due to increased use of medication and decreased pig performance. Economic losses are also influenced by the impact that other infections of the respiratory tract have on production parameters. Several strategies can be used in order to manage *M. hyopneumoniae* infections and partial control can be achieved by vaccination, antibiotic treatment or application of management practices. However, there is a growing need for examining novel methods to generate earlier protection against *M. hyopneumoniae* in young pigs and to reduce the usage of antimicrobial drugs in livestock.

Recent investigations in the area of immune responses and their relationship with the gut microbiota have opened the door to new approaches for disease treatment and control. In this study, we explored the possibility of using a non-pathogenic oral inoculum to decrease the detrimental effect of *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) infection.

### Materials and Methods:

•All protocols for this experiment were approved and animals were cared for following the guidelines from the IACUC and IBC of the University of Illinois at Urbana-Champaign.

•Twelve piglets naturally farrowed to a SIV, PRRSV, and *M. hyopneumoniae* negative gilt were housed in controlled research units.

•Piglets were artificially raised and were fed bovine colostrum during the first 2 days of life, and a medicated milk replacer thereafter until week 4. An automated feeding system was put in place in order to deliver milk replacer to piglets every 60 min at a daily rate of 360mg/Kg of live weight. A partial view of the automated feeding system is shown in Fig 1. Pigs were gradually introduced to a commercial dry feed formula, which eventually replaced the liquid feeding.

•The experimental design for the study is depicted in Fig 2. Piglets were split into 2 groups at weaning. One group was orally exposed (E) to a non-pathogenic inoculum 1x/day over 1 week. The second group was left un-exposed (UE). The non-pathogenic inoculum consisted of a 1:1 mixture containing a fresh fecal slurry (obtained from a boar in a high health herd) and phosphate buffer saline. Four weeks after the oral exposure was completed, pigs were experimentally infected with 20 ml of 1x10<sup>5</sup> CCU/ml of *M. hyopneumoniae* strain 232 (Iowa State University, Ames, IA), using the intra-tracheal route of inoculation.



Fig. 1: Automatic feeding system for piglets.

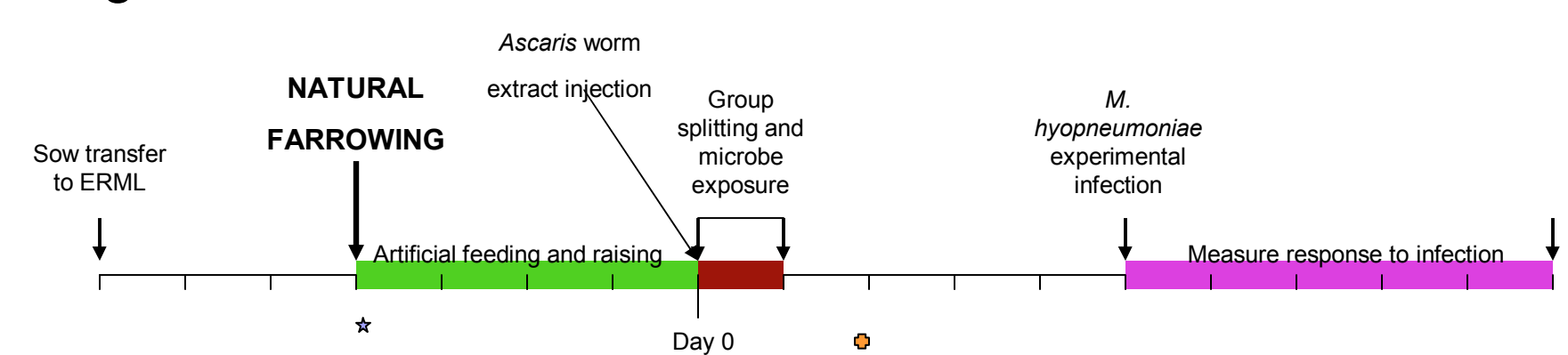


Fig. 2: Study experimental design.

•Experimental groups were evaluated daily for clinical signs (dry cough). Serum samples and nasal swabs were obtained from all pigs during the first weeks after infection. Weights were measured at 0, 15 and 22 dpi. Pigs were humanely euthanized 5 weeks after infection and lung lesions were blindly scored. Lung tissue samples, bronchial swabs and lung lavage were collected at euthanasia.

•Antibodies to *M. hyopneumoniae* and to porcine C reactive protein were identified in serum samples using the ELISA tests DAKO (Feld et al., 1992) and Tridelta Development Limited (Bray, Ireland), respectively.

Porcine specific cytokines were determined in lung lavage using a custom multiplex ELISA test (Aushon Biosystems Inc., Billerica, MA).

•DNA was extracted from nasal and bronchial swabs using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Bacterial load was quantified from swabs using a Real Time PCR test (VDL, University of Minnesota).

•Data was evaluated using the Students *t* test and Kruskal-Wallis one way analysis of variance, where appropriate. The proportion of seropositive pigs was compared using a Hypothesis test. The pig was the experimental unit for all comparisons.

### Results:

•All animals were negative to *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), porcine respiratory and reproductive virus (PRRSv) and swine influenza virus (SIV), as confirmed by a herd history of high health and the absence of antibodies by ELISA for such agents at the beginning of the study. All pigs remained negative to PRRSV and SIV throughout the experiment. This was demonstrated by the lack of clinical signs and the negative ELISA test results for the detection of antibodies for both pathogens.

•No GI tract clinical signs were observed in pigs before or after the oral inoculation.

•Experimentally infected pigs in group E seroconverted to *M. hyopneumoniae* as early as 9 dpi, while all pigs in groups E and UE had seroconverted by day 14 after infection, as shown in Fig 3. Onset of clinical signs differed between the groups, being earlier for the UE group (9 dpi) than for the E group (12 dpi).

•Microscopically examined lungs of all pigs showed classical *M. hyopneumoniae* lesions, evidenced by a perivascular and peribronchiolar lymphocyte infiltration, increased number of mononuclear and polymorphonuclear cells in alveoli and lymphoid nodules associated with the airways.

•The number of dry coughs suggestive of *M. hyopneumoniae* infection was greater ( $p < 0.005$ ) in the UE group than in the E group (Fig. 4).

•Microscopic lung lesions suggestive of *M. hyopneumoniae* infection were apparent in all research subjects. Pigs in the UE group tended to have greater ( $p = 0.07$ ) lung lesion scores than pigs in the E group, as shown in Fig 5.

•The bacterial load assessed in the nasal and bronchial swabs and broncho-alveolar lung lavage (BALF) was similar between experimental groups throughout the study ( $p > 0.05$ ).

•Concentration of porcine IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$  in BALF were similar between the two groups. However, TNF- $\alpha$  concentration was very similar in pigs from the E group, while it was more spread and tended to be higher in pigs from the UE experimental group (Fig. 6).

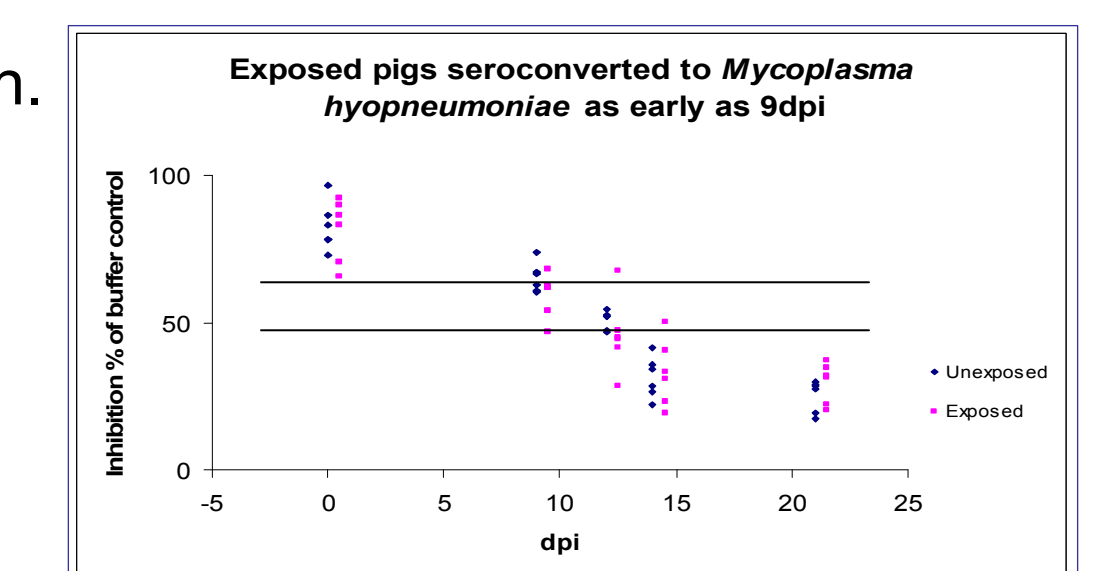


Fig 3: Seroconversion to *M. hyopneumoniae* in experimentally infected pigs. Inhibition between 50% and 65% is suspect, < 50% denotes seropositivity.

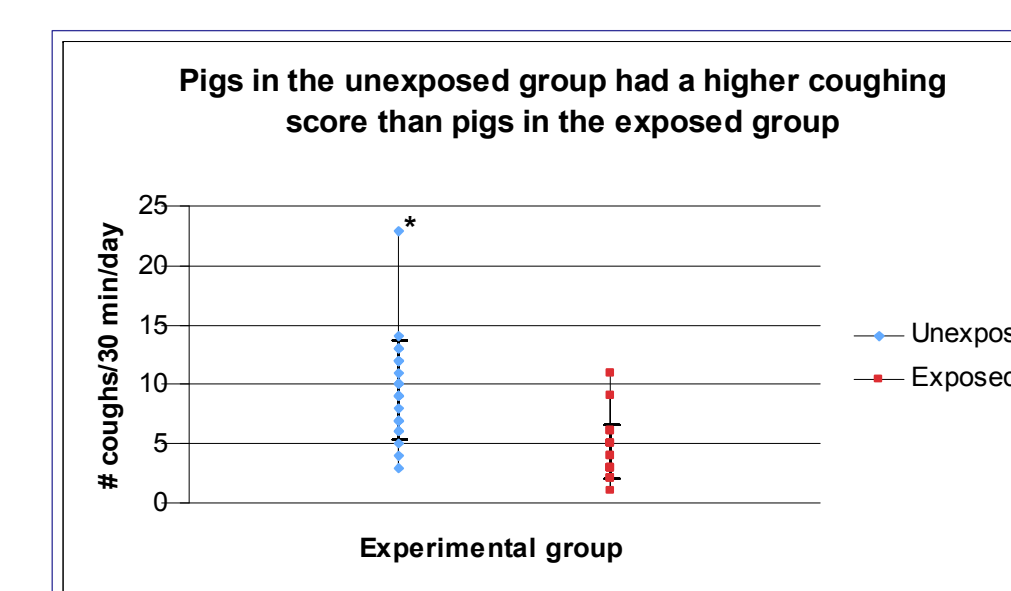


Fig. 4: Coughing score of experimentally infected pigs ( $p < 0.05$ ).

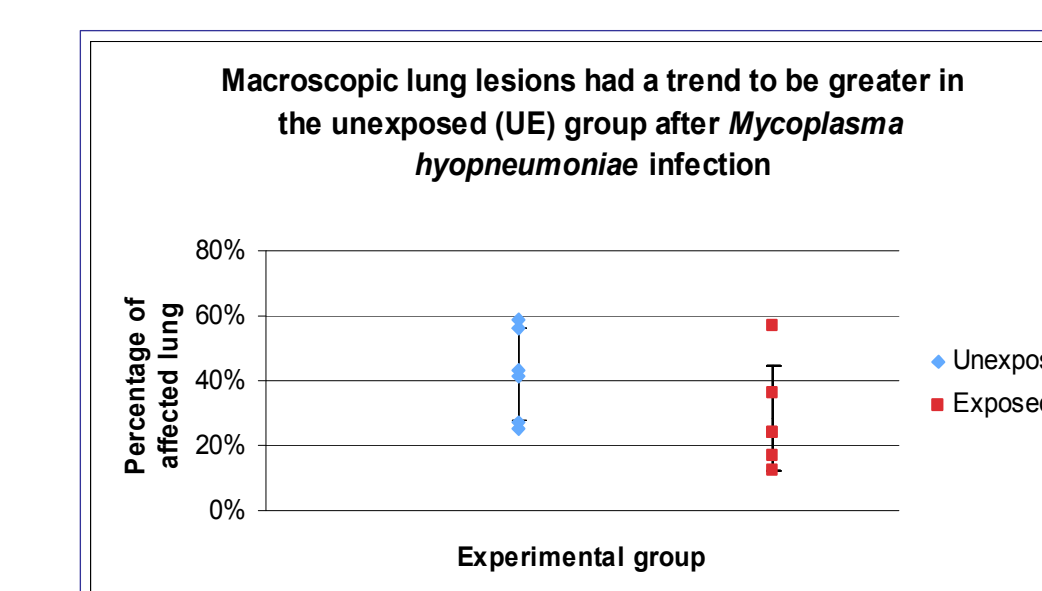


Fig. 5: Macroscopic lung lesions of pigs infected with *M. hyopneumoniae* ( $p = 0.07$ ).

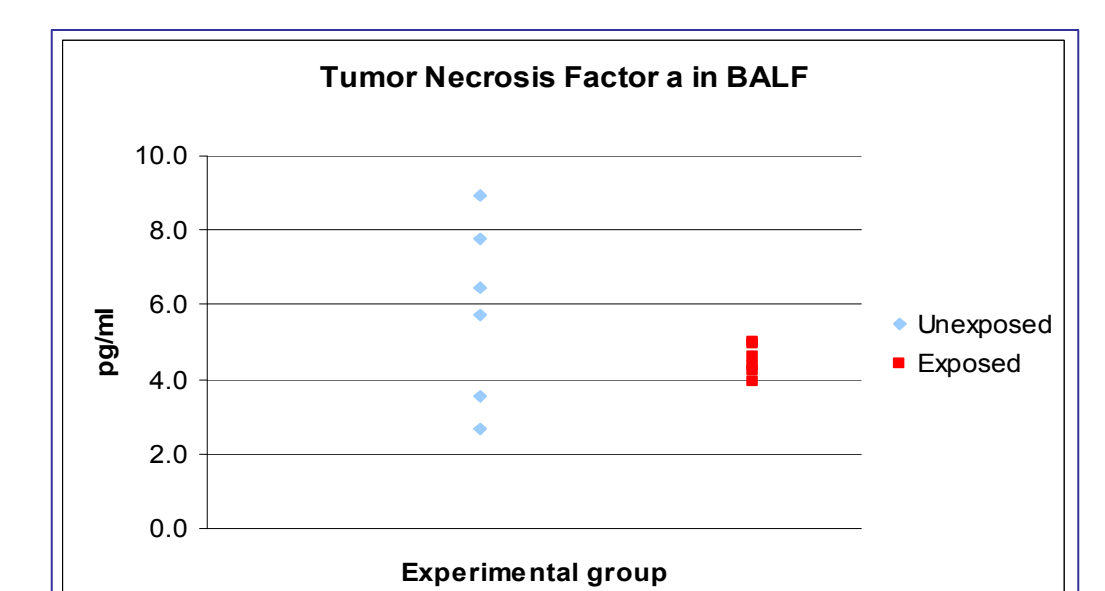


Fig. 6: TNF- $\alpha$  concentration in BALF (35 dpi).

### Conclusions and Implications:

Under the conditions of this study, pigs in the E group had less respiratory clinical signs and lesions, and seroconverted earlier than pigs in the UE group, suggesting a beneficial effect of the oral non-pathogenic inoculation on the severity of mycoplasmal pneumonia. In this investigation, we have tested the effect of gut microbiota manipulation involving a solely respiratory infection. Therefore, other studies involving multiple infectious agents may be needed before field implementation of this technique. It is also important to note that this model of oral non-pathogenic inoculation does not go against all-in/all-out management practices, neither suggests stopping its application, but implies a feedback from older animals (extremely healthy), during early stages of pig life.

### References:

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