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PathoCHIP Identification of Genes Critical in Host/Pathogen Interactions During *H parasuis* Infection

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Introduction and Objective

Haemophilus parasuis is becoming increasingly important as a swine pathogen as high-health, multi-site production systems expand. In the Americas, *H. parasuis* is a "high health" disease even in conventional health sow herds. Current management and vaccination strategies often break down, and alternative solutions are necessary. The **PathoCHIP** (from *pathos*: disease and chip: microchip) project is set up to identify genes of the host and pathogen that are critical during infection with *Haemophilus parasuis* and eventually with other swine pathogens.

Material and Methods

Colostrum-deprived pigs originating from a commercial herd positive to *H. parasuis* antibodies were raised as previously described (1) and challenged intratracheally with a pathogenic *H. parasuis* serovar 5 strain. Experiments took place following EC welfare regulations. Preliminary studies were performed to identify the optimal challenge dose. Doses of 10^4, 10^6, and 10^8 CFU of bacteria were tested and a final dose of 10^8 CFU of *H. parasuis* was chosen for further inoculations. Experimental animals were challenged at 21 days of age in groups of 20 pigs in consecutive months. Each experiment included pigs either challenged with bacteria or mock inoculated controls. Pigs were killed at days 1, 2, and 3 post-infection. Clinical signs were recorded from infection to death. From each pig, bacterial detection was attempted using an *H. parasuis* PCR (2) and standard bacterial isolation from tonsil, lung, serosas, and brain/meninges. Lesions were recorded during the post mortem evaluation. Susceptibility to *H. parasuis* disease was assessed for all pigs. Fully susceptible animals were defined as those pigs that were positive for PCR and bacterial isolations in all tissues and presenting clinical signs and lesions characteristic of disease. Tissues were kept in RNAlater (Ambion) for further recovery of host and bacterial RNA. Normalized and SSH libraries will be used in conjuction with microarray technology to find differentially expressed genes. Sequencing of these differentially expressed genes, both in the pathogen and the host, together with bioinformatics, will be used to identify genes, encoded proteins and the biological pathways that they control.

Results and Discussion

Antibodies play an important role in protection against *H. parasuis* disease. To identify underlying genetic factors involved in resistance to disease, pigs free of *H. parasuis* antibodies were required and therefore a colostrum-deprived model was established. The average survival rate of colostrum-deprived pigs tested was 88%. One hundred and forty experimental pigs have been tested so far (challenge experiments are ongoing). As expected, pigs were susceptible to *H. parasuis* infection showing typical responses such as fever, lameness, respiratory, and neurological signs and lesions. Pigs that met the criteria of fully susceptible were identified. However, a large number of pigs presented a broad range of responses to the challenge, suggesting different grades of susceptibility. Tissues from infected and control pigs have been stored and will be used for gene expression studies using microarrays to identify genes involved with susceptibility to disease at different times post infection.

The *H. parasuis* array so far consists of 5500 clones selected from a genomic library of 10,000 clones. This is expected to give 2-3 fold coverage of the bacterial genome that has about 1,800 gene products. The *H parasuis* library has been printed with success on the array and some preliminary testing has been performed (Figure II). A host library comprising 9,000 clones has been printed on an array. A normalized library has been constructed according to methods previously described (3,4) and will also be used in host array. This library will be used to reduce high abundance genes and increase low abundance genes to ensure thorough analysis of gene expression. Tissues of pigs challenged with bacteria and control pigs will be tested on the bacterial and host arrays. An additional SSH library of genes differentially expressed during *H. parasuis* infection will also be made and fully sequenced. The experimental design is shown in Figure I. This work is in progress and an update will be given during the presentation.

In the absence of maternal antibodies pigs showing various degrees of susceptibility were found up to 3 days post infection. These findings suggest that there may be genetic factors involved in the development of *H.* parasuis disease. The genes and biological pathways involved are under investigation. Microarrays are a

recent development in genomics and the PathoCHIP project will be one of the first applications of this technology in pigs. This innovative technology allows the study of thousands of genes simultaneously on a glass microscope slide. This is a large step forward over past methods where only few genes could be studied in any single experiment. Microarrays developed in this project could be useful for investigation of other important pig pathogens, including PRRSv, *Mycoplasma hyopneumoniae* and others. Genes identified using microarray technology could play an important role in improving pig health and therefore pig welfare.

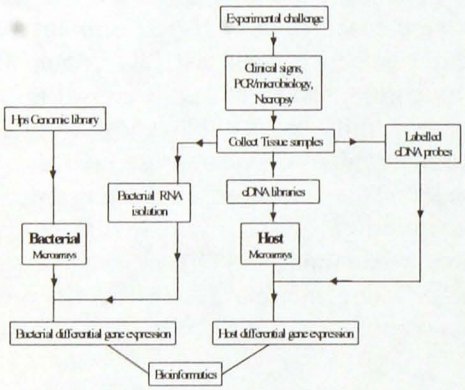

Figure I. Experimental design of PathoCHIP project.

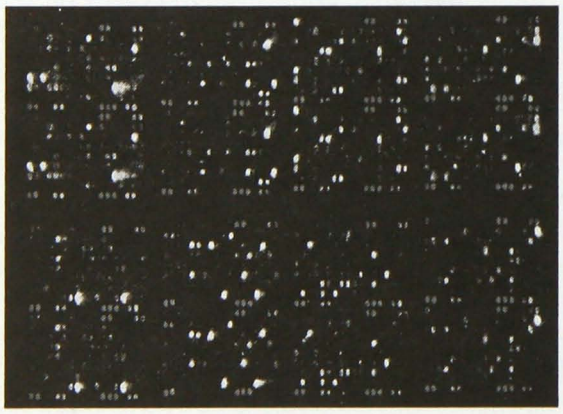

Figure II *H. parasuis* array comparing *H.parasuis* control RNA vs. iron-limited *H.parasuis* RNA. Iron limited bacteria have much higher induced expression, giving the array an overall 'green' appearance. The yellow spots reflect similar levels of gene expression between control and experimental condition. Red spots and adjacent green spots are the array controls, indicating the subsections of the grid. Each subarray is in duplicate.

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