Characterization of the bacterial gut microbiota in new neonatal porcine diarrhoea - DTU Orbit (14/10/2019)

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During the last decade farmers and veterinarians have reported the emergence of a new neonatal porcine diarrhoea (NNPD) affecting piglets up to 7 days old. Routine laboratory testing for common pathogens are inconclusive and vaccination and treatment with antibiotics or alternative zootechnical interventions have limited effect. NNPD is not associated with an increased mortality, but have been reported to cause significant morbidity within herds and litters.

Piglets born to gilts are in particularly affected by NNPD. NNPD impairs the welfare of the piglets, and results in decreased weight gain which is of economic importance to the farmer. Despite the limited effect of antibiotics, farmers often treat affected piglets with antibiotics to prevent secondary infections to NNPD resulting in increased consumption of antibiotics. Thus, there are several encouraging reasons for identifying the aetiology behind NNPD. Consequently an interdisciplinary project called: “New neonatal porcine diarrhoea in Denmark. Elucidation of aetiology, diagnostics, and effect of treatments” (freely translated) was initiated. The project enrolled three PhD students with different approaches and hypotheses. The aim of this project was to investigate whether the aetiology to NNPD could be identified in the bacterial gut microbial changes.

In order to be able to characterize the bacterial gut microbiota of numerous samples simultaneously the Gut Microbiotassay was developed. This is an assembly of 24 different primer sets targeting 16S or 23S rRNA genes of the major bacterial groups constituting the gut microbiota. This approach was applied due to the limited number of intestinal bacteria that currently can be cultivated. Primers were found in published literature, tested in silico and modified or designed if necessary. The Gut Microbiotassay was optimized for the high-throughput quantitative real-time PCR-based 48.48 Access Array™ Integrated Fluidic Circuit (Fluidigm). The efficiency and sensitivity of the primer sets were tested against 15 different pure-cultured bacterial strains. Finally the Gut Microbiotassay was tested on DNA extracted from ileal or colonic contents from piglets with or without NNPD and verified via 454 next generation sequencing of the PCR amplicons. Bioinformatics was conducted using BION-meta customized for this specific setup.

With the Gut Microbiotassay in place gut microbial profiles of ileal and colonic contents of 50 control piglets and 52 case piglets from four Danish pig farms affected by NNPD were obtained and deep taxonomic insight was acquired by sequencing the PCR amplicons. Statistic results from qPCR data revealed that the gut microbiota of NNPD-affected piglets differed from that of control piglets by a depletion of the phyla Firmicutes, Bacteroidetes, and Actinobacteria, while the numbers of genus Enterococcus and the class Beta- and Gammaproteobacteria (including family Enterobacteriaceae and species Escherichia coli), but also phylum Fusobacteria were elevated. Moreover, piglet born to gilts possessed more members from family Enterobacteriaceae including species E. coli and a reduced number of bacteria from phylum Firmicutes. Piglets born to gilts were estimated to have 25 higher odds of being affected by NNPD. Sequence results revealed genus Enterococcus to be comprised of high read numbers of species Enterococcus hirae but also Enterococcus durans. Conversely, particularly Lactobacillus acidophilus was scarcely represented in piglets suffering from NNPD.

As part of one of the other enrolled PhD projects a NNPD-infection model was established by inoculating healthy neonate piglets with intestinal NNPD Material (case piglets) or healthy intestinal material (control piglets), while some piglets not inoculated. Diarrhoea was successfully reproduced in case piglets while control piglets remained healthy. In order to assess whether the diarrhoea was characterized by similar gut microbial changes as detected for field cases of NNPD, ileal and colonic intestinal contents from 49 case piglets (13 un-inoculated) and 32 control piglets (18 un-inoculated) were analyzed using the Gut Microbiotassay. The corresponding regulation of selected intestinal genes involved in diarrhoea was examined for a subset of piglets by qPCR using the 96.96 Dynamic Array™ Integrated Fluidic Circuits (Fluidigm). Similar to NNPD-field cases the gut microbiota of case piglets were characterized by reduced numbers of the phyla Firmicutes, Bacteroidetes, and Actinobacteria. Furthermore, they were inhabited by increased numbers of genus Enterococcus as well as class Beta- and Gammaproteobacteria including species E. coli. The expression of several genes involved in recognition of pathogen-associated molecular patterns, inflammation, and intestinal barrier function were significantly up- or down-regulated reflecting the complex immunological response to being inoculated and/or infected with NNPD-material. Finally, a high abundance of genus Enterococcus (characteristic of case piglets) was associated with high expressions of several transcripts involved in epithelial integrity.

Altogether, the results of the studies included in this thesis reveal that NNPD is associated with a disturbed gut microbial composition, and all points towards members from the genus Enterococcus are involved in the pathogenesis of NNPD.

**General information**

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