

A microscopic image of intestinal tissue, likely from a pig, showing a cross-section of the mucosal layer. The surface is covered with a single layer of columnar epithelial cells. A specific region of this epithelium is highlighted with a black and red outline, showing a more complex, possibly folded or damaged structure. The background is filled with numerous small, clear, circular structures, likely mucus or secretions, and some larger, more irregular structures. The overall color palette is dominated by shades of pink, red, and orange.

**New Neonatal Porcine Diarrhea  
under the microscope**

**(Herd 2, 3, 4)**

**Beata Jonach**

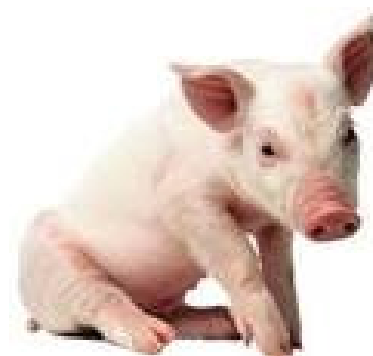
**DTU National Veterinary Institute**

## Ph.D project

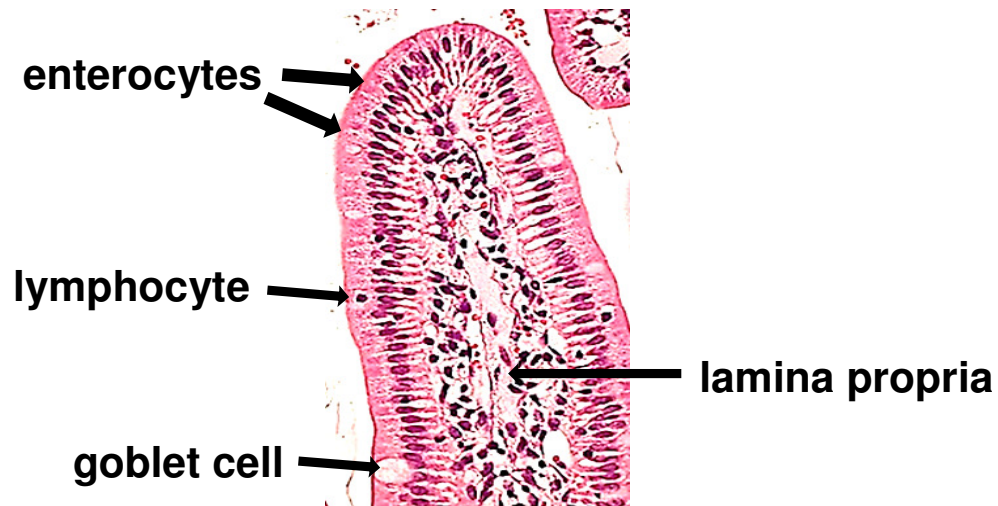
supervisors: Tim Kåre Jensen, Mette Boye (DTU VET)

### **New Neonatal Porcine Diarrhea in Denmark. Characterization of the intestinal lesions and identification of the etiology.**

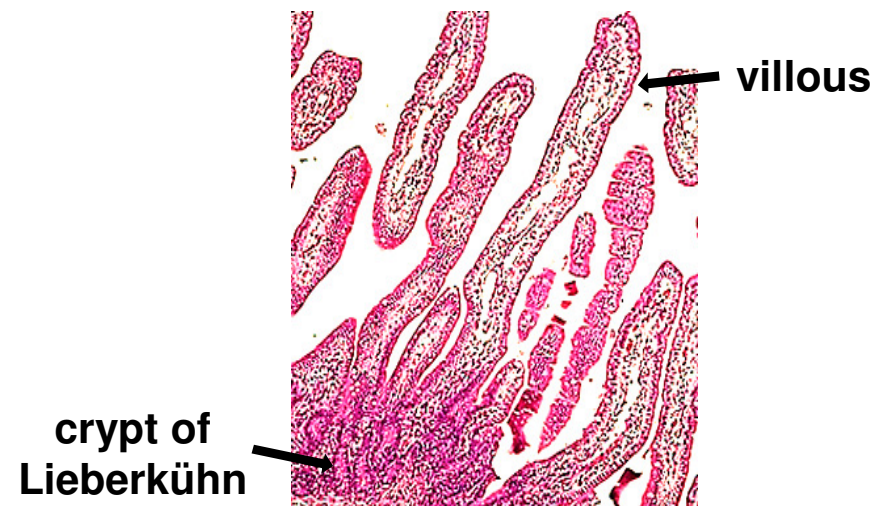
- Histopathology
- Morphometry
- Fluorescence In Situ Hybridization (FISH)



## Histopathological examination



- villous and crypts structure
- epithelium structure
- infiltration of the lamina propria
- goblet cells, intraepithelial lymphocytes
- inflammation
- necrosis



## Pathological findings: Villous atrophy (62% diarrheic piglets)



**diarrheic piglet**

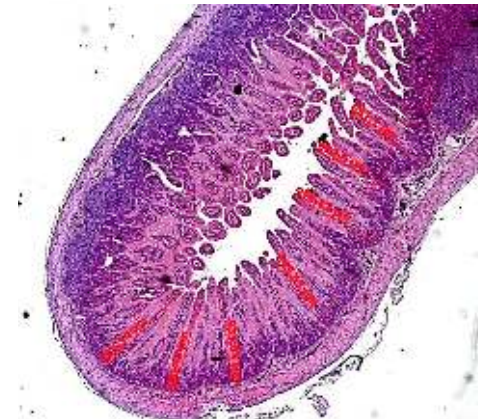
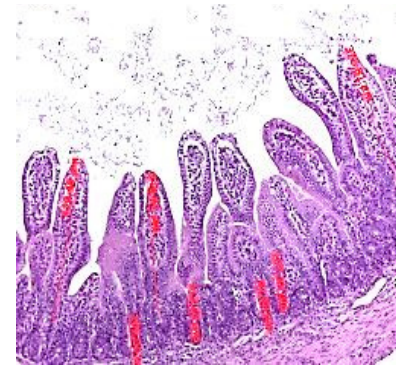
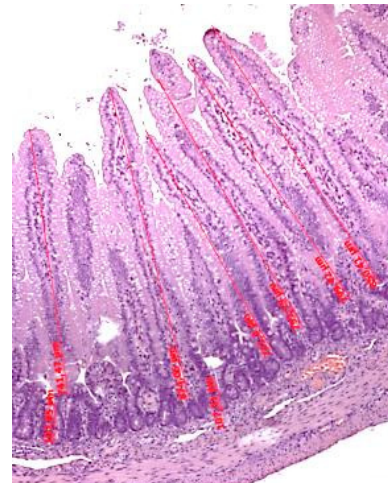


**control piglet**

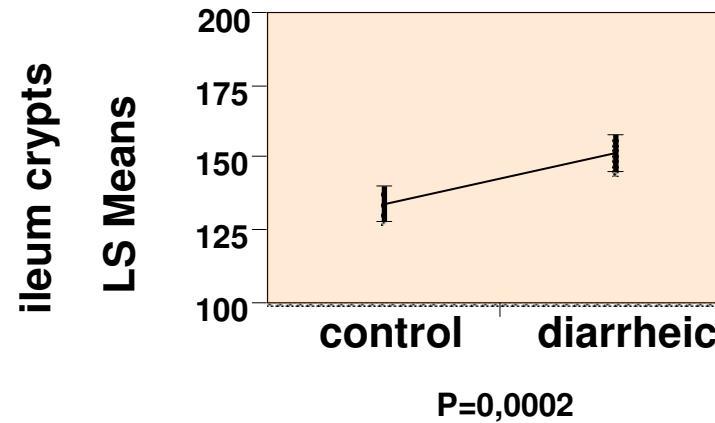
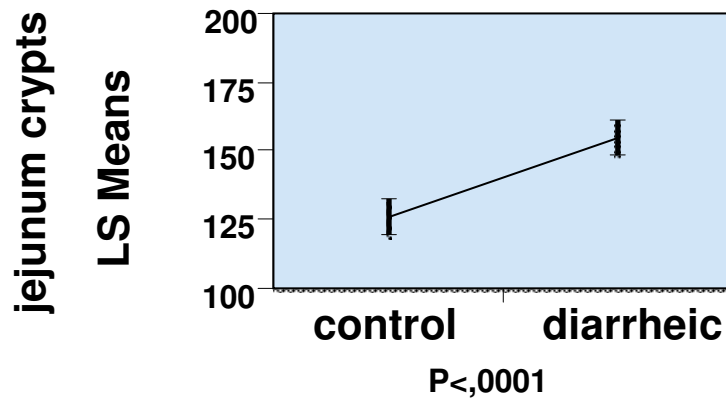
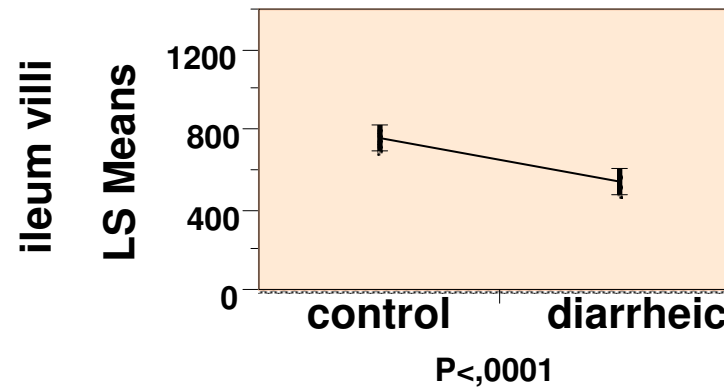
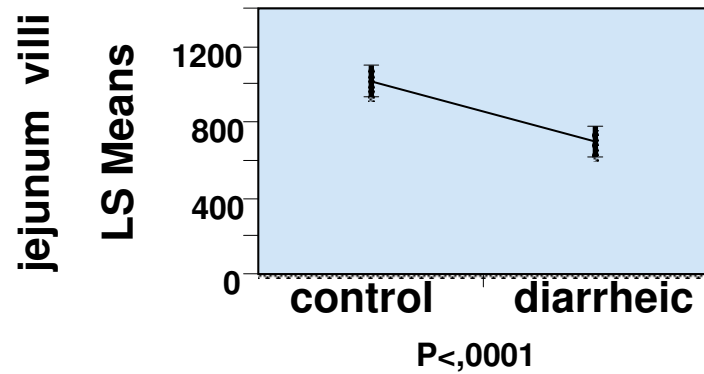
- ✓ Short, blunt villi
- ✓ Flattened- low columnar/cuboidal/squamous epithelium
- ✓ Increased cellularity of the lamina propria
- ✓ Hyperplastic crypts
- ✓ Other pathological changes (congestion, edema, lacteal dilatation, hemorrhages, enterocyte vacuolation)

## Morphometry

- Villous length
- Crypt depth
- Mucosa thickness
- Mean villi length
- Villous/crypt ratio



# Morphometry



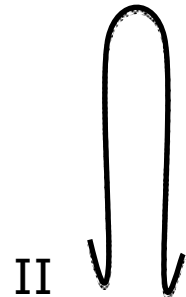
## Villi and crypts length in jejunum and ileum



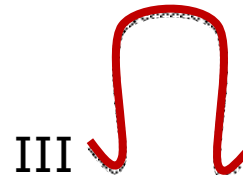
# Morphometry



**I**  
**Normal**



**II**  
**Hypotrophy**



**III**  
**Mild atrophy**



**IV**  
**Moderate-severe atrophy**

Jejunum 1000-1200 $\mu$ m  
Ileum 700-900 $\mu$ m

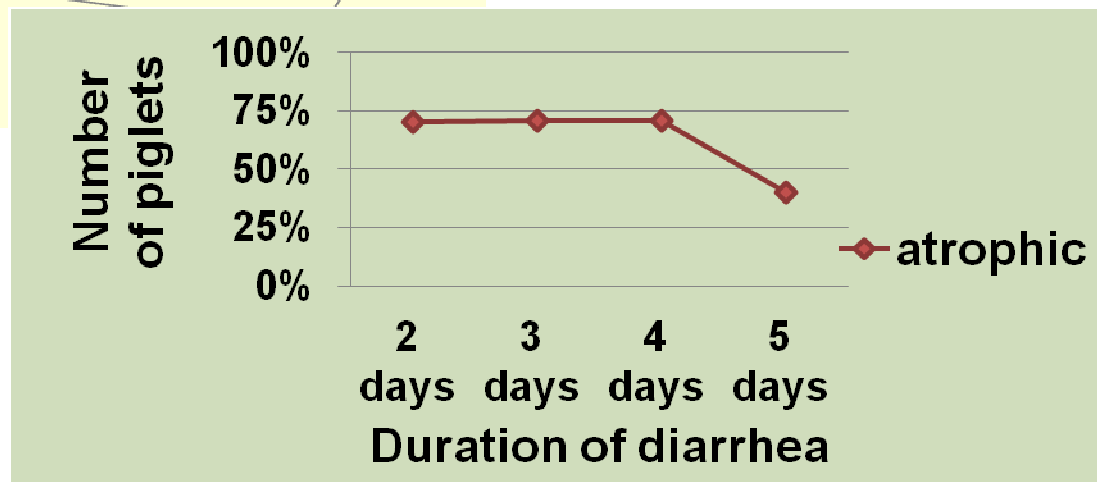
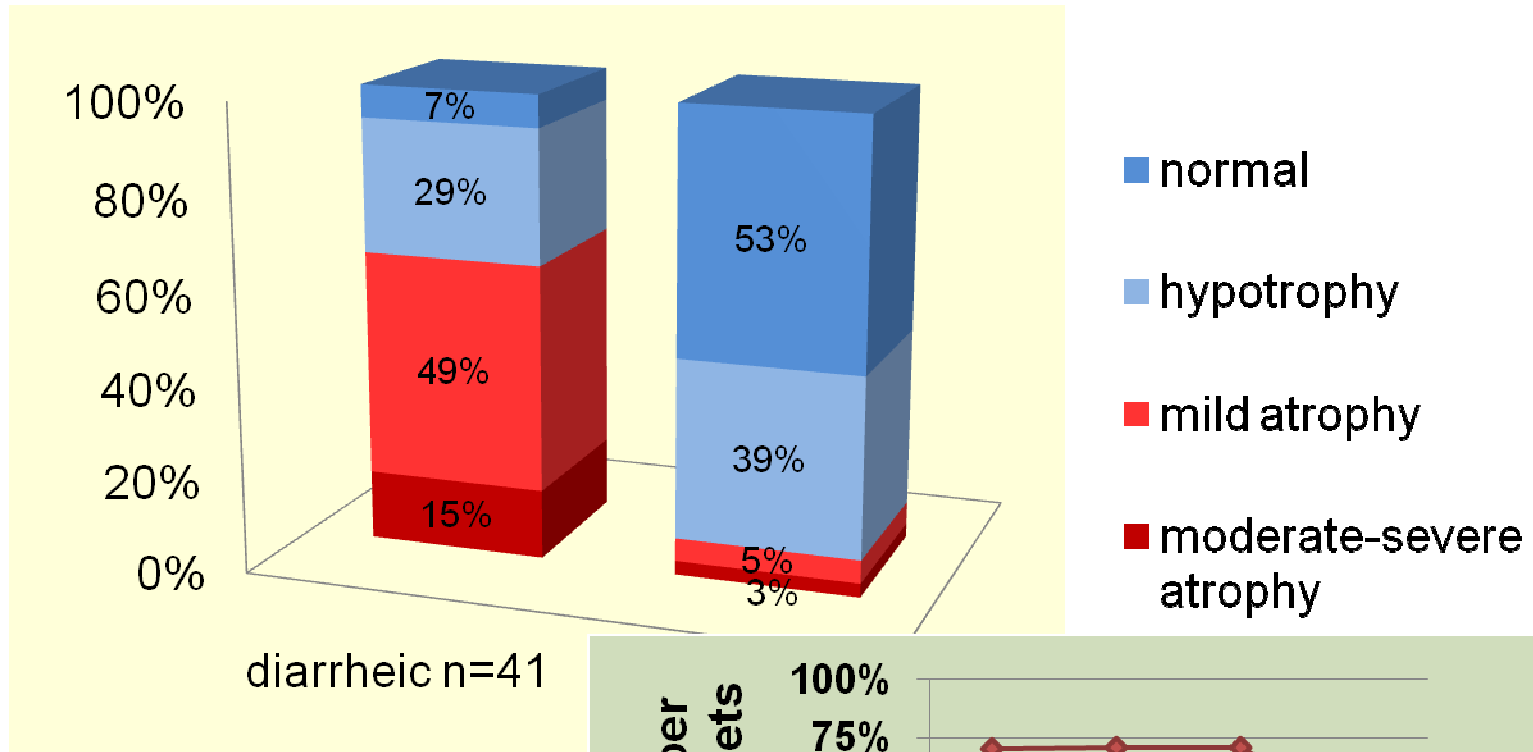
60-90%

30-60%

<30%

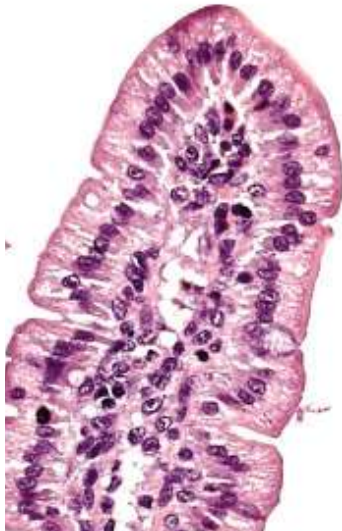
## Classification of villi based on mean villi length and structural lesions

## Villi length alterations in piglets (based on the higher/worse classification score)

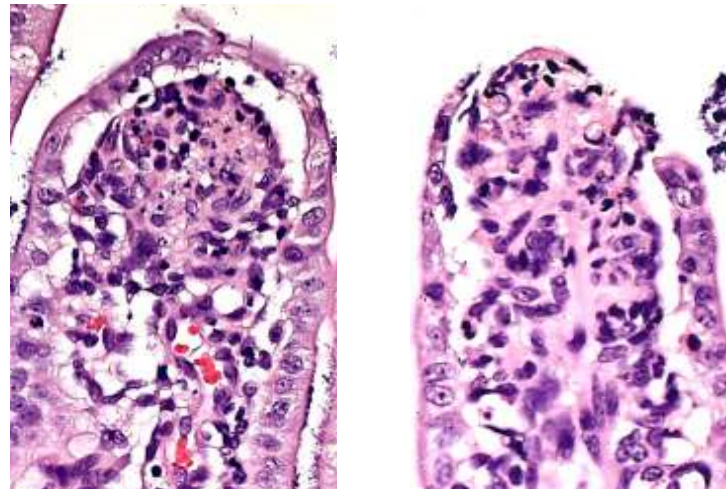




## Pathological findings



**normal epithelium**

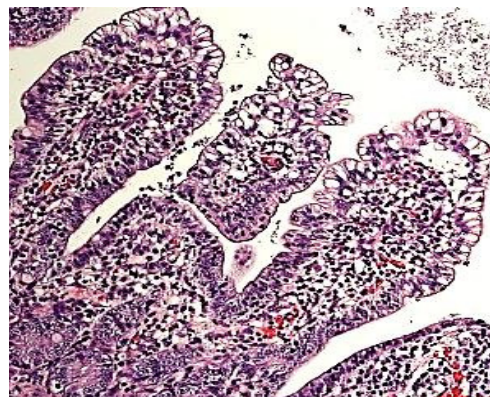


**Epithelial lesions  
(31% diarrheic, 16% control piglets)**

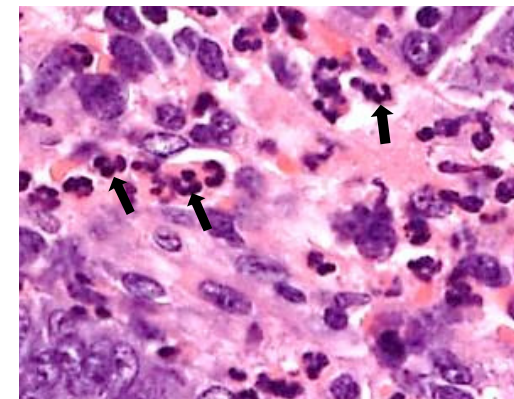
## Pathological findings



**normal cellularity**



**increased cellularity**



**neutrophil granulocytes**

**Slight local infiltration with neutrophils  
(38% diarrheic, 24% control piglets)**

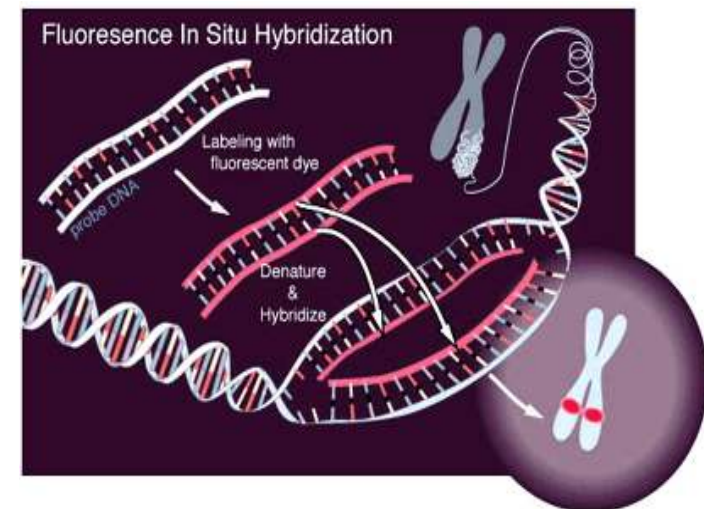
<b><u>Pathological changes</u></b>	<b><u>Herd 2</u></b>		<b><u>Herd 3</u></b>		<b><u>Herd 4</u></b>		<b><u>total</u></b>			
	<b>D</b> <i>n=16</i>	<b>C</b> <i>n=16</i>	<b>D</b> <i>n=12</i>	<b>C</b> <i>n=12</i>	<b>D</b> <i>n=14</i>	<b>C</b> <i>n=14</i>	<b>D</b> <i>n=42</i>	<b>C</b> <i>n=42</i>	<b>D</b> ~%	<b>C</b> ~%
<b>Villous atrophy</b>	<b>9</b>	<b>2</b>	<b>9</b>	<b>1</b>	<b>8</b>	<b>0</b>	<b>26</b>	<b>3</b>	<b>62</b>	<b>7</b>
<b>Neutrophils infiltration</b>	<b>7</b>	<b>6</b>	<b>6</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>16</b>	<b>10</b>	<b>38</b>	<b>24</b>
<b>Other changes in lamina propria</b>	<b>5</b>	<b>4</b>	<b>9</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>17</b>	<b>7</b>	<b>40</b>	<b>16</b>
<b>Epithelial lesions in small intestines</b>	<b>6</b>	<b>4</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>13</b>	<b>7</b>	<b>31</b>	<b>16</b>
<b>Mucosal necrosis</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>
<b>Epithelial lesions in colon</b>	<b>2</b>	<b>1</b>	<b>5</b>	<b>1</b>	<b>5</b>	<b>1</b>	<b>12</b>	<b>3</b>	<b>28</b>	<b>7</b>

**Histopathology results**  
**(42 diarrheic and 42 control piglets from herd 2,3,4)**

## Fluorescence In Situ Hybridization (FISH)

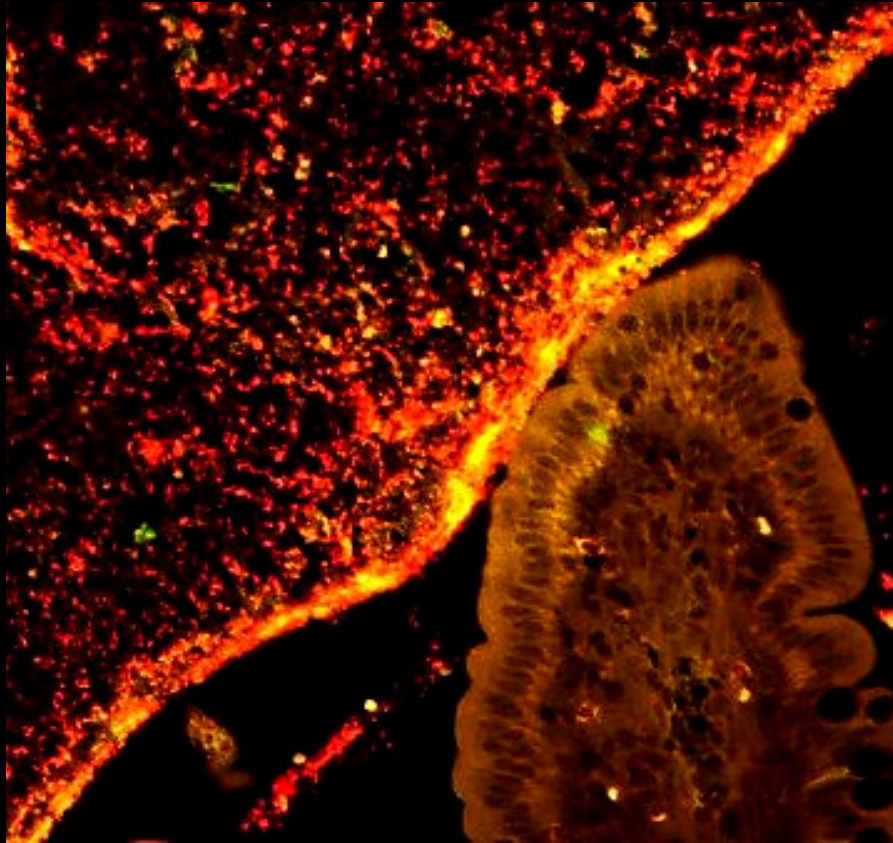
- Detection of bacteria in formalin-fixed, paraffin-embedded tissue samples
- 16s rRNA-targeted fluorescence-labelled oligonucleotide probes
- Characterization of bacterial adhesion to the epithelium

- **Escherichia coli**
- **Clostridium perfringens**
- **Costridium difficile**
- **Eubacteria**

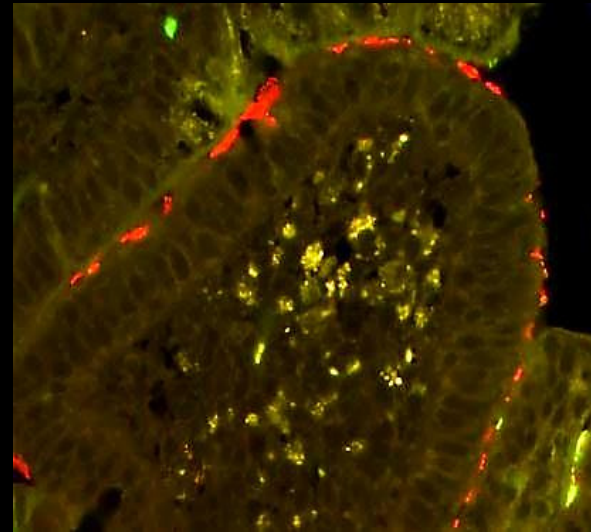




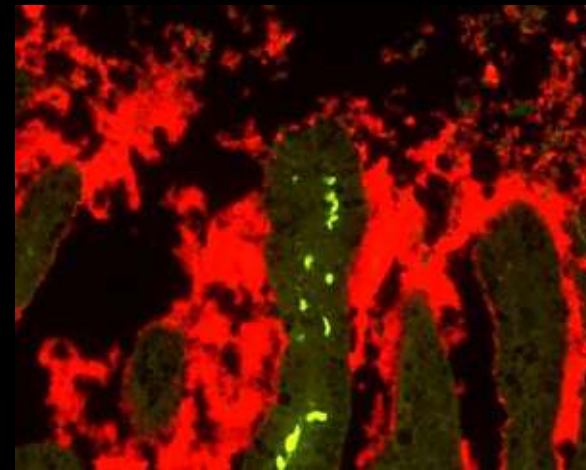
1



2



3



FISH *E. coli* (red)

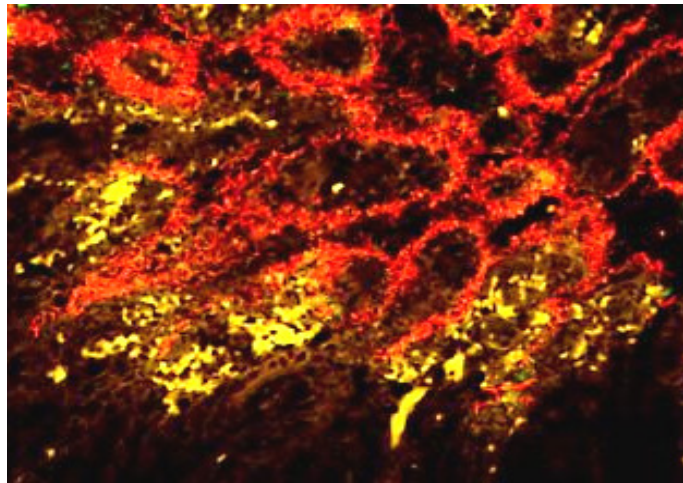
## FISH results

### *Escherichia coli*

Moderate- large amounts **adherent** to the epithelium in 14 diarrheic piglets (33%)  
In 10 cases associated with villous atrophy

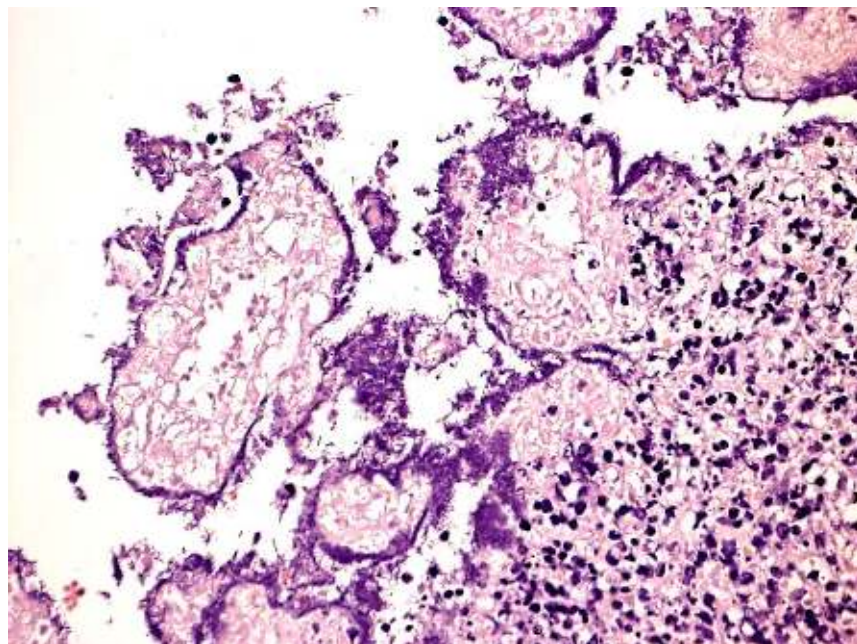
### *Clostridium perfringens*

Very small-moderate amounts in 31 diarrheic piglets (74%)  
In 1 control piglet associated with necrotic mucosa in the ileum



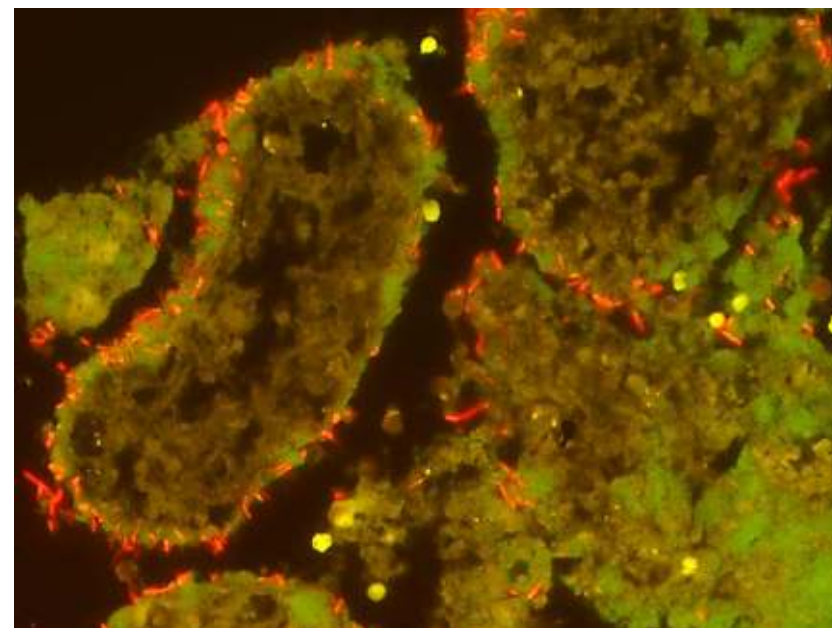
*Cl. perfringens* (red)

## **Cl. Perfringens type C (Klassisk Tarmbrand?)**



**HE**

**Ileum of diarrheic piglet from herd 5**



**FISH**

***Cl. perfringens* (red)  
*Eubacteria* (green)**



## FISH results

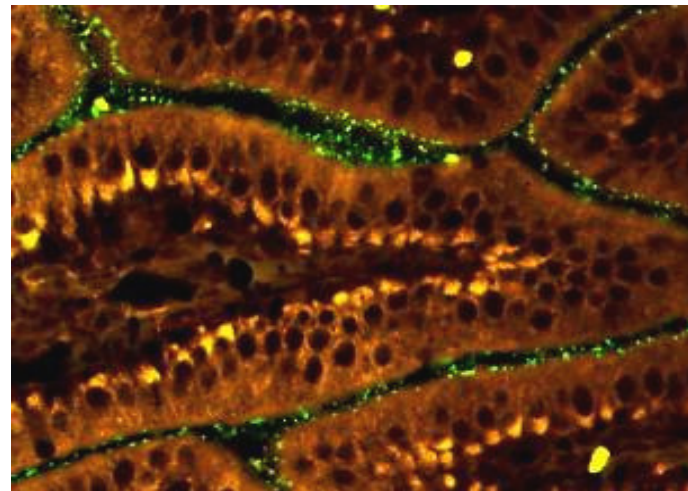
### *Clostridium difficile*

Positive signals within the intestinal content in the colon of 19 diarrheic piglets

### *Other bacteria*

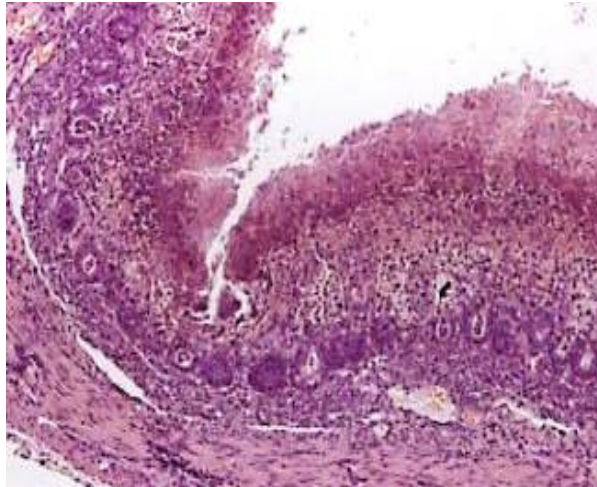
Present in 27 diarrheic piglets

In 1 case associated with necrotic mucosa in the ileum

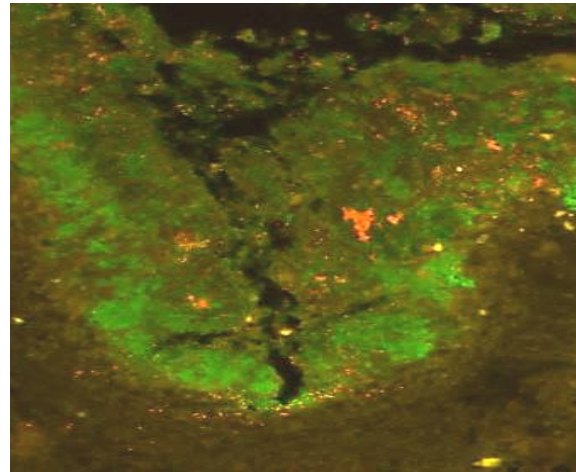


**Eubacteria (green)**

## Bacteria in necrotic tissue



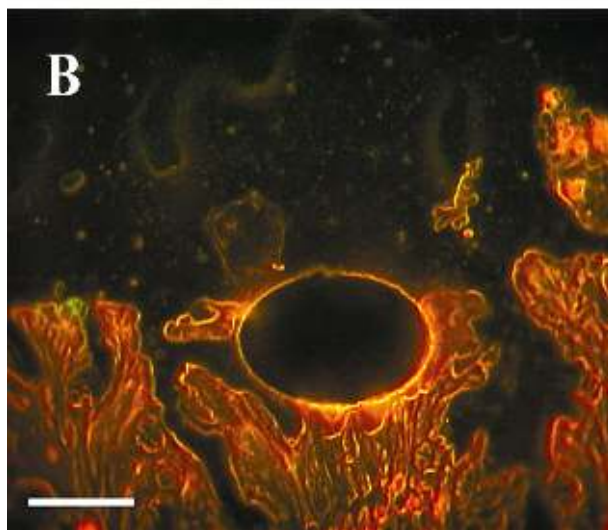
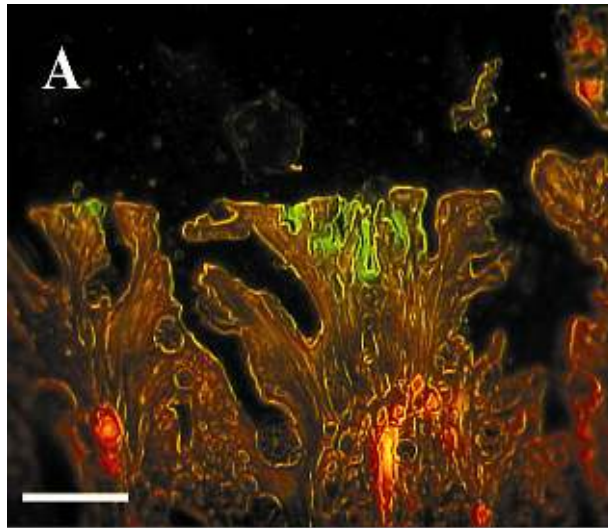
HE



FISH

**Necrotic ileal mucosa heavily colonized by bacteria.**  
**Double hybridization for *Domain bacteria* (green) and *E. coli* (red).**

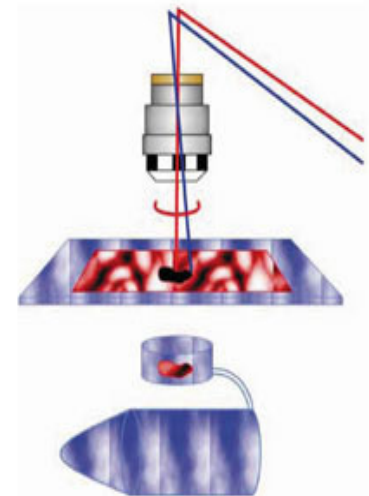
Case piglet from herd 2, 5 days old, diarrhea days- 2+3+4+5  
 Microscopically- superficial necrosis of the mucosa in the ileum

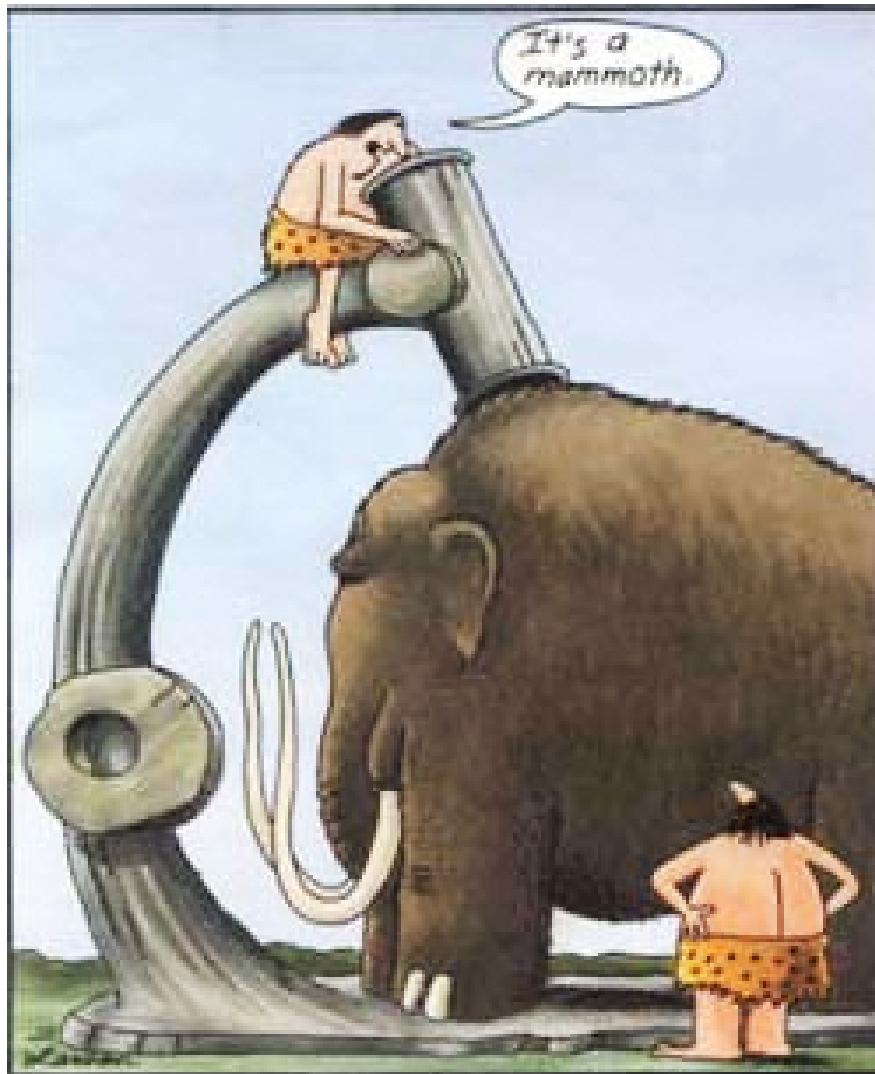


## Laser Capture Microdissection LCM

-allows for the removal from tissue of small structures in a manner that preserves the important molecules we are interested (RNA and protein)

-to analyze pathogenic bacteria in histological tissues





**Early microscopes**

***“The eye of a human being is a microscope, which makes the world seem bigger than it really is.”***

***Kahlil Gibran***