



## Brachyspirainfektioner hos svin og in situ identifikation

Jensen, Tim Kåre

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# Brachyspirainfektioner hos svin og in situ identifikation

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**Tim K. Jensen**

Seniorforsker,  
DTU-VET  
København  
E-mail: [tije@vet.dtu.dk](mailto:tije@vet.dtu.dk)  
WWW.vet.dtu.dk





# Diagnostic Pathology

## *In situ* detection methods

Detection of bacteria in formalin-fixed  
paraffin embedded tissue sections

- Classic, indicative stainings (e.g. Giemsa, Warthin-Starry)
- Specific stainings
  - immunohistochemistry, phenotype depending
  - in situ* hybridization targeting ribosomal RNA, genotype depending

# Fluorescent *in situ* hybridisation for identification of intestinal pathogens

## Specific in situ probes

- *Domain bacterium*
- *Gammaproteobacteria*
  
- genus *Brachyspira*
- genus *Campylobacter*
- genus *Helicobacter*

## *Brachyspira*

- *B. hyodysenteriae*
- *B. pilosicoli*
- *B. intermedia*
- *B. innocens*
- *B. murdochii*
  
- *Fusobacterium necrophorum*
- *Campylobacter jejuni/coli*
- *Clostridium perfringens*
- *Clostridium difficile*
- *Salmonella enterica*
- *Lawsonia intracellularis*

# Intestinal diseases and differential diagnostic problems

- Proliferative enteropathy: *Lawsonia intracellularis*
- Post weaning diarrhoea / edema disease: *Escherichia coli*
- Swine dysentery : *Brachyspira hyodysenteriae*
- Spirochaetal colitis: *Brachyspira pilosicoli*
  - *Brachyspira intermedia*: pathogenic?
  - *Brachyspira innocens*: non-pathogenic?
  - *Brachyspira murdochii*: non-pathogenic?
  - "*Brachyspira suanatina*": colitis
- Salmonellosis
- Porcine Circovirus type 2 (PCV2)
- Other agents?

# Svinedysenteri

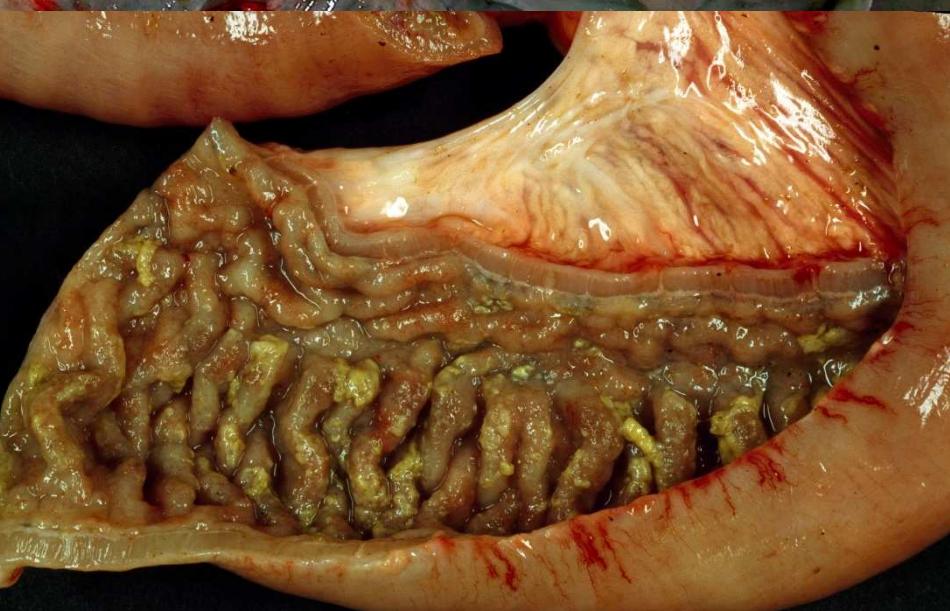
- Smitsom mukohæmmorrhagisk colitis
- Brachyspira hyodysenteriae

# Andre Brachyspira

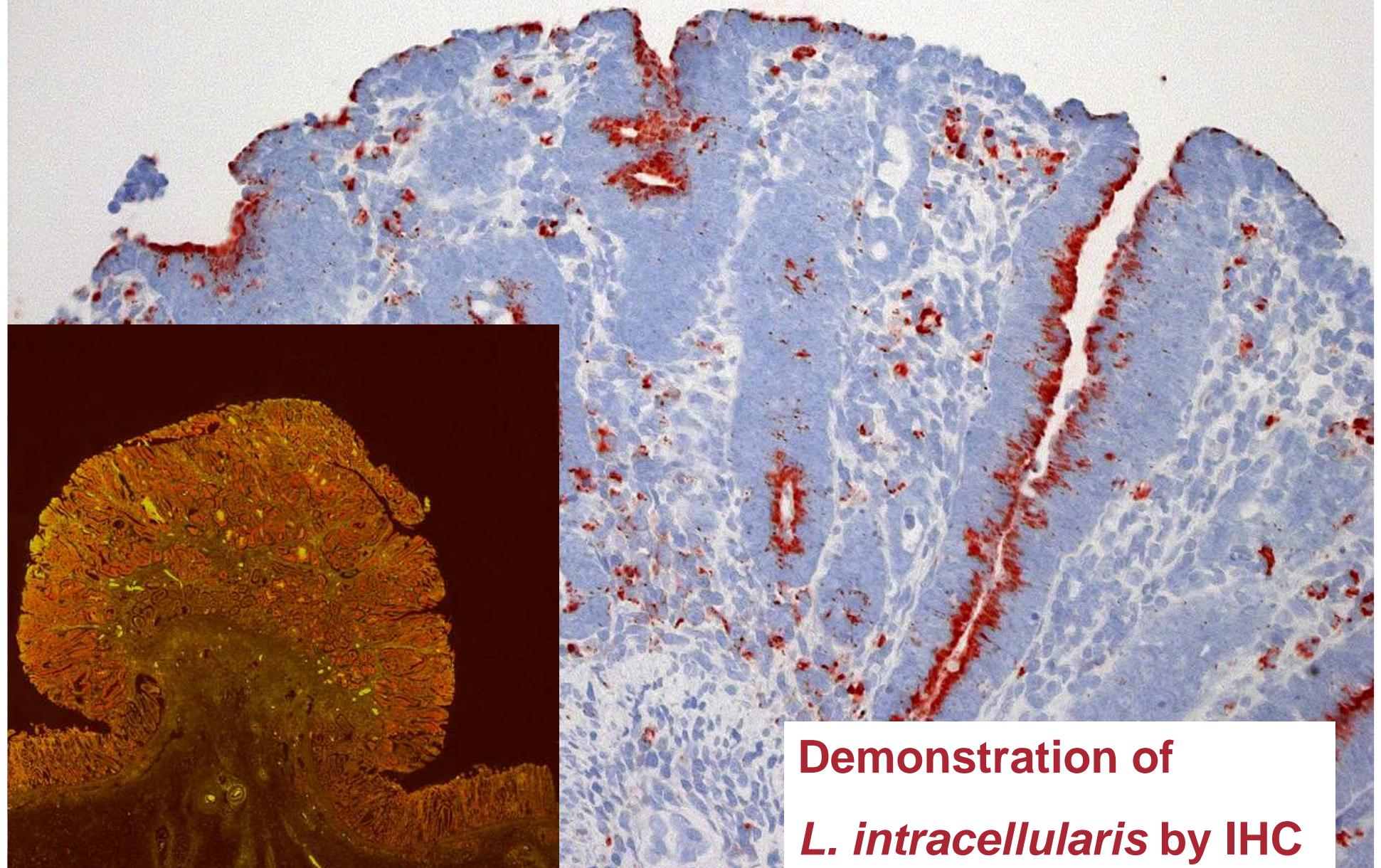
- *B. innocens*, non-patogen
- *B. intermedia*, patogen?
- *B. pilosicoli*, colitis, flere dyr. intestinal spirokætose
- *B. murdochii*, non-patogen?
- "B. suanantina", kraftig hæmolyse, andefugle, svin (colitis)
- "B. hampsonii", kraftig hæmolyse, svin, colitis, nordamerika



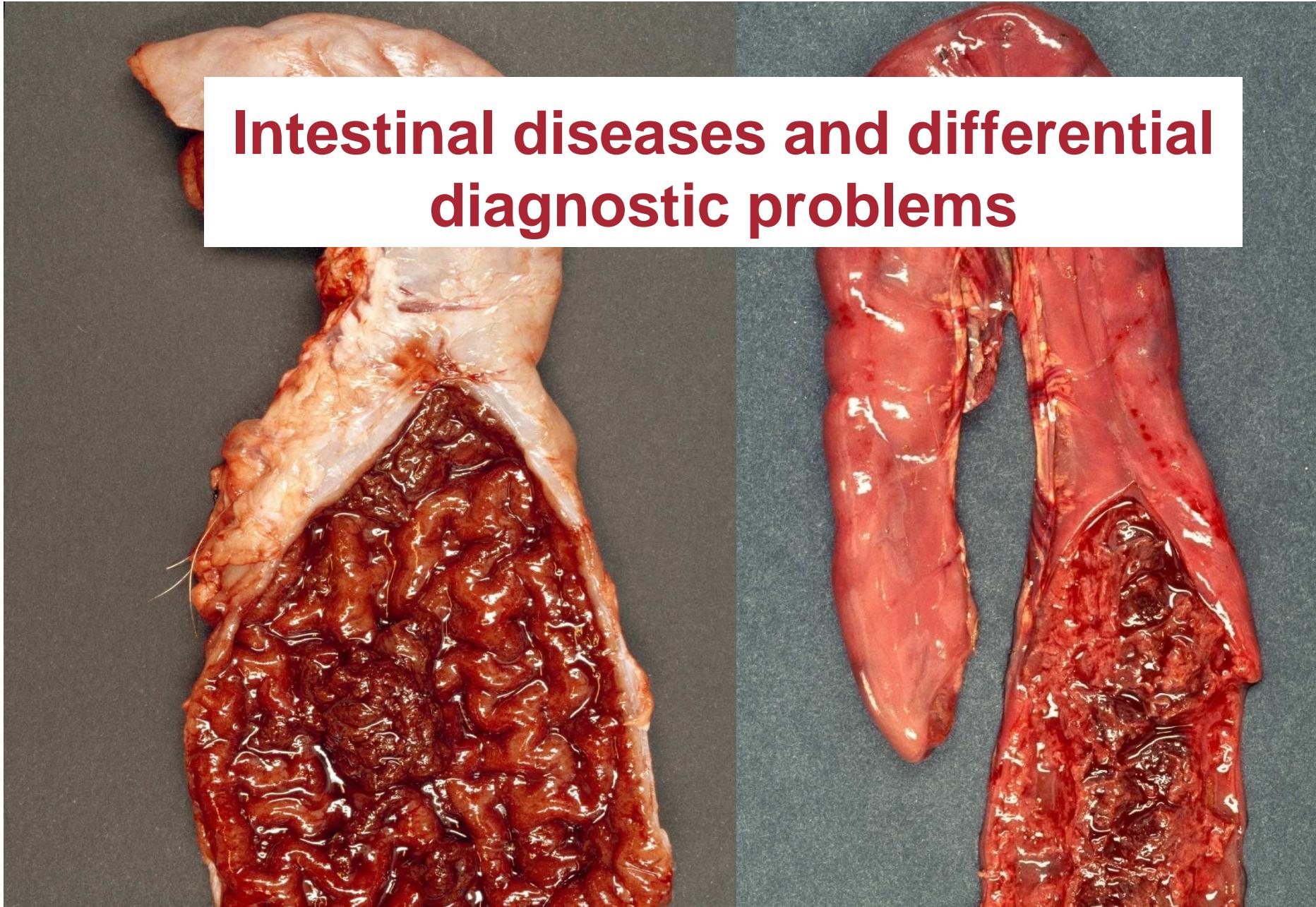
# Intestinal diseases and differential diagnostic problems



# Proliferative enteropathy – *L. intracellularis*



Demonstration of  
*L. intracellularis* by IHC

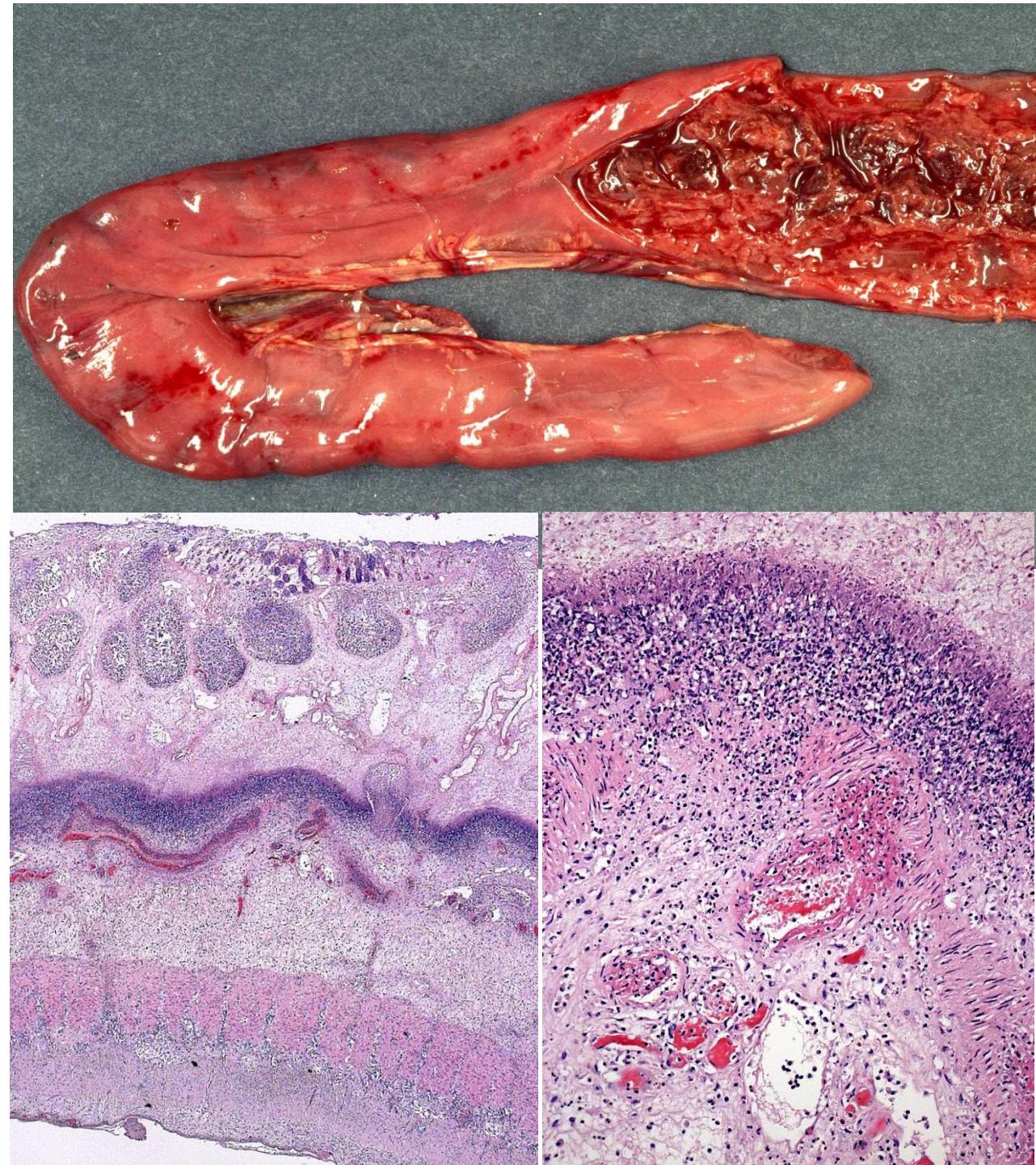


# Intestinal diseases and differential diagnostic problems

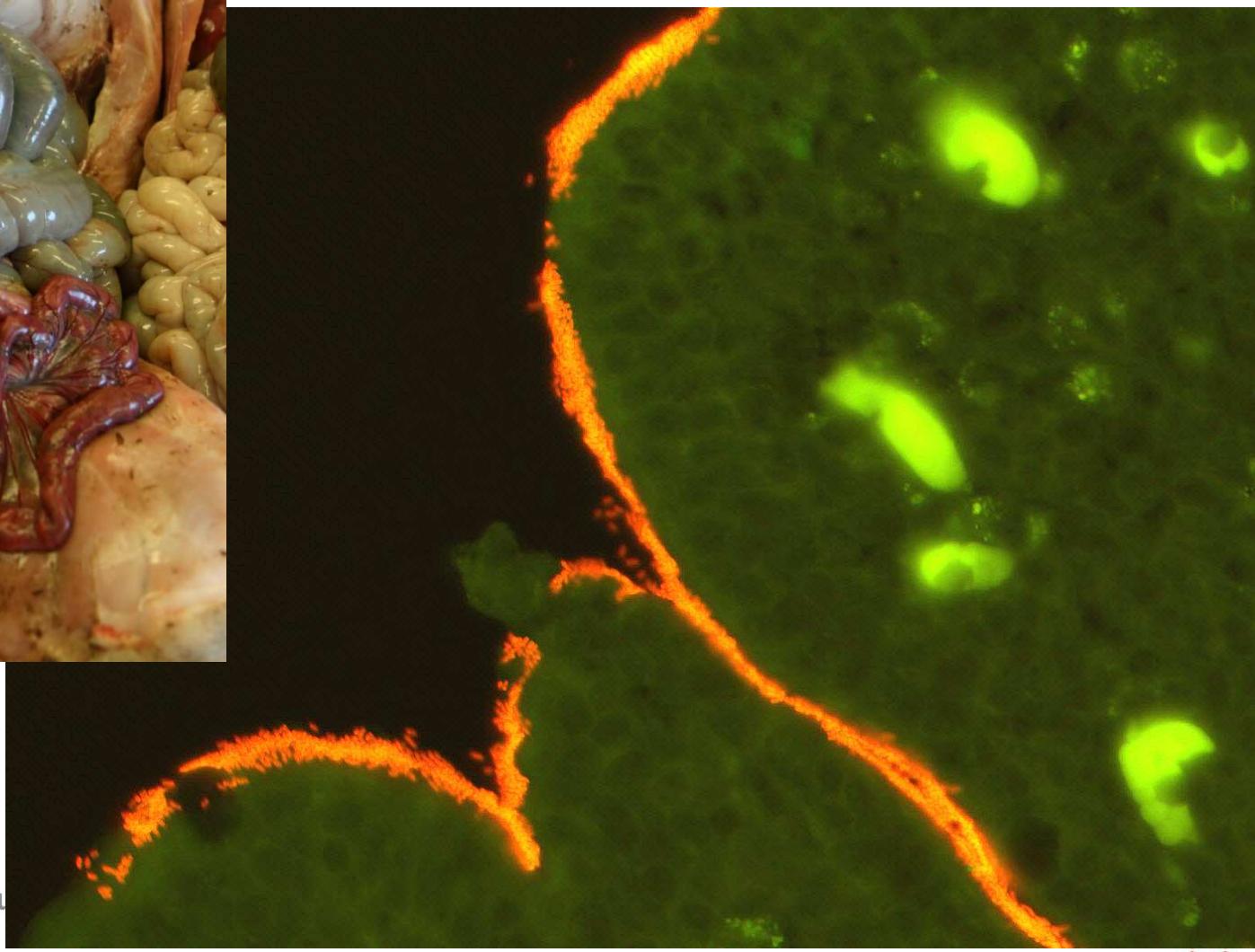
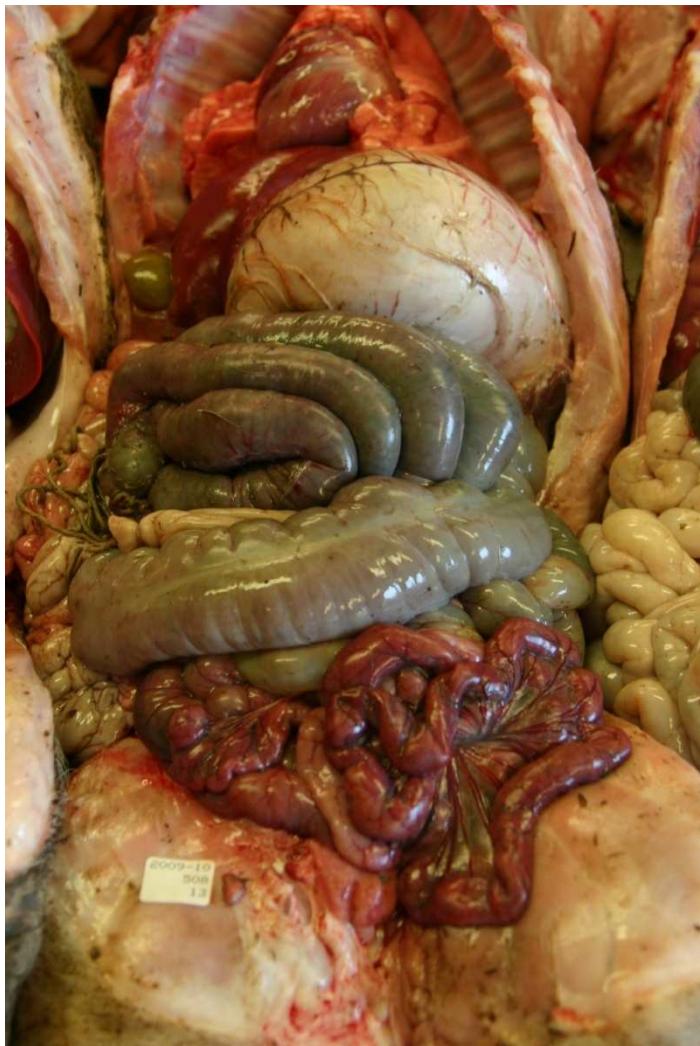
National Veterinary Institute

DIU  
≡

**Edema disease**  
*E. coli* positive for  
verotoxin 2e and  
fimbria (F18).



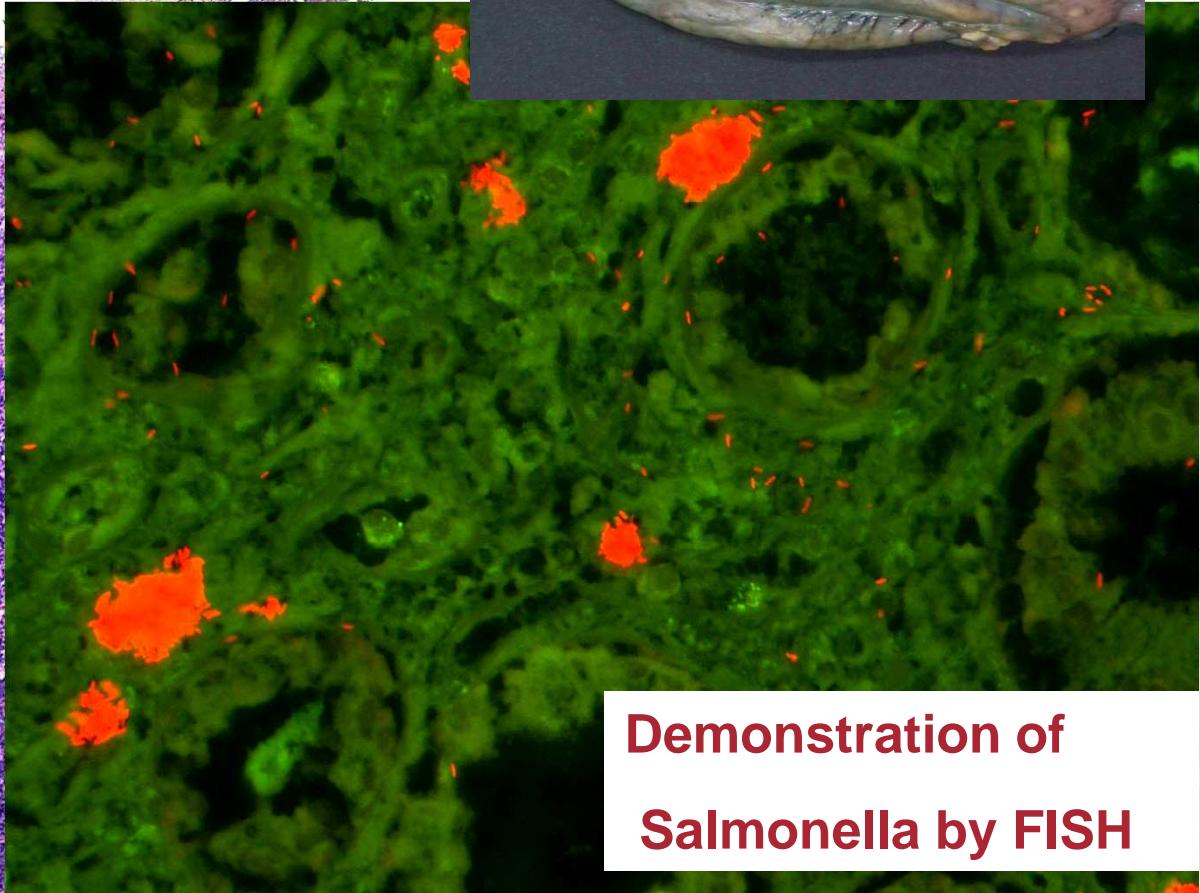
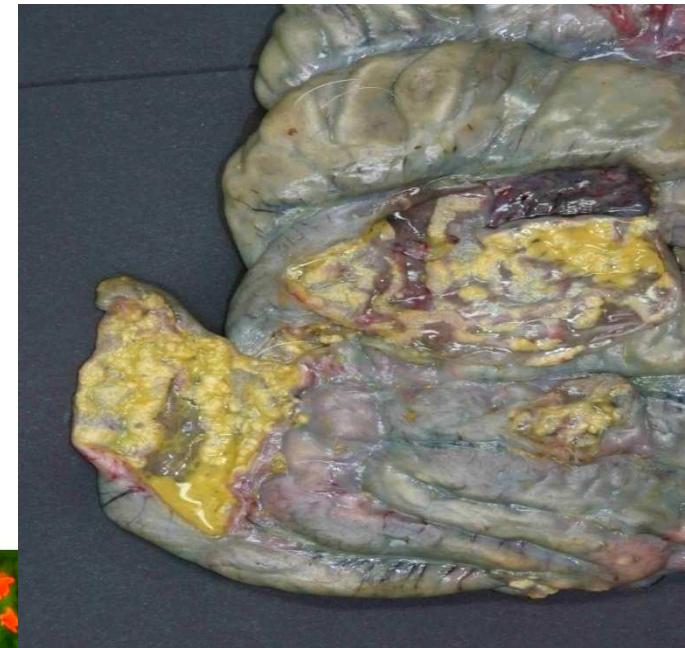
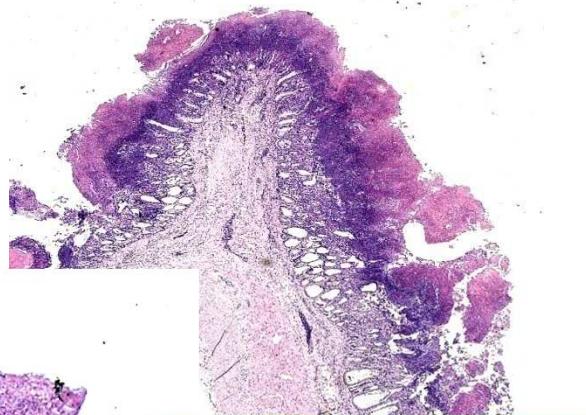
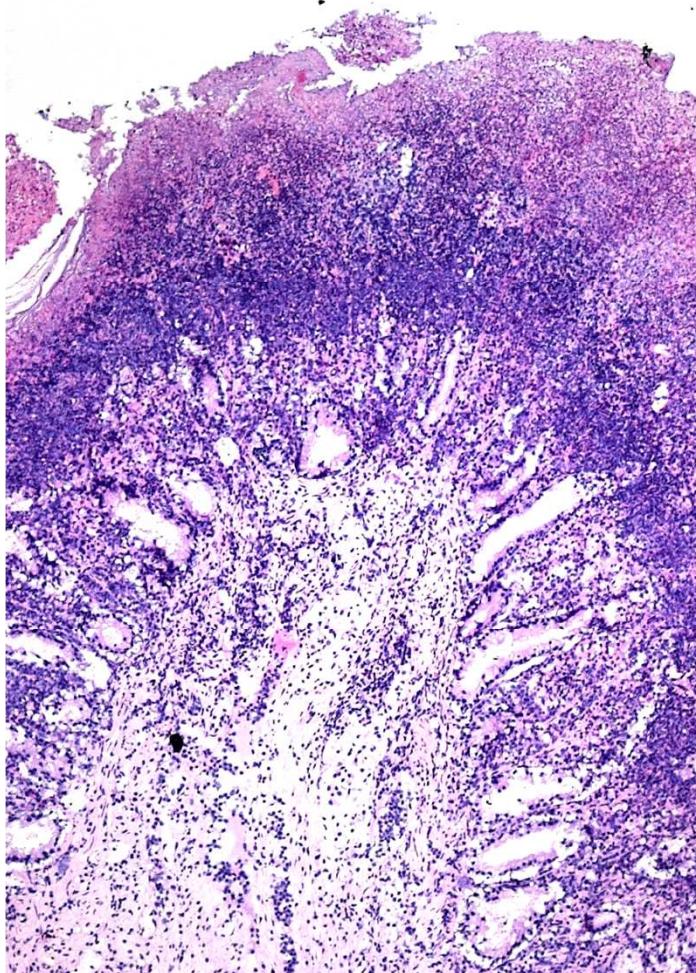
Demonstration of *E. coli* attached to villus epithelium in jejunum, FISH



# Intestinal diseases and differential diagnostic problems



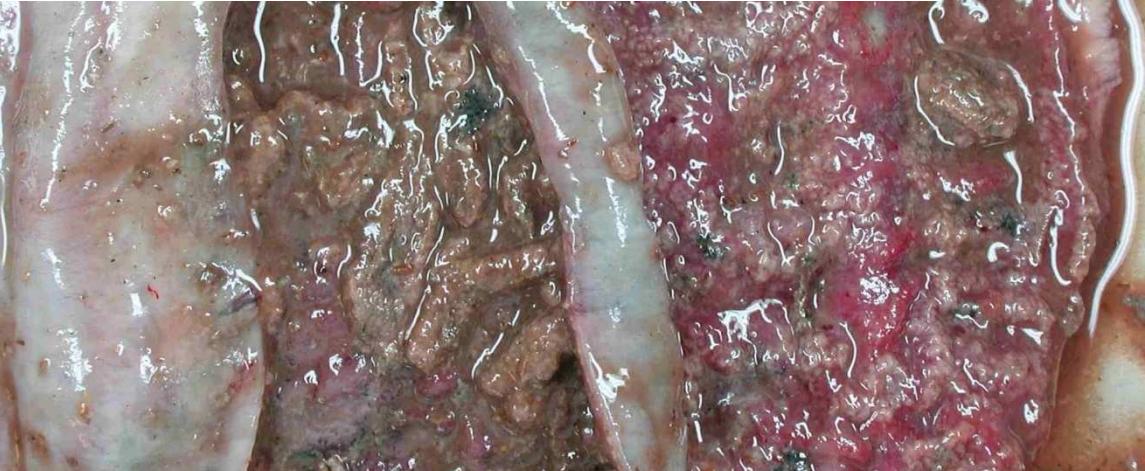
# Salmonellosis



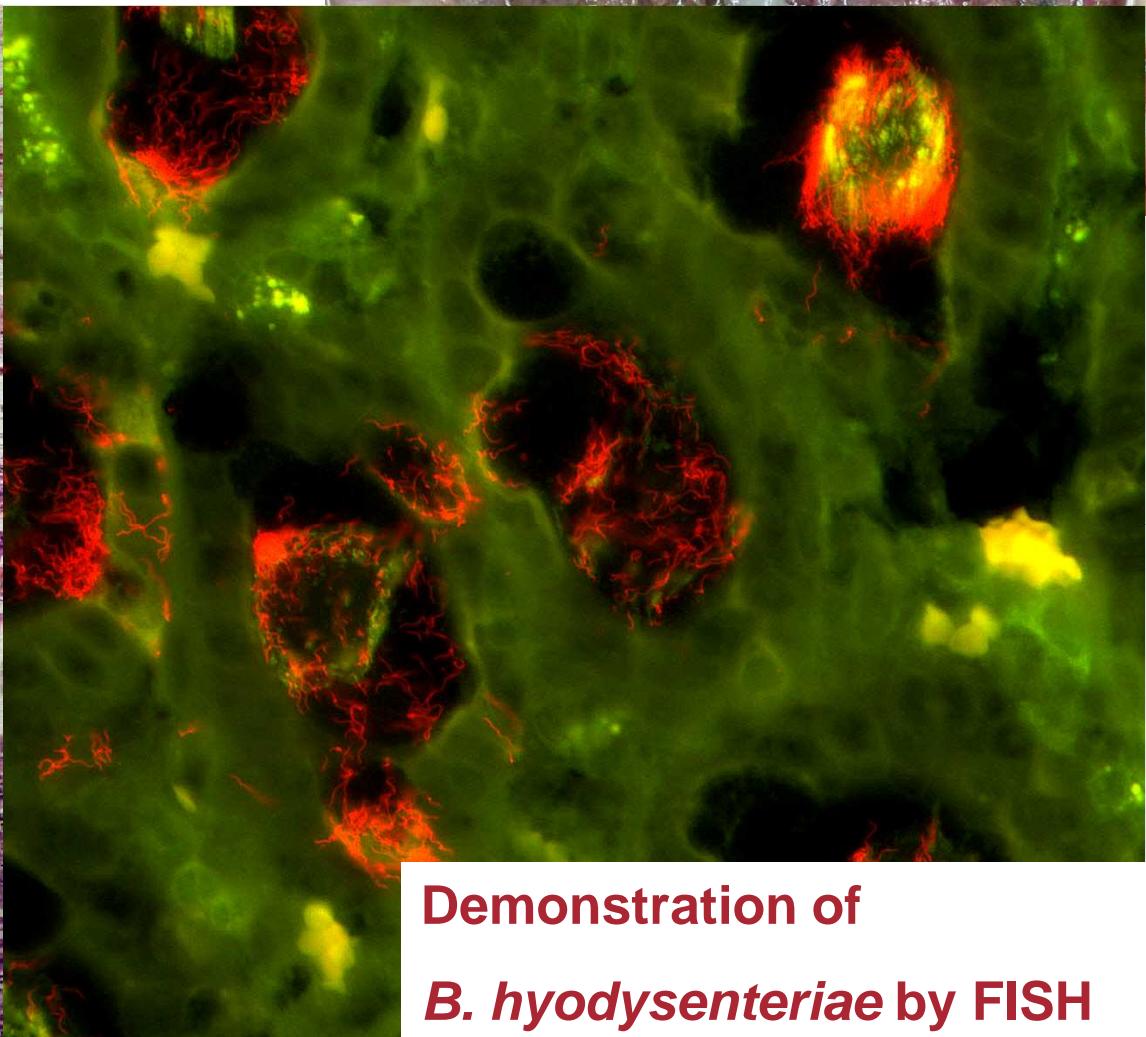
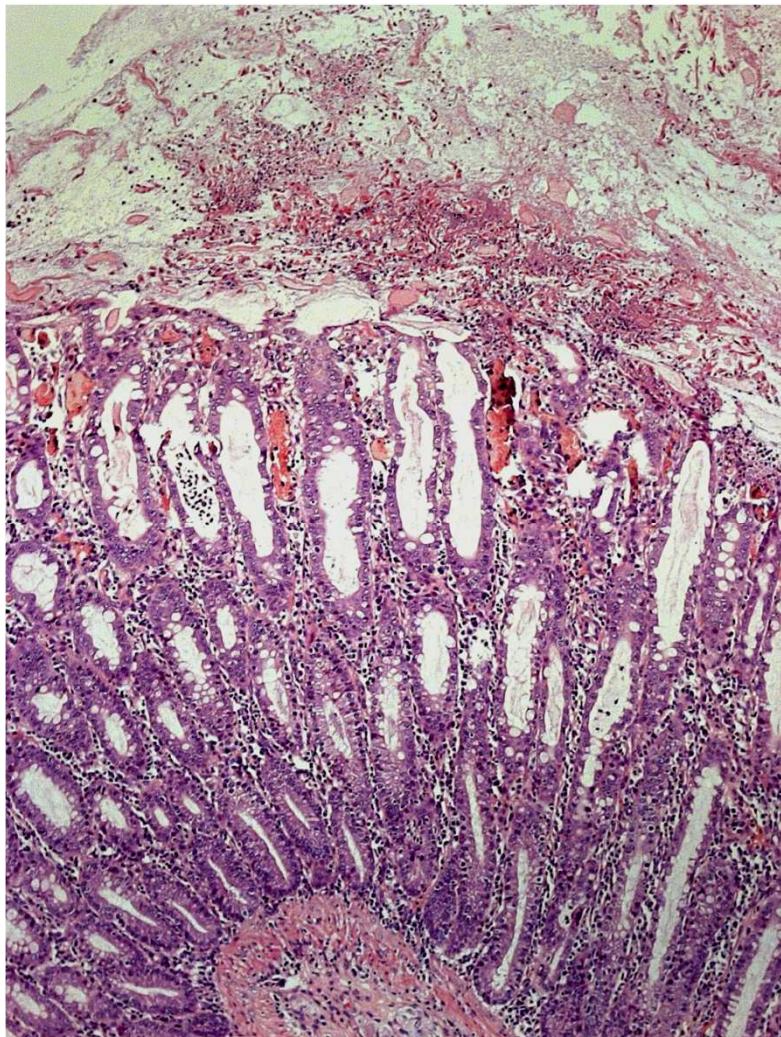
Demonstration of  
*Salmonella* by FISH



# Intestinal diseases and differential diagnostic problems

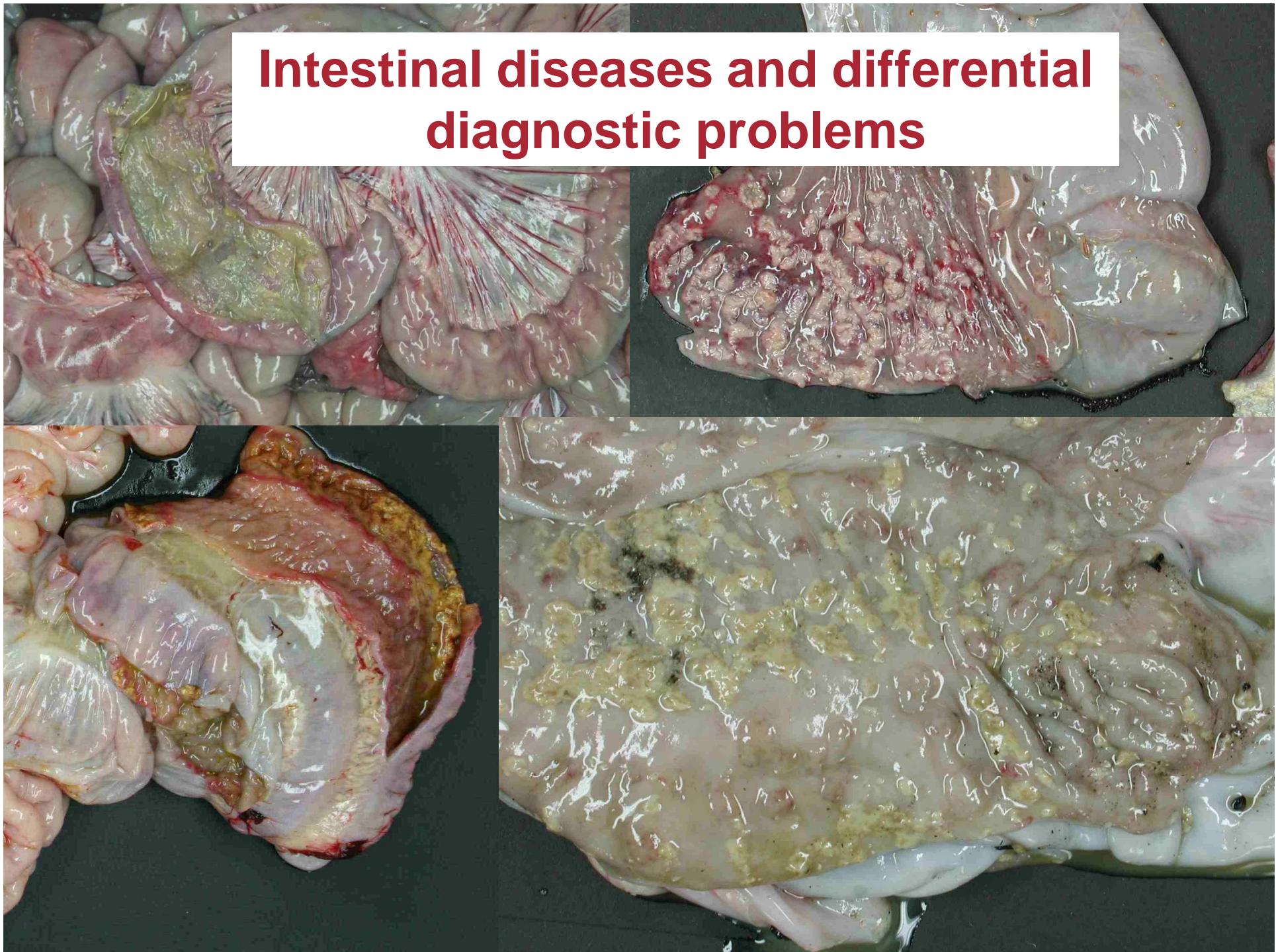


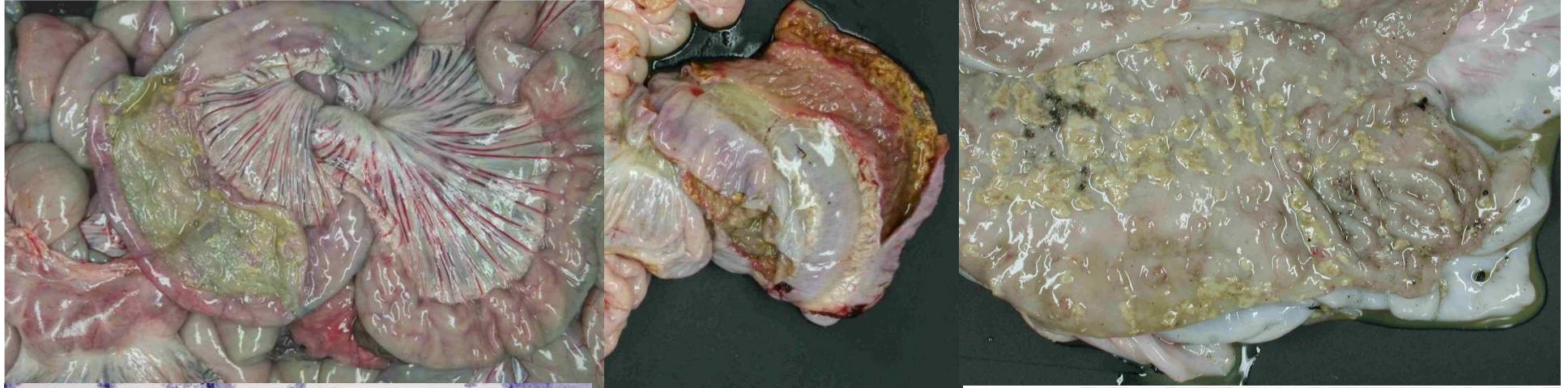
## Swine dysentery *B. hyohysenteriae*



Demonstration of  
*B. hyohysenteriae* by FISH

# Intestinal diseases and differential diagnostic problems





## PCV2 enteritis

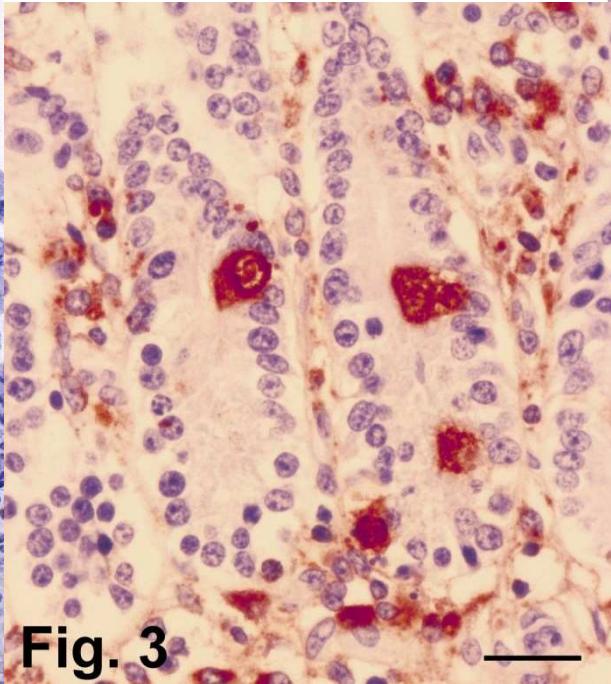
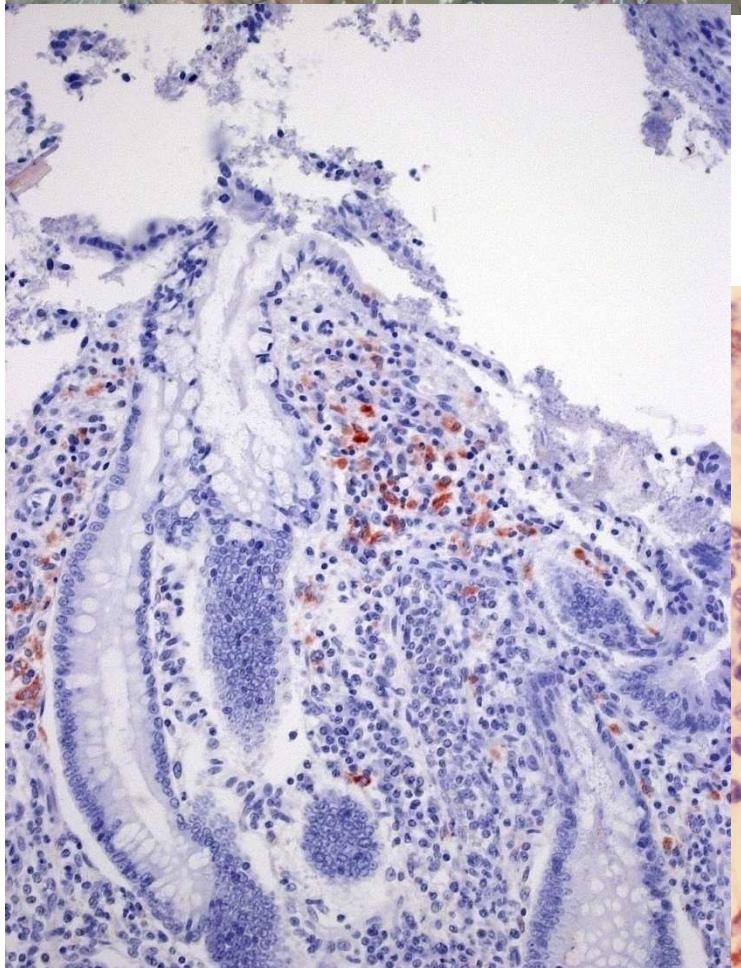
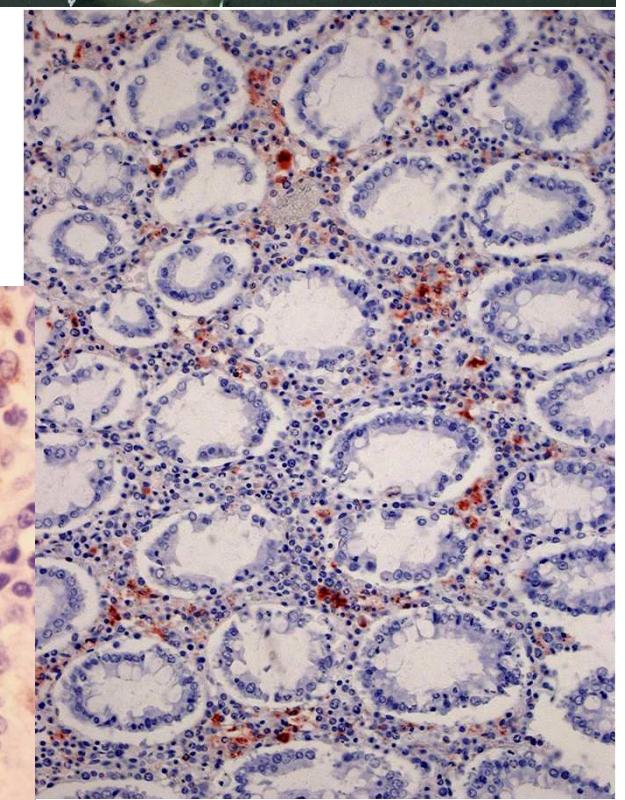


Fig. 3



Demonstration of  
PCV2 by IHC

# Detection of large intestinal pathogens in pigs by in situ methods

Intestines from 140 pigs submitted for routine laboratory examination with suspicion of spirochaete associated diarrhoea/colitis. FISH and IHC.

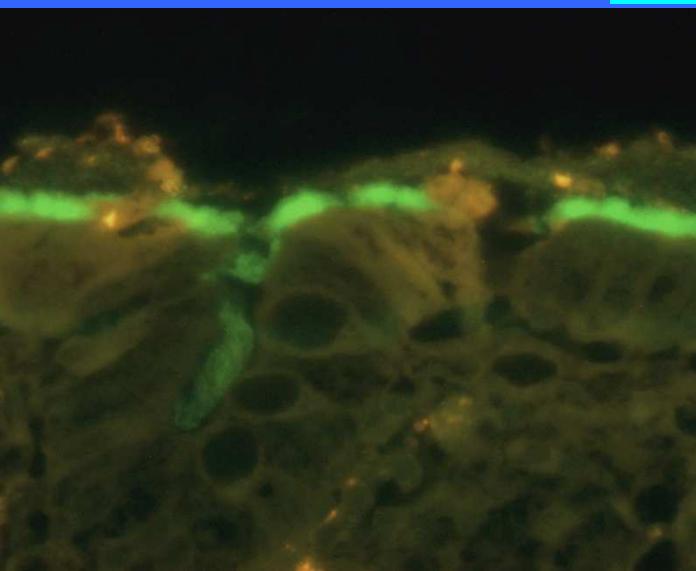
Demonstration of mono- and polyinfections in pigs

	<i>L.intra</i>	<i>Bhy+Bpil</i>	<i>B.hyo</i>	<i>B.pilo</i>	<i>B.inter</i>	B. inn	B. mur	PCV2
<i>L.intra</i>	28	2	7	1	1	2	3	5
<i>B.hyo</i>			22	4		2		
<i>B.pilo</i>				3		1		2
<i>B.inter</i>					2	1		
B. inn						3		2
B. mur							3	
PCV2								14

# *In situ* detection of large intestinal pathogens in pigs

Demonstration of mono- and polyinfections in pigs

	<i>L.intra</i>	<i>Bhy+Bpil</i>	<i>B.hyo</i>	<i>B.pilo</i>	<i>B.inter</i>	<i>B. inn</i>	<i>B. mur</i>	PCV2
<i>L.intra</i>	28	2	7	1	1	2	3	5
<i>B.hyo</i>			22	4		2		
<i>B.pilo</i>				3		1		2
<i>B.inter</i>								
<i>B. inn</i>								
<i>B. mur</i>								
PCV2								

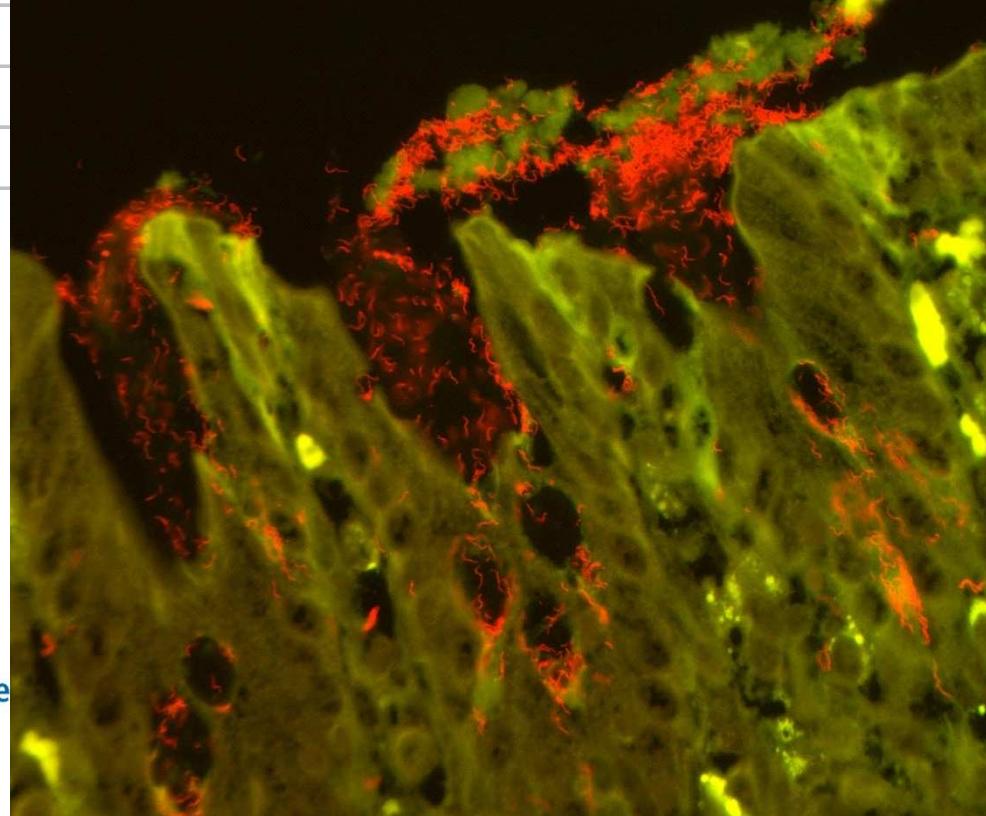



National Veterinary Institute

# *In situ* detection of large intestinal pathogens in pigs

Demonstration of mono- and polyinfections in pigs

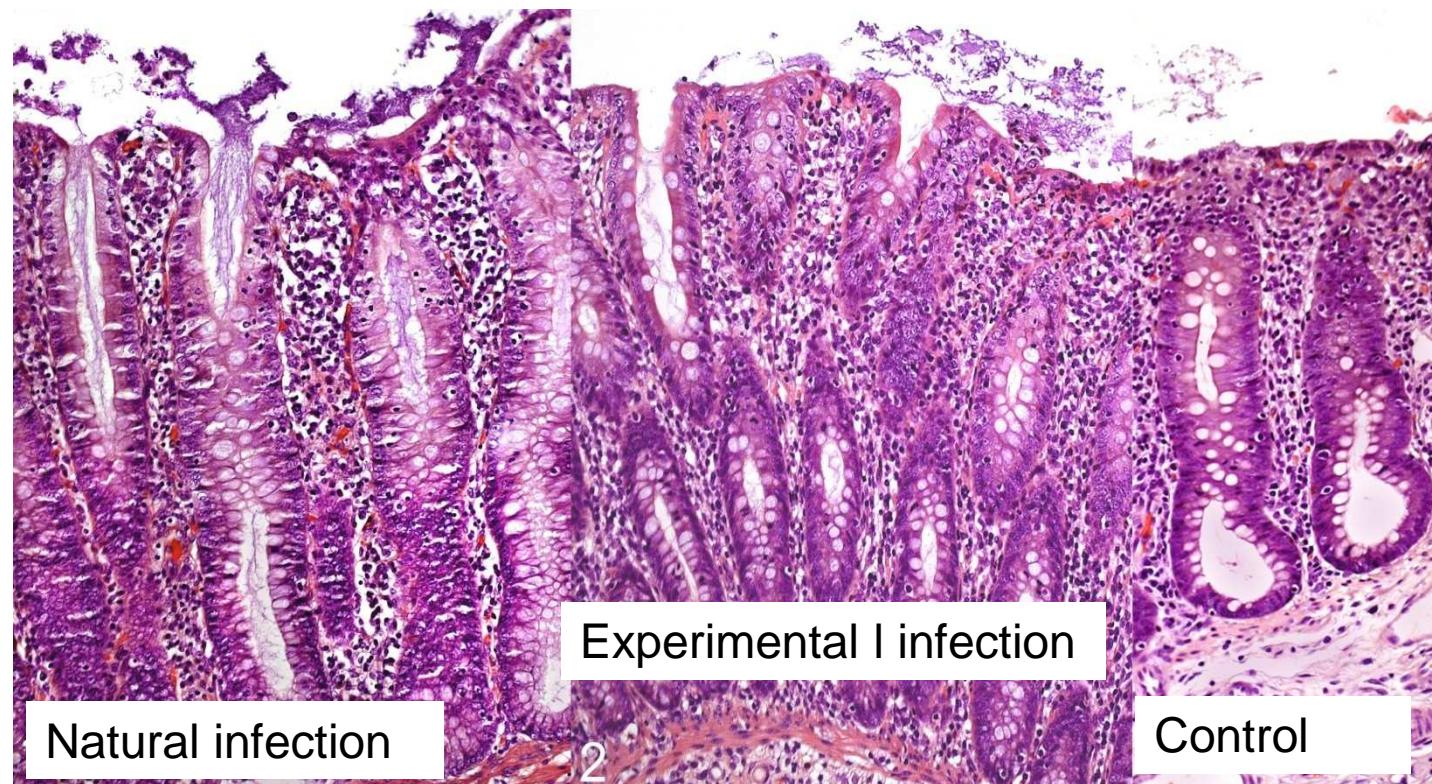
	<i>L.intra</i>	<i>Bhy+Bpil</i>	<i>B.hyo</i>	<i>B.pilo</i>	<i>B.inter</i>	B. inn	B. mur	PCV2
<i>L.intra</i>	28	2	7	1	1	2	3	5
<i>B.hyo</i>			22	4		2		
<i>B.pilo</i>				3		1		2
<i>B.inter</i>					2	1		
B. inn						3		2
B. mur							3	
PCV2								14



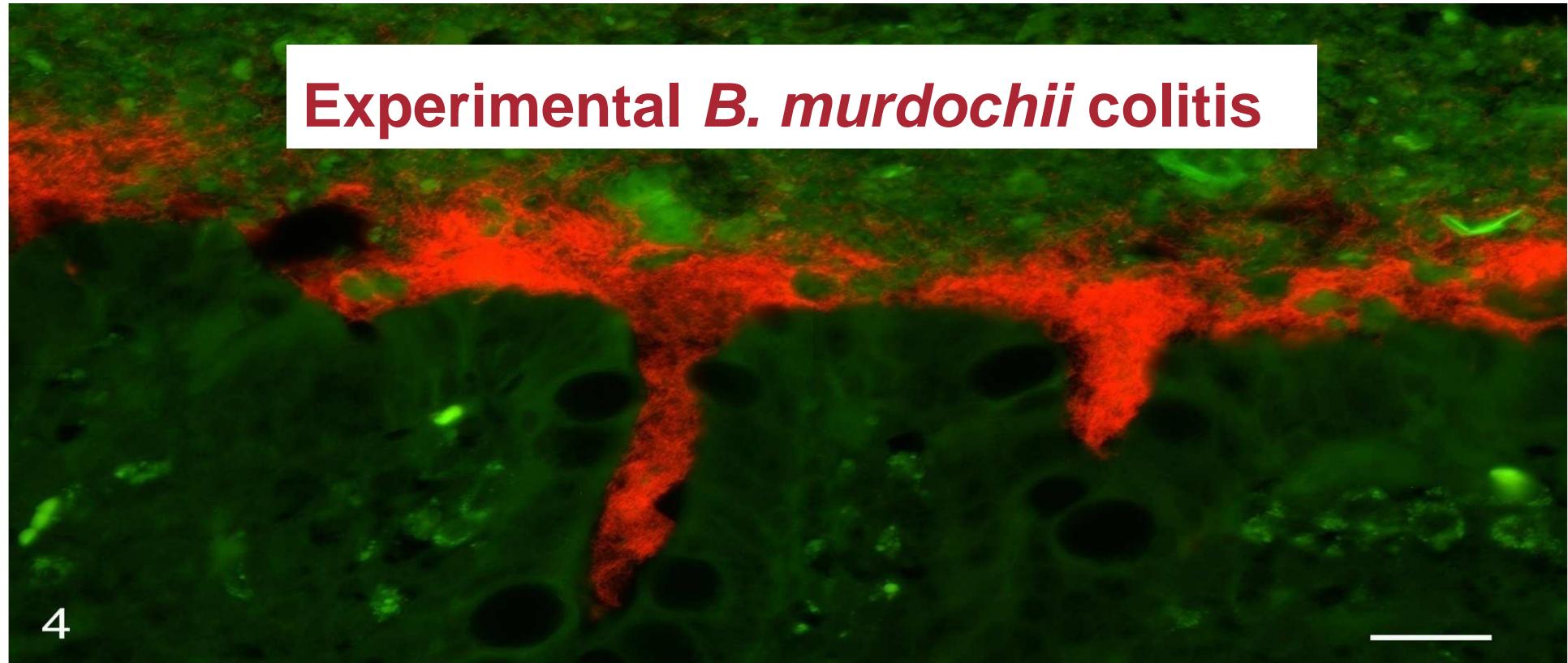
## Experimental *B. murdochii* colitis

Two out of 8 pigs showed catarrhal colitis 3 weeks after challenge with *B. murdochii*.

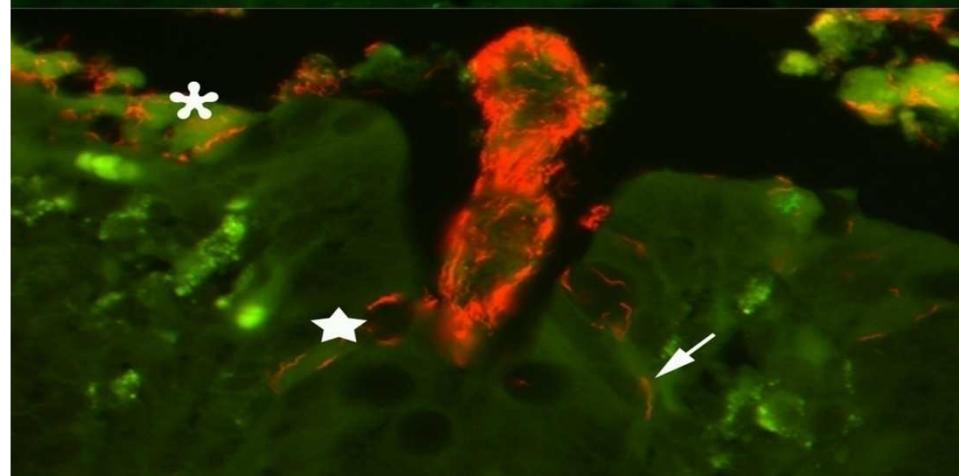
Diarrhoea was not observed.



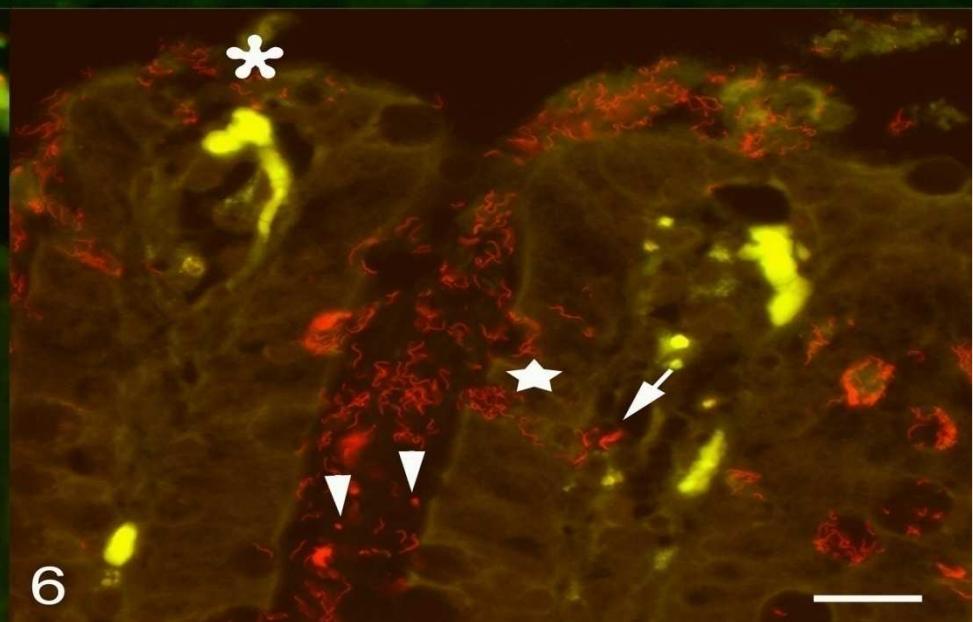
## Experimental *B. murdochii* colitis



4



*B. murdochii* by in situ hybridization



6

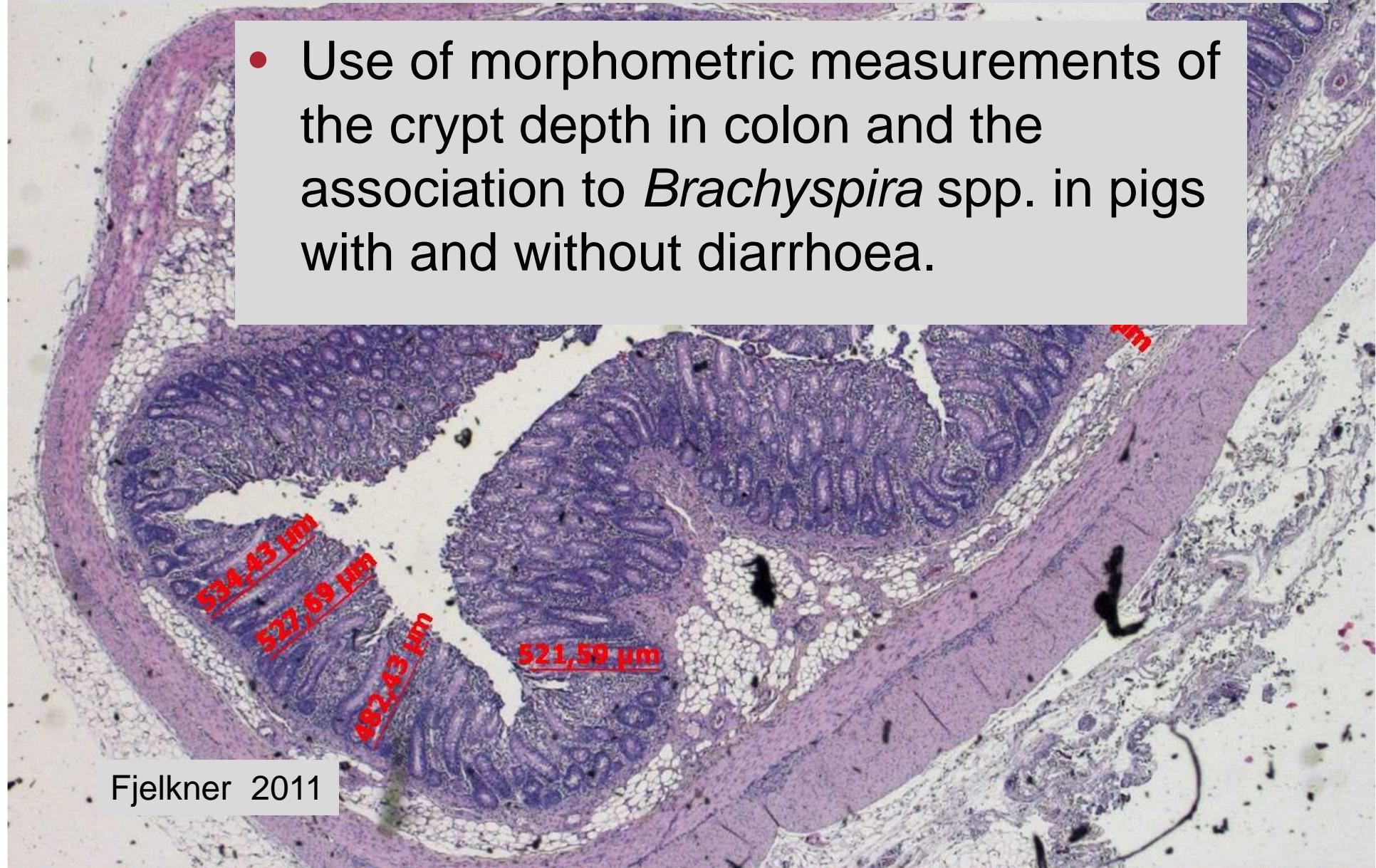
# Prevalence of microbiological findings in nursery pigs selected for antibiotic medication of diarrhoea by the farmer (20 herds, 312 pigs)

## Pig prevalences (%)

• <i>L. intracellularis</i>	19
• PCV2	4
• <i>Brachyspira spp</i>	40
<i>B. pilosicoli</i>	7
<i>B. intermedia</i>	10
<i>B. innocens</i>	16
<i>B. murdochii</i>	21
• Hemolytic <i>E. coli</i>	15

## Prevalence of microbiological findings in nursery pigs selected for antibiotic medication of diarrhoea by the farmer (20 herds, 312 pigs)

- Use of morphometric measurements of the crypt depth in colon and the association to *Brachyspira* spp. in pigs with and without diarrhoea.



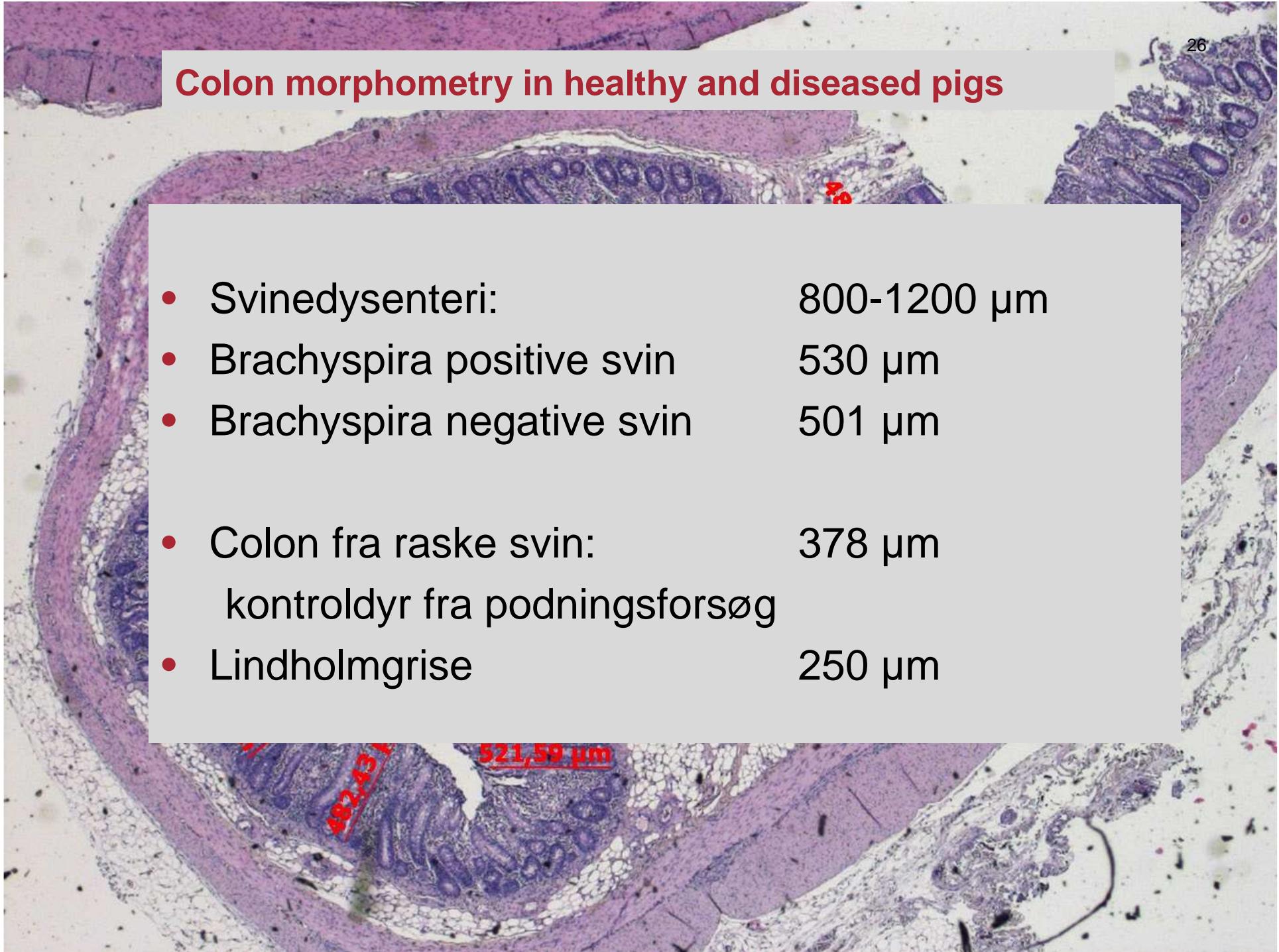
Fjelkner 2011

## Prevalence of microbiological findings in nursery pigs selected for antibiotic medication of diarrhoea by the farmer (20 herds, 312 pigs)

- An increased thickness of the colonic mucosa was associated with demonstration of *Brachyspira* spp. (*B. pilosicoli*, *B. intermedia*, *B. innocens* and/or *B. murdochii*) ( $p=0.04$ ).
- The mean thickness of the colonic mucosa in pigs positive for *Brachyspira* spp. was  $529.5 \mu\text{m}$  compared to  $501.4 \mu\text{m}$  in *Brachyspira* spp. negative pigs.
- Increased thickness of the colonic mucosa was associated with small pig size ( $p = 0.05$ ) and increasing age ( $p= 0.03$ ).

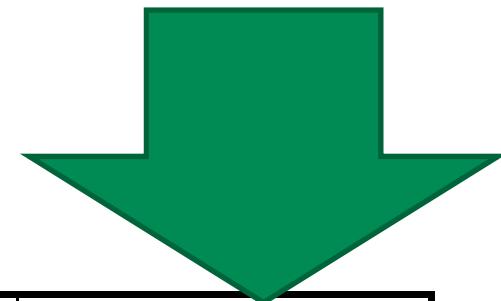
## Colon morphometry in healthy and diseased pigs

- Svinedysenteri: 800-1200 µm
- Brachyspira positive svin 530 µm
- Brachyspira negative svin 501 µm
- Colon fra raske svin:  
kontroldyr fra podningsforsøg 378 µm
- Lindholmgrise 250 µm



# Porcine necrotizing enterocolitis

**Gross lesions and detection of the agents: *L. intracellularis* and PCV2  
in 64 pigs with enteritis**



Law(n=28)	Law+PCV2 n=6)	PCV2 (n=23)	None (n=7)
<b>Uncomplicated PE (15) Necrotizing enterocolitis (10) PHE (3)</b>	<b>Uncomplicated PE (3) Necrotizing enterocolitis (3)</b>	<b>Necrotizing enterocolitis (12) Other lesions (11)</b>	<b>Necrotizing enterocolitis (6) Other lesions (1)</b>



## Porcine necrotizing enterocolitis

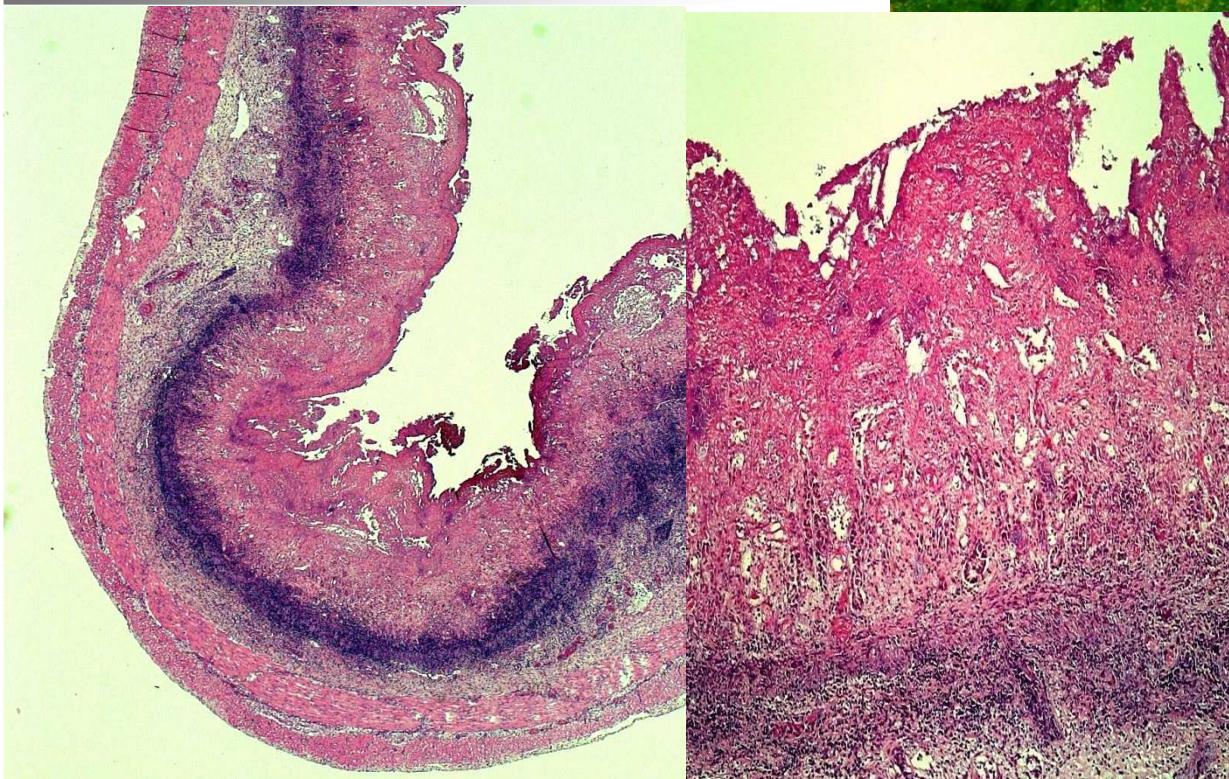
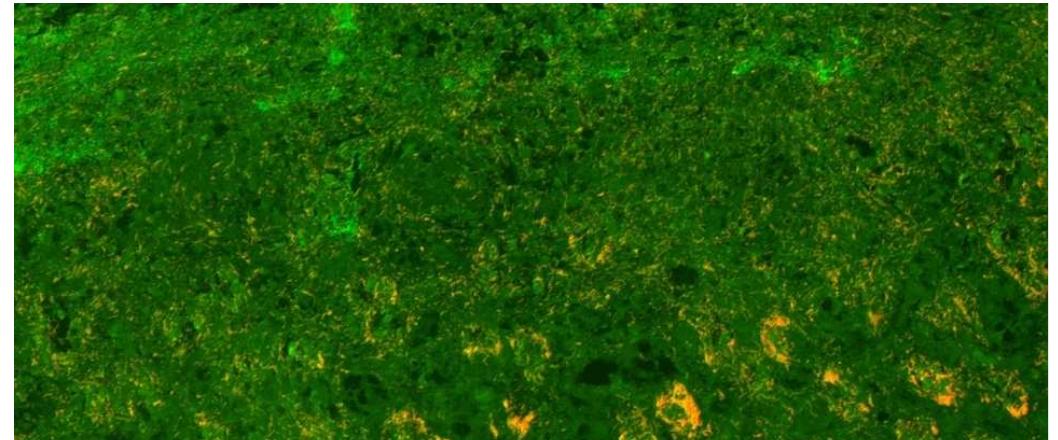
- Necropsy  
Severe, acute necrotizing enterocolitis was found in both animals while all other organs appeared normal.



# Porcine *Fusobacterium necrophorum* necrotizing enterocolitis

Histopathology:

Acute to subacute  
necrotizing enterocolitis.  
High number of slender rods  
observed.



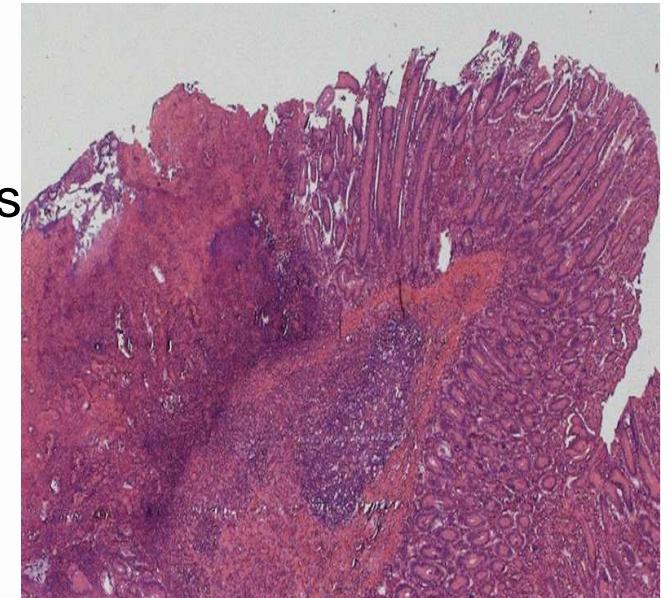
# Porcine *Fusobacterium necrophorum* necrotizing enterocolitis

30

Retrospective FISH study on 84 intestines  
from pigs with suspicion of *L. intracellularis*  
infection (33+/51-)

Necrotic mucosa associated with  
*F. necrophorum* colonization detected in 28 pigs  
(33%)

- *L. intracellularis* +: 18 cases (55%)
- *L. intracellularis* -: 10 cases (20%)
  - Including 4 severely affected cases



Knowing how *F. necrophorum* looks like: Nine  
out nine positive! Age: 6 days to 6 weeks

# Intestinal diseases in pigs

## Conclusion

- The results suggest that *F. necrophorum* in some cases may act as a primary intestinal pathogen.
- Large scale studies to be initiated to investigate the importance of *F. necrophorum* as an intestinal pathogen.
- *B. intermedia*, *B. murdochii* and *B. innocens* should be regarded as low pathogenic for pigs.

# Intestinal diseases in pigs

## Conclusions

- The diagnostic importance, however, of culturing *B. intermedia*, *B. murdochii* and *B. innocens* from feces only is uncertain, as the method is not quantitative!
- Diagnostic pathology is difficult – not all cases are straight forward!
- Application of different in situ detection methods is a requirement for research as well as diagnostic laboratories.



# **Diarrhoea in fattening pigs**

**Mette Boye  
Henriette Hvass  
Ulla Riber  
Rikke Lindecrona  
Henriette Boesen  
Anja Schmidt  
Helle Stege  
Øystein Angen  
Marie Ståhl  
Charlotte Hjulsager**

**Kristian Møller  
Sven Erik Jorsal  
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Torsten Boutrup  
Thomas Leser  
Gregers Jungersen  
Lars E. Larsen  
Roberto Guedes  
Markku Johansen  
Birgitta Svensmark  
Svend Haugegaard  
Godelind Wolf-Jäckle  
Ken Pedersen  
Jens Peter Nielsen  
Johanna Fjelkner**

**Thank you for your attention**