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Histopathological, morphometrical and FISH investigation of New Neonatal Porcine Diarrhea in Denmark

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In recent years a new kind of neonatal diarrhea of unknown etiology has been identified in swine herds in Denmark[1]. The aim of the study was to clarify the pathomechanism of this disease by characterization of the morphological lesions in the intestinal mucosa with respect to determination of possible infectious or non-infectious nature.

Materials and methods

Intestinal tissue samples were collected from 51 diarrheic and 50 control piglets aged two-seven days (age matched) from four Danish pig herds affected by New Neonatal Porcine Diarrhea (NNPD). Sections of duodenum, jejunum, ileum and colon from each piglet were examined histopathologically, morphometrically and by fluorescence in situ hybridization (FISH) with 16S rRNA targeted oligonucleotide probes for *Domain bacteria*, *Escherichia coli*, *Clostridium perfringens* and *Clostridium difficile.*

Histopathology results

1. 63% of diarrheic piglets had villous atrophy of various degrees with intestinal crypts hyperplasia (Fig.1B,D).
2. Villous atrophy was occasionally associated with mild epithelial lesions (Fig.1F).
3. 33% of diarrheic and 30 % of control piglets had local slight neutrophil infiltration in the lamina propria.

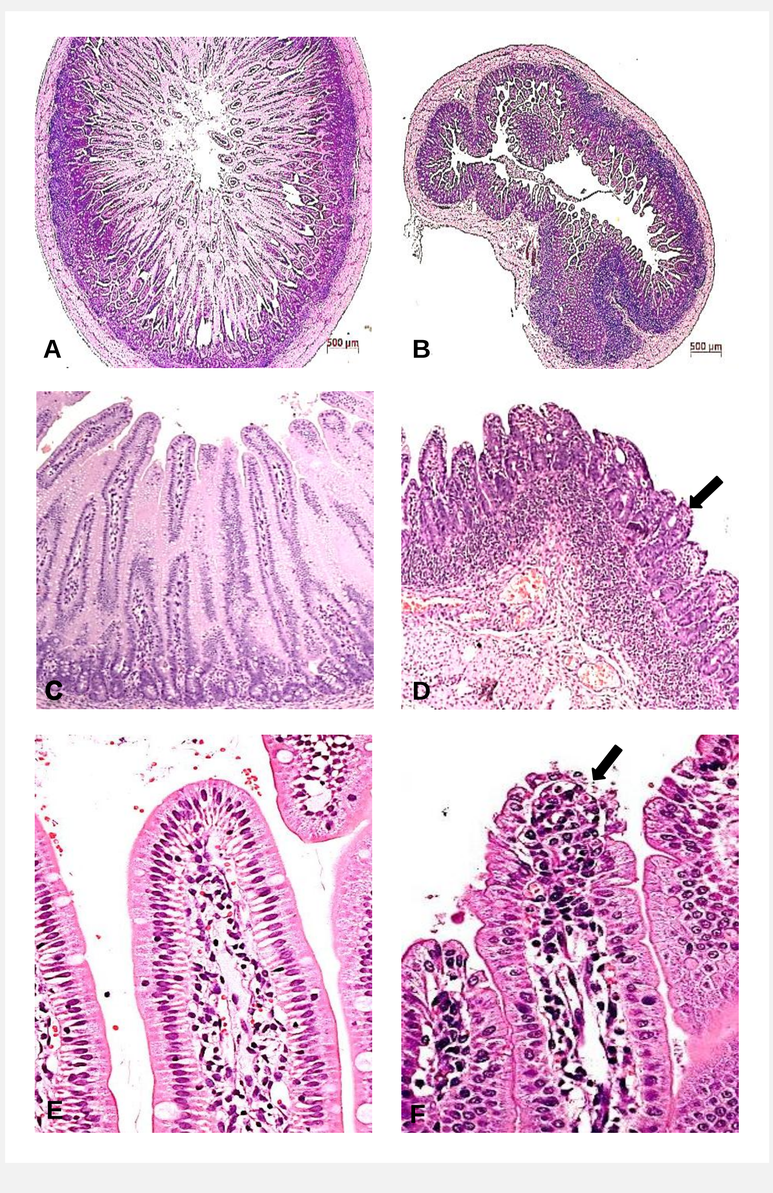

Fig.1 **A-D** Light micrographs of the intestinal mucosa in the ileum of 5-day-old piglets, HE, obj. 2,5x (A,B) and 10x (C,D).
A,C. Normal ileal mucosa in a control piglet.
B,D. Short and blunt villi (arrow) in a diarrheic piglet.
E,F. Light micrographs of the intestinal villous from the duodenum. HE,obj. 40x.
E. Normal simple columnar epithelium in a control piglet.
F. Flattened, necrotic epithelium lining the tip of the villous in a diarrheic piglet (arrow).

Morphometry results

Diarrheic piglets had significantly shorter villi, deeper crypts of Lieberkühn, thinner mucosa, lower villous/crypt ratios in jejunum and ileum and longer crypts in colon compared to control piglets (Fig.2)

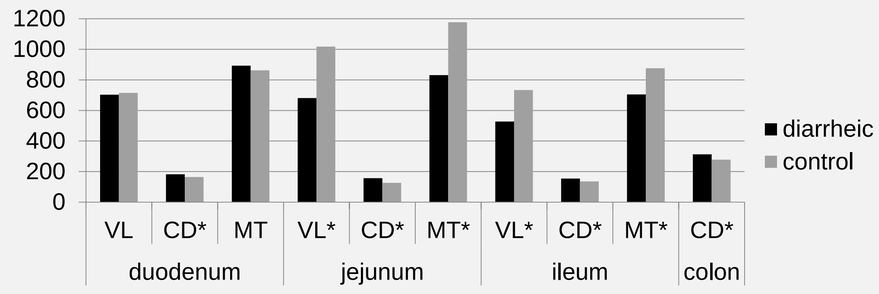

Fig.2 Mean values (µm) of villi length (VL), crypts depth (CD) and mucosa thickness (MT) in piglets. * p< 0,05)

FISH results

1. The prevalence and quantities of *E. coli*, *Cl. perfringens* and *Cl. difficile* in diarrheic piglets was similar to those in control piglets (Fig.3).
2. Slightly higher number of diarrheic piglets had moderate-large amounts of *E. coli* compared to control piglets.
3. There was no obvious correlation between the pathological changes and presence of these bacteria in the intestinal tissue.

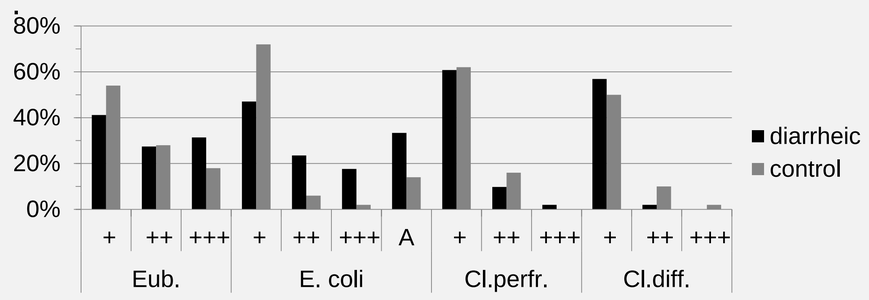

Fig.3 Results of semiquantitative analyses of fluorescence signals for *Domain bacteria (Eub)*, *E. coli* and *Cl. perfringens* in small intestines and *Cl. difficile* in colon.
(+) small, (++) moderate, (+++) large amounts of bacteria, (A) adherent *E. coli.*

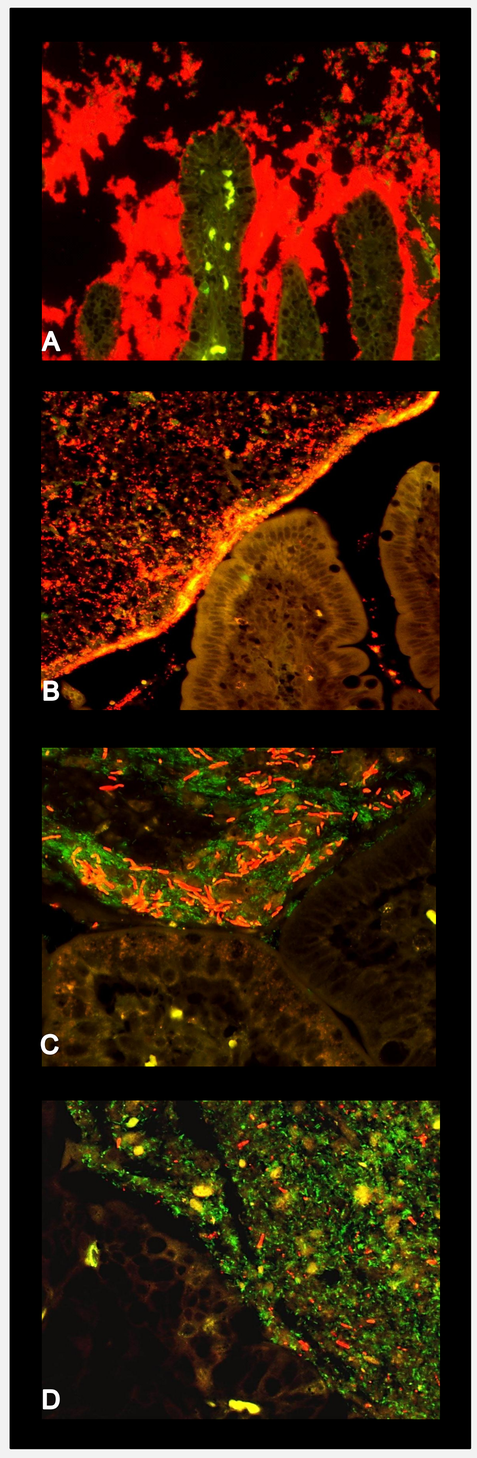

Fig.4 **A-D** Fluorescent in situ hybridization of intestinal mucosa.
A,B. Double hybridization for *Domain bacteria* (green) and *E. coli* (red) in ileum.
A- diarrheic, **B**- control piglet.
C. Double hybridization for *Domain bacteria* (green) and *Cl. perfringens* (red), ileum, control piglet.
D. Double hybridization for *Domain bacteria* (green) and *Cl. difficile* (red), colon, diarrheic piglet.

Conclusions

The present study indicates an association between clinical symptoms and shortening of the intestinal villi and shows that the piglets affected with NNPD differ from healthy individuals in the intestinal morphology. However, the quantity of potentially pathogenic bacteria identified by fluorescence in situ hybridization was lower than one would associate with bacterial infection in diarrheic condition. In order to determine the exact nature and certain etiology of NNPD further investigations are necessary.

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1. Svensmark, B. 2009. New neonatal diarrhoea syndrome in Denmark. Proceedings of 1st European Symposium on Porcine Health Management, Copenhagen, Denmark, August 2009. p27.