New Neonatal Porcine Diarrhea Syndrome in Denmark Characterization of the intestinal lesions and identification of the etiology - DTU Orbit (28/09/2019)

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In recent years, a new form of porcine neonatal diarrhea, termed 'New Neonatal Porcine Diarrhea Syndrome' (NNPDS) has been reported from several European countries including Denmark. This condition is characterized by watery to creamy, non-hemorrhagic diarrhea occurring during the first week of life, high morbidity and relatively low mortality and weak or no response to the antibiotic treatment. This diarrhea affects pig farms despite good management practice and routine sows vaccination. None of well-known enteric pathogens causing neonatal porcine diarrhea has so far been associated with this syndrome. The main focus of the work presented in this PhD thesis was to characterize the morphological changes in the intestinal mucosa of the piglets affected by NNPDS in respect to elucidation of the pathological mechanism and etiology. This was based on systematically performed microscopic investigation of the intestinal tissue from the diarrheic and non-diarrheic piglets collected from four herds affected by NNPDS. This investigation included histopathological, morphometrical, immunohistochemical and fluorescence in situ hybridization examination of the intestinal mucosa. In the second part of the PhD project, an animal model of NNPDS has been established. This model can provide basis for future studies on the pathogenesis of this new syndrome.

The results of the histopathological investigation performed on 51 diarrheic and 50 non-diarrheic piglets showed that 63% of the diarrheic piglets developed villus atrophy of various degrees with concomitant crypt hyperplasia in the jejunum and ileum (Chapter 4.1). Villus atrophy is a common pathological feature seen in numerous infectious intestinal conditions and is associated with malabsorptive diarrhea due to insufficient absorption of water and nutrients from the small intestine. The morphometric study performed on the intestinal tissue showed that the diarrheic piglets had significantly shorter villi, deeper crypts, and thinner mucosa in the jejunum and ileum compared to the non-diarrheic piglets (Manuscript I). Reduction in villus height led to decrease in villus/crypt ratios which suggest an adverse effect on gut absorptive functions. Intestinal tissue samples from 24 representative piglets were subjected for immunohistochemical study on cell proliferation and apoptosis (Manuscript II). The results of this investigation showed that villus atrophy was associated with enlargement of the proliferative compartment in the crypts and that epithelial cell turnover was enhanced in the diarrheic piglets. Potentially pathogenic bacteria such as Escherichia coli, Enterococcus spp., Clostridium perfringens and Clostridium difficile have been proposed to be involved in NNPDS. In order to elucidate their role in the pathogenesis of the syndrome, fluorescence in situ hybridization (FISH) with oligonucleotide probes targeting rRNA specific for these bacteria was applied on the formalin fixed, paraffin embedded intestinal tissue samples (Manuscript III). The results of this investigation showed that adherent E. coli and Enterococcus spp. were involved in NNPDS. These bacteria were present in 37% of the diarrheic piglets and were associated with villus atrophy and epithelial lesions in the small intestine. No clear association between the presence of C. perfringens and C. difficile and diarrhea was shown by FISH investigation. In order to provide basis for further studies on the pathogenesis of the syndrome, an animal model of NNPDS has been established (Manuscript IV). Eleven litters of newborn piglets were orally challenged with intestinal tissue homogenate derived from four diarrheic field herds previously investigated in the project and one healthy herd. Diarrhea was successfully reproduced in piglets from six litters and these piglets developed pathological lesions similar to those seen in the natural infection. Neither piglets challenged with the homogenate from the healthy herd, nor with the filtrate deprived of bacteria of three case homogenates developed diarrhea. This indicates that NNPDS is of a bacterial, rather than a viral nature. The course of experimentally induced diarrhea differed in severity of the symptoms and histological lesions depending on the inoculum used in the experiment, suggesting that herd-related factors may influence the clinical outcome of NNPDS. Taken together, the work described in this thesis contributed to the knowledge on the pathogenesis of NNPDS, however the exact etiology of the syndrome has not been determined. Furthermore, this study has provided a reproducible model of NNPDS that can be the basis for future investigations on this emerging syndrome in newborn piglets.