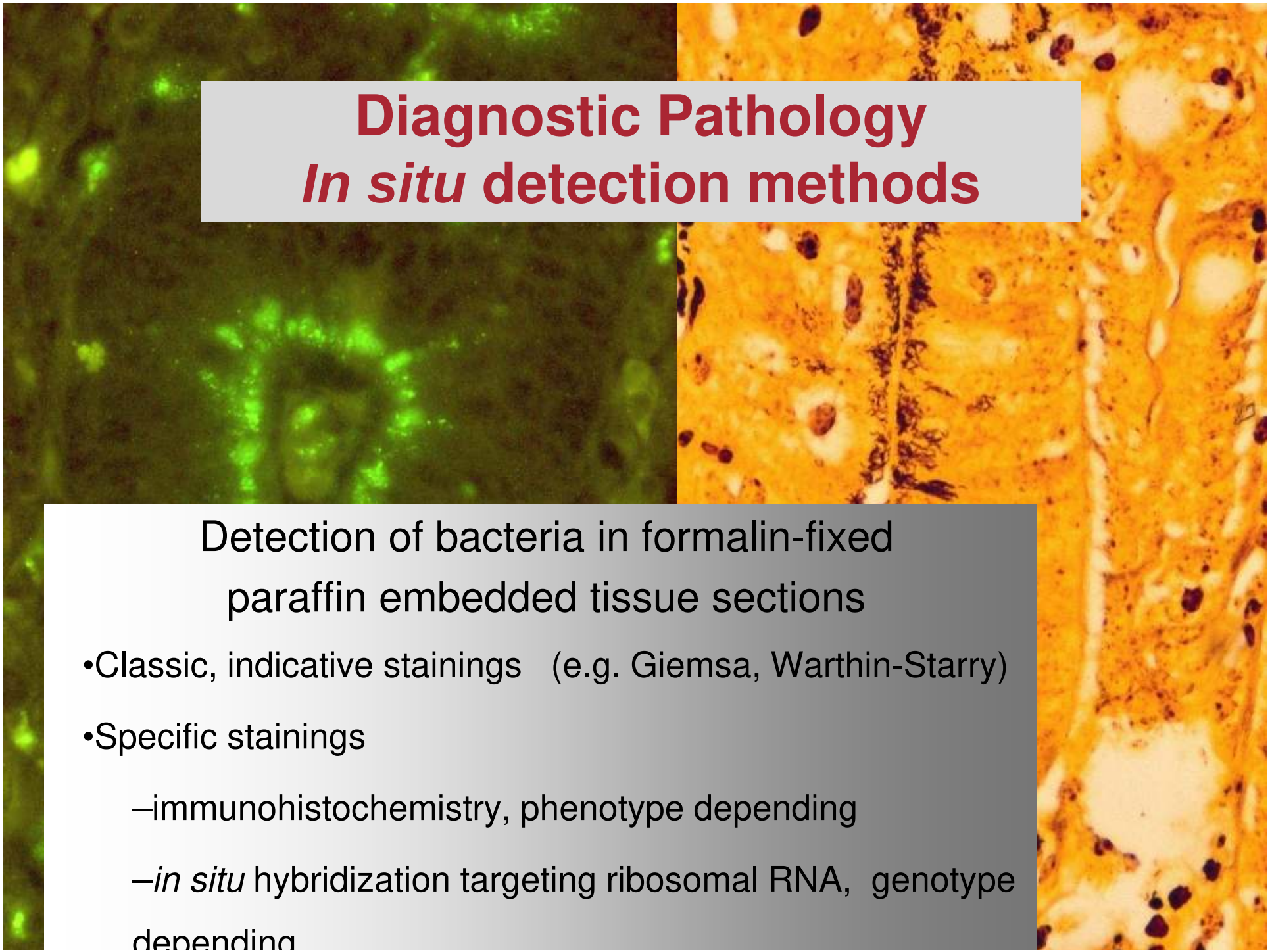
National Veterinary Institute

Technical University of Denmark

Brachyspirainfektioner hos svin og in situ identifikation

DVHS møde, Kolding 2/11 2012

Tim K. Jensen
Seniorforsker,
DTU-VET
København
E-mail: 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?

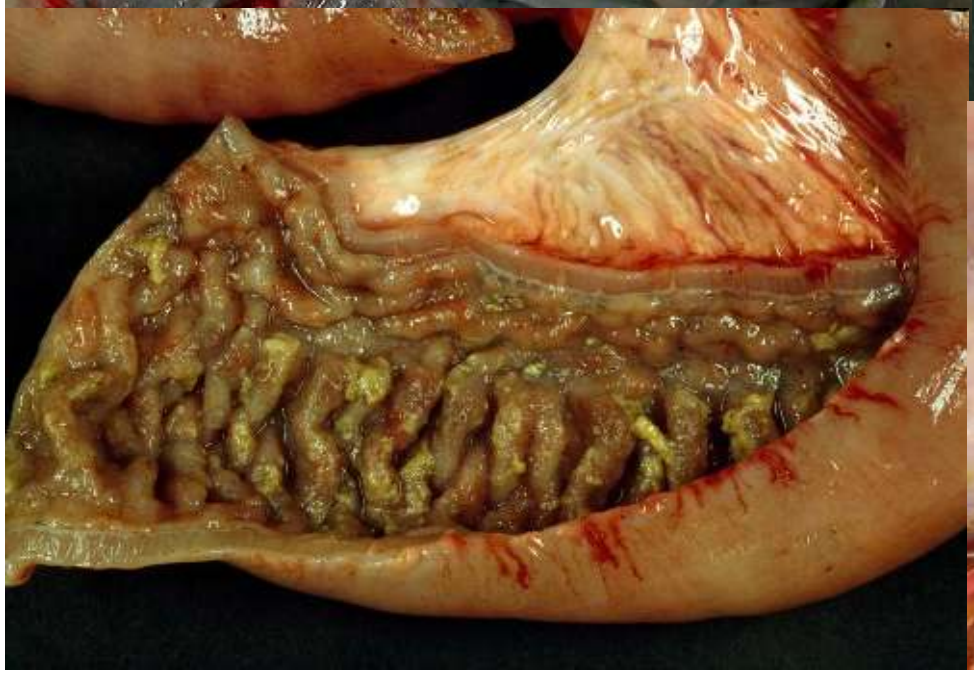
Svinedysenteri

- Smitsom mukohæmmorrhagisk colitis
- Brachyspira hyodysenteriae

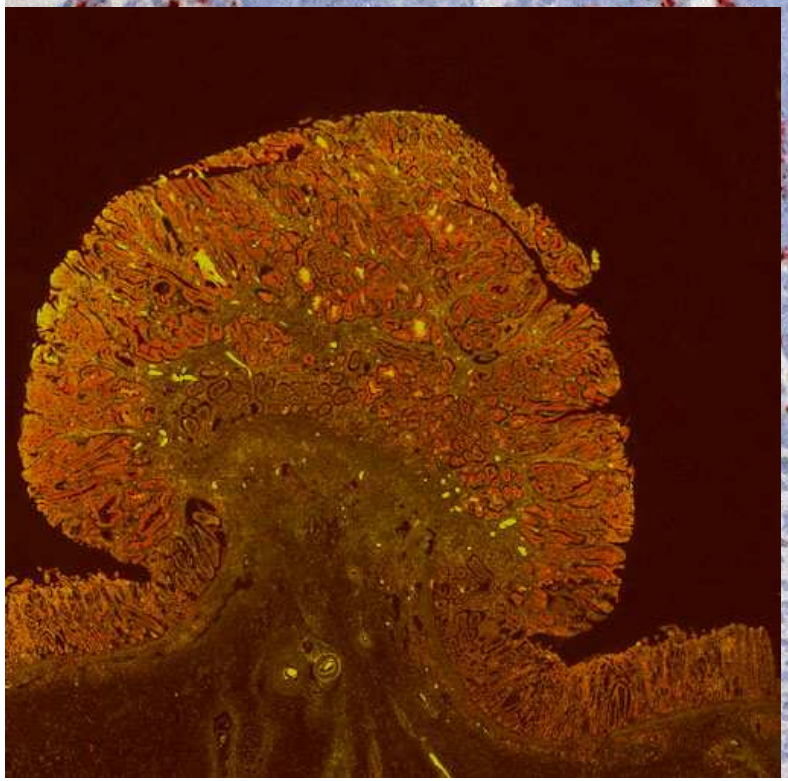
Andre Brachyspira

- 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



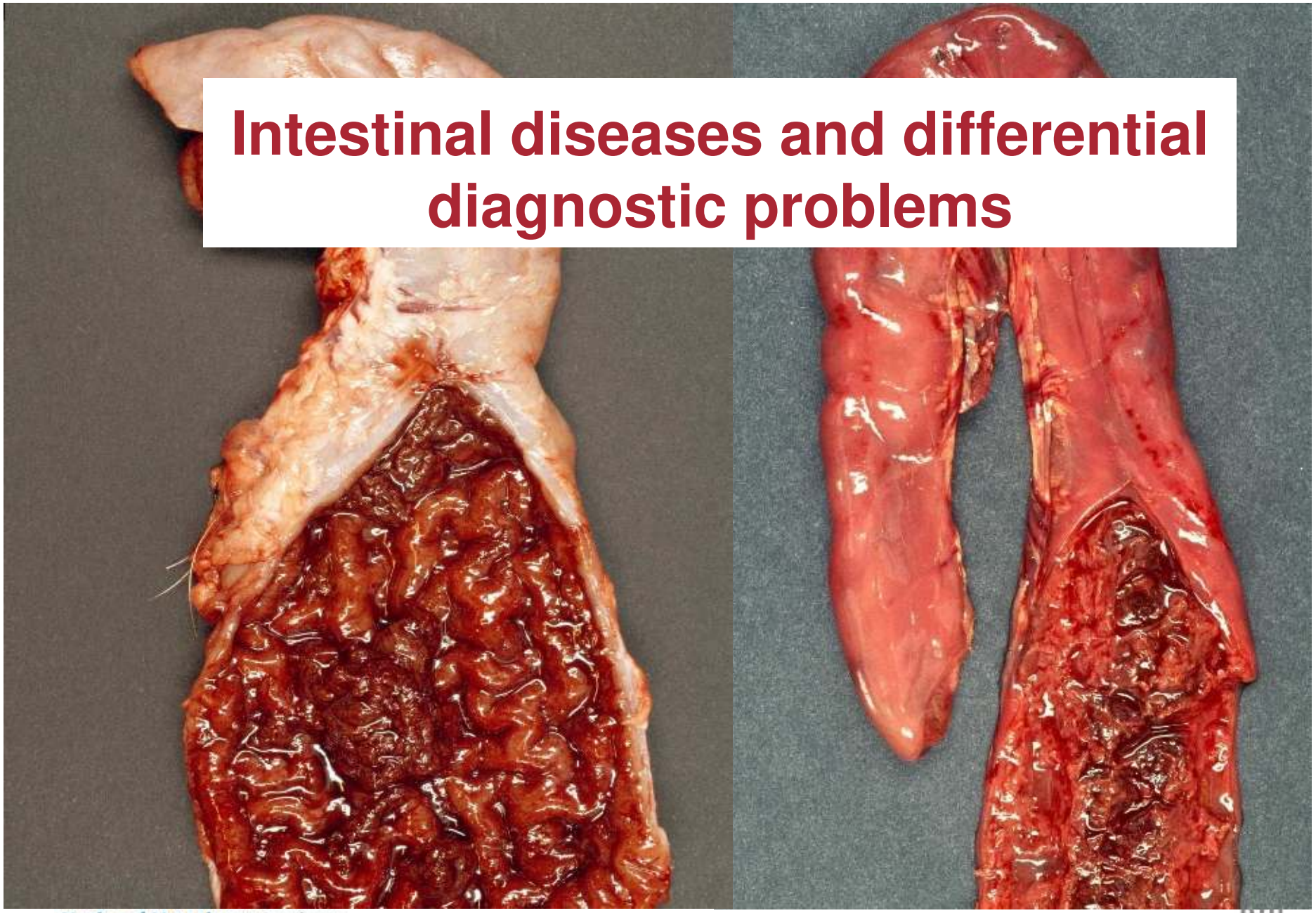
Proliferative enteropathy – *L. intracellularis*



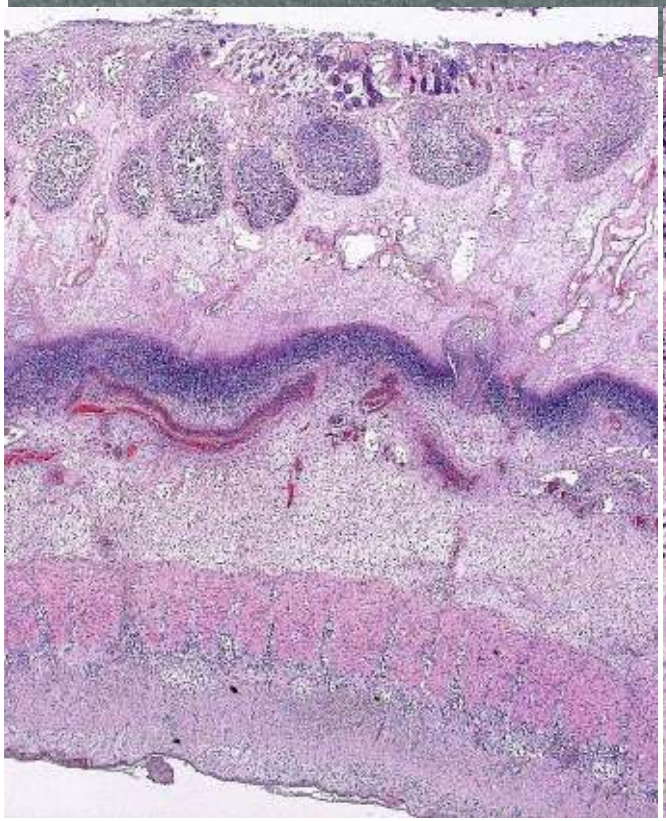
Demonstration of

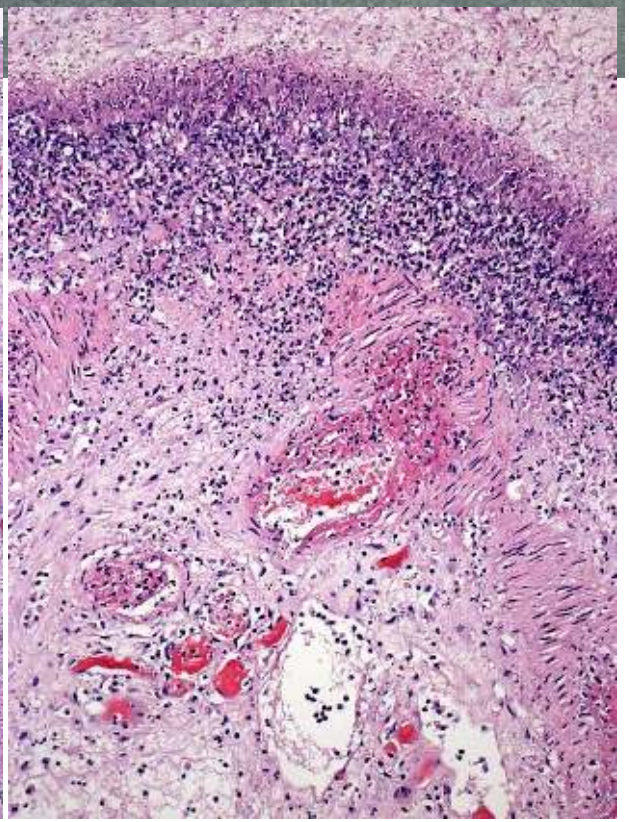
L. intracellularis by IHC

Intestinal diseases and differential diagnostic problems



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





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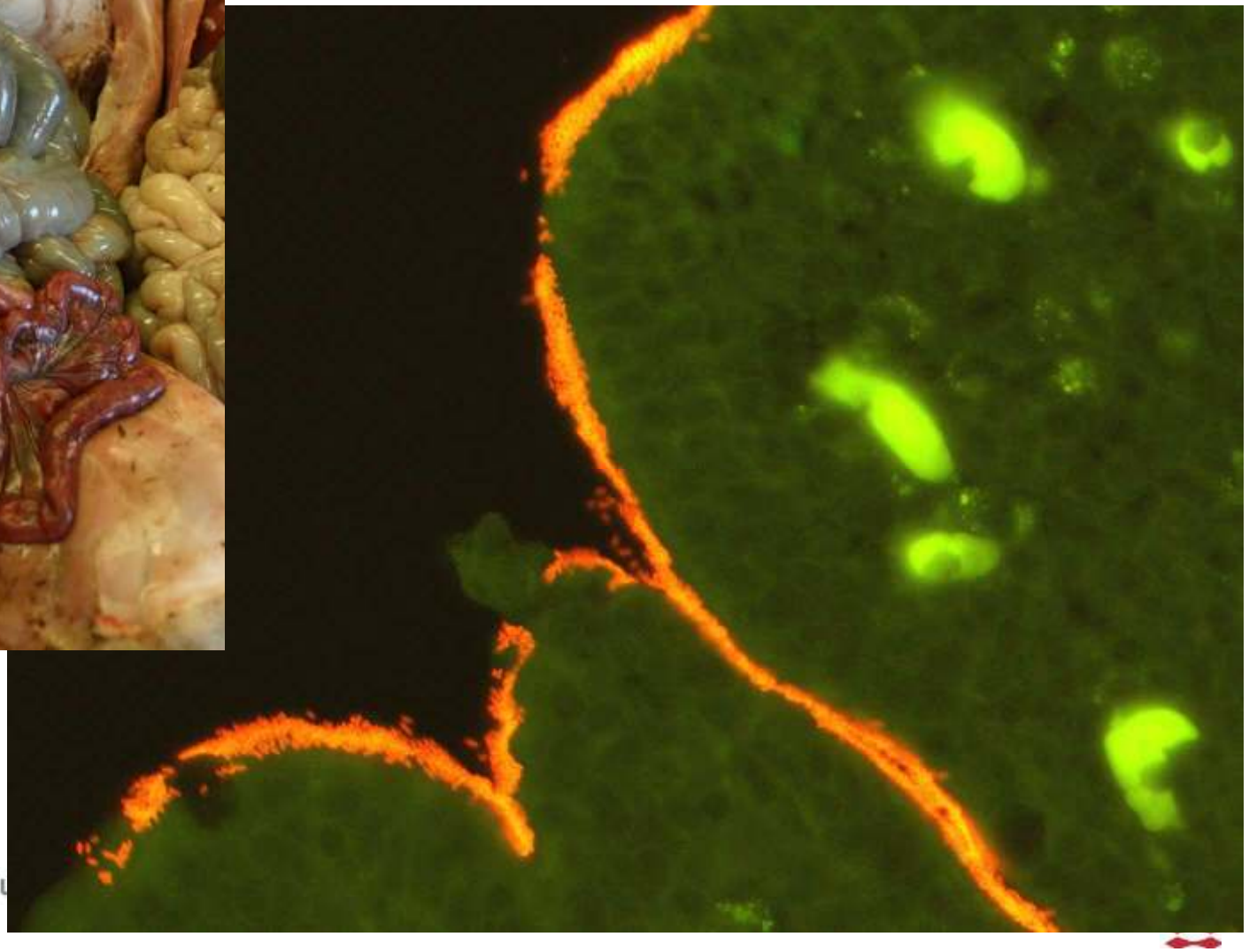
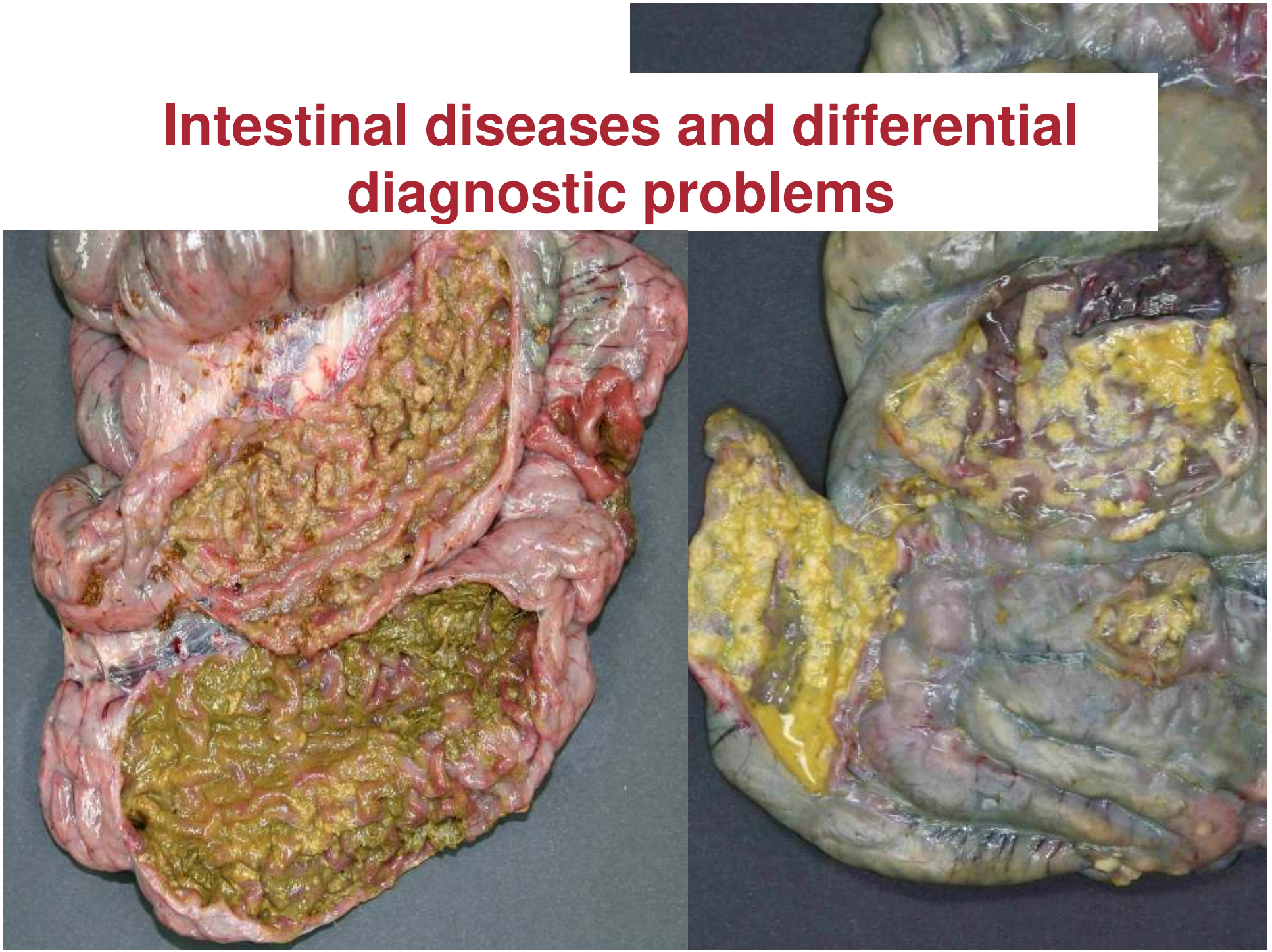
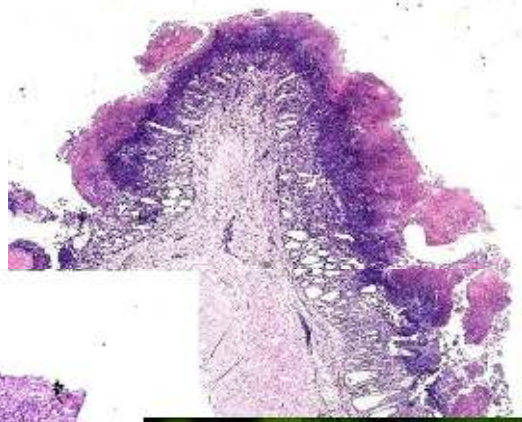
Demonstration of *E. coli* attached to villus epithelium in jejunum, FISH

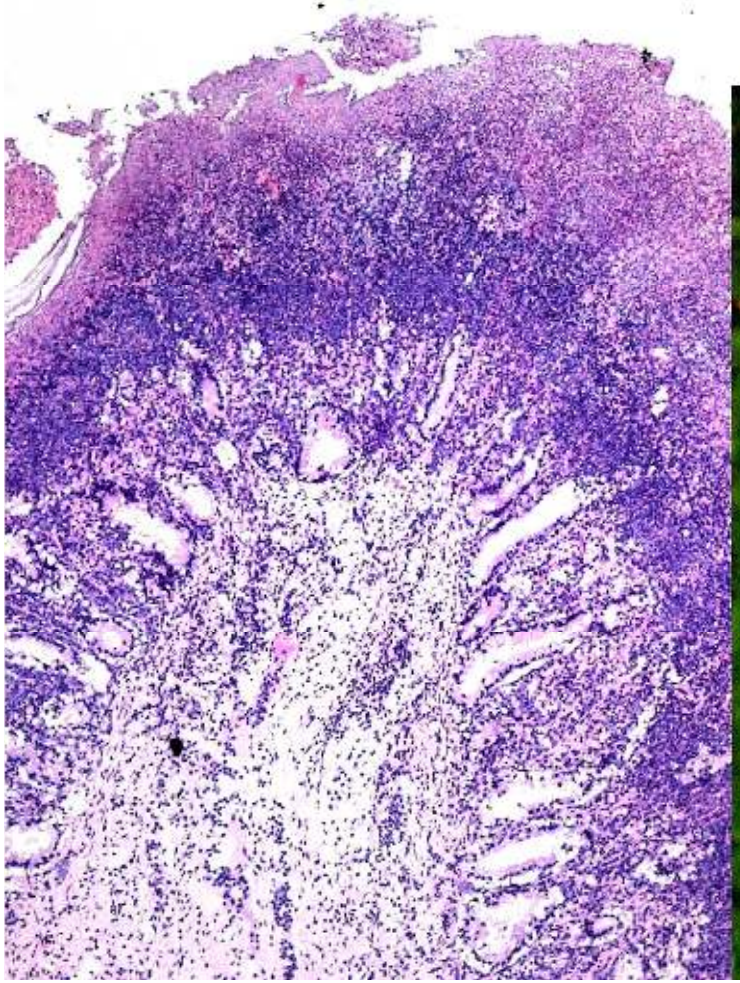


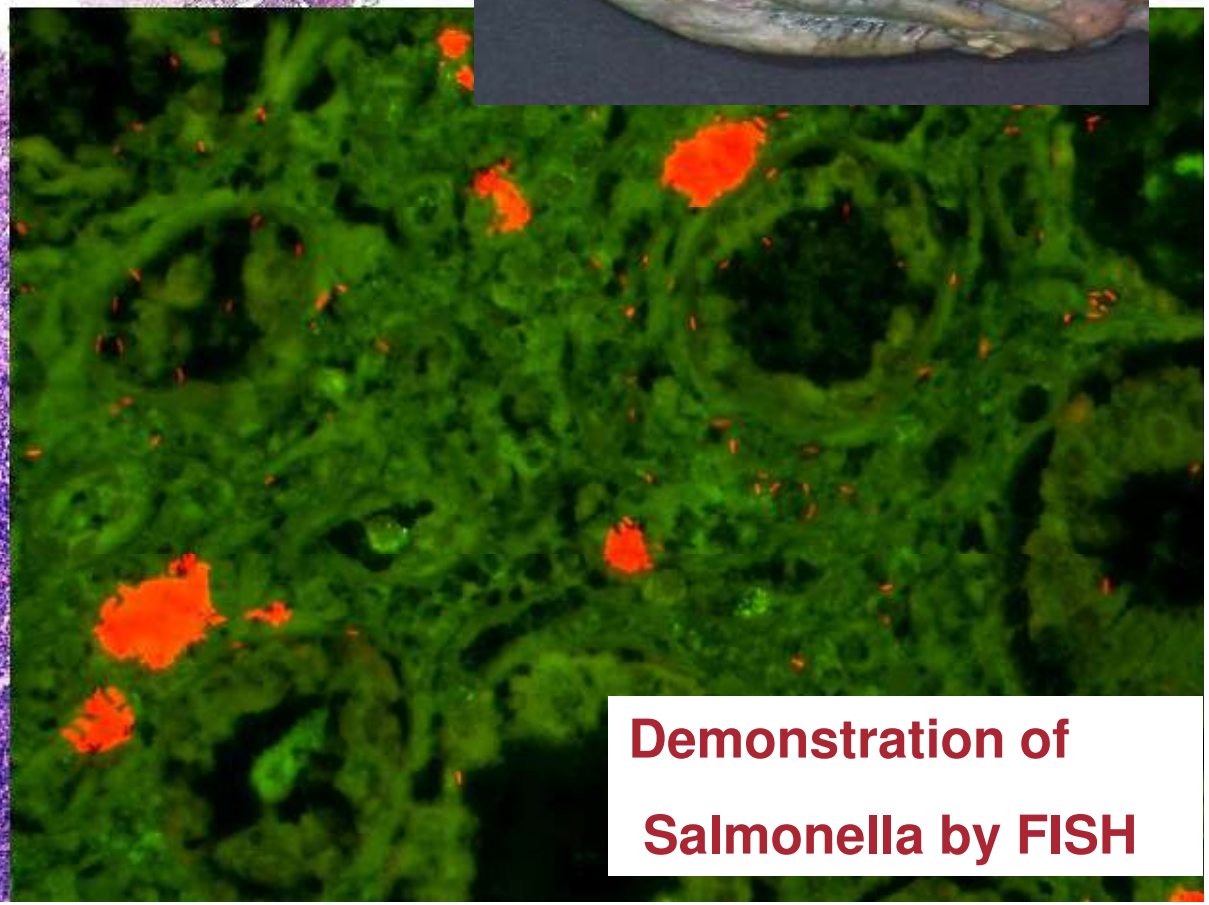
Intestinal diseases and differential diagnostic problems



Salmonellosis

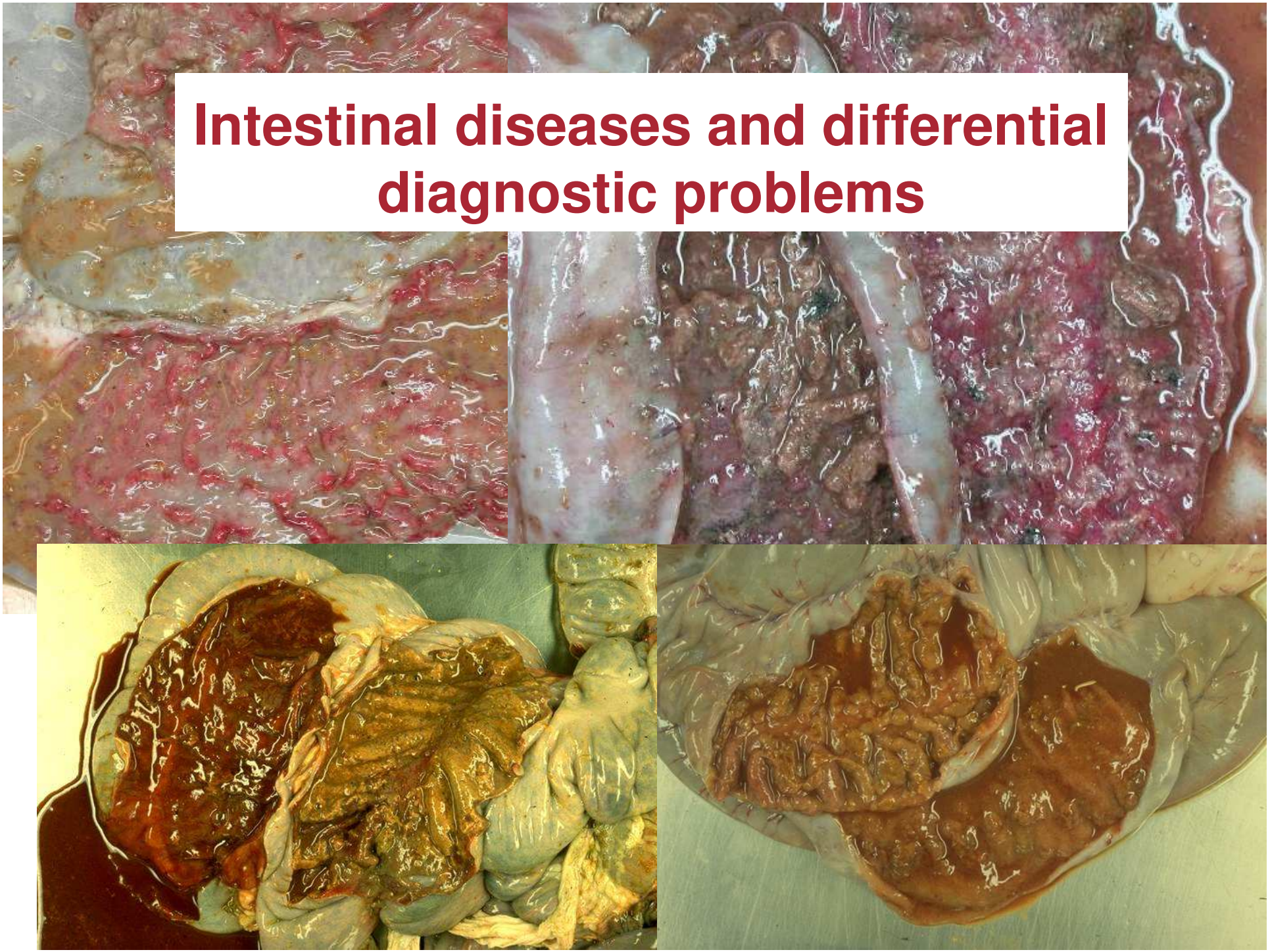




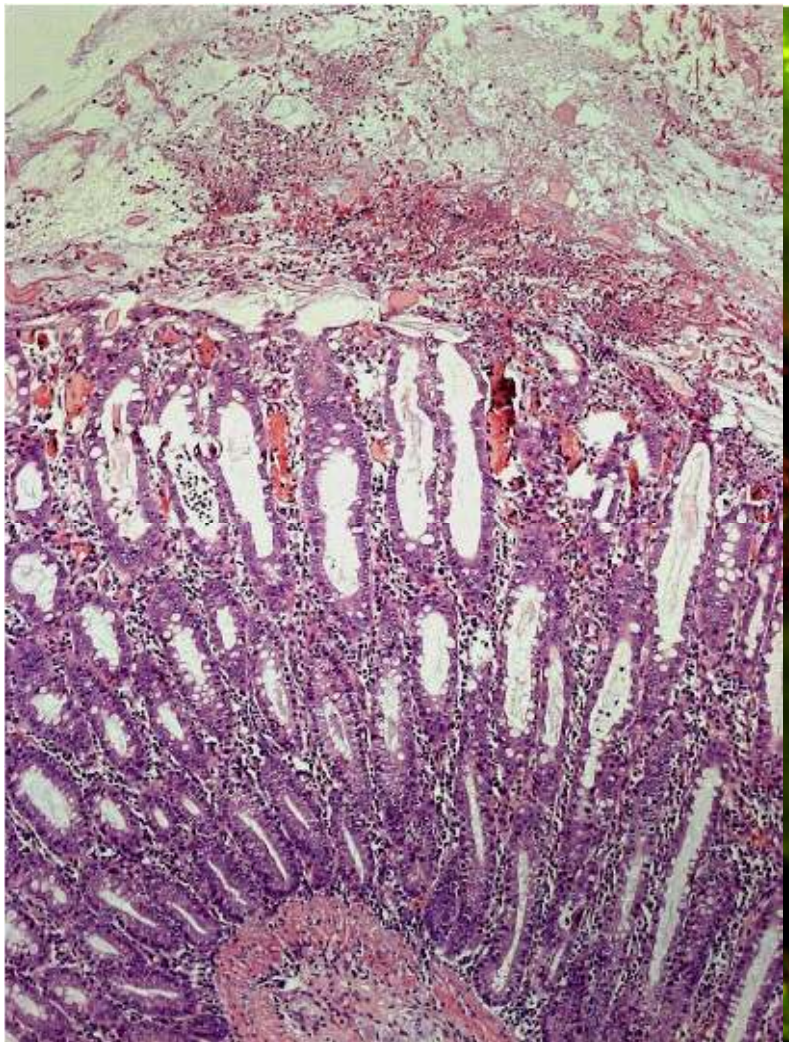


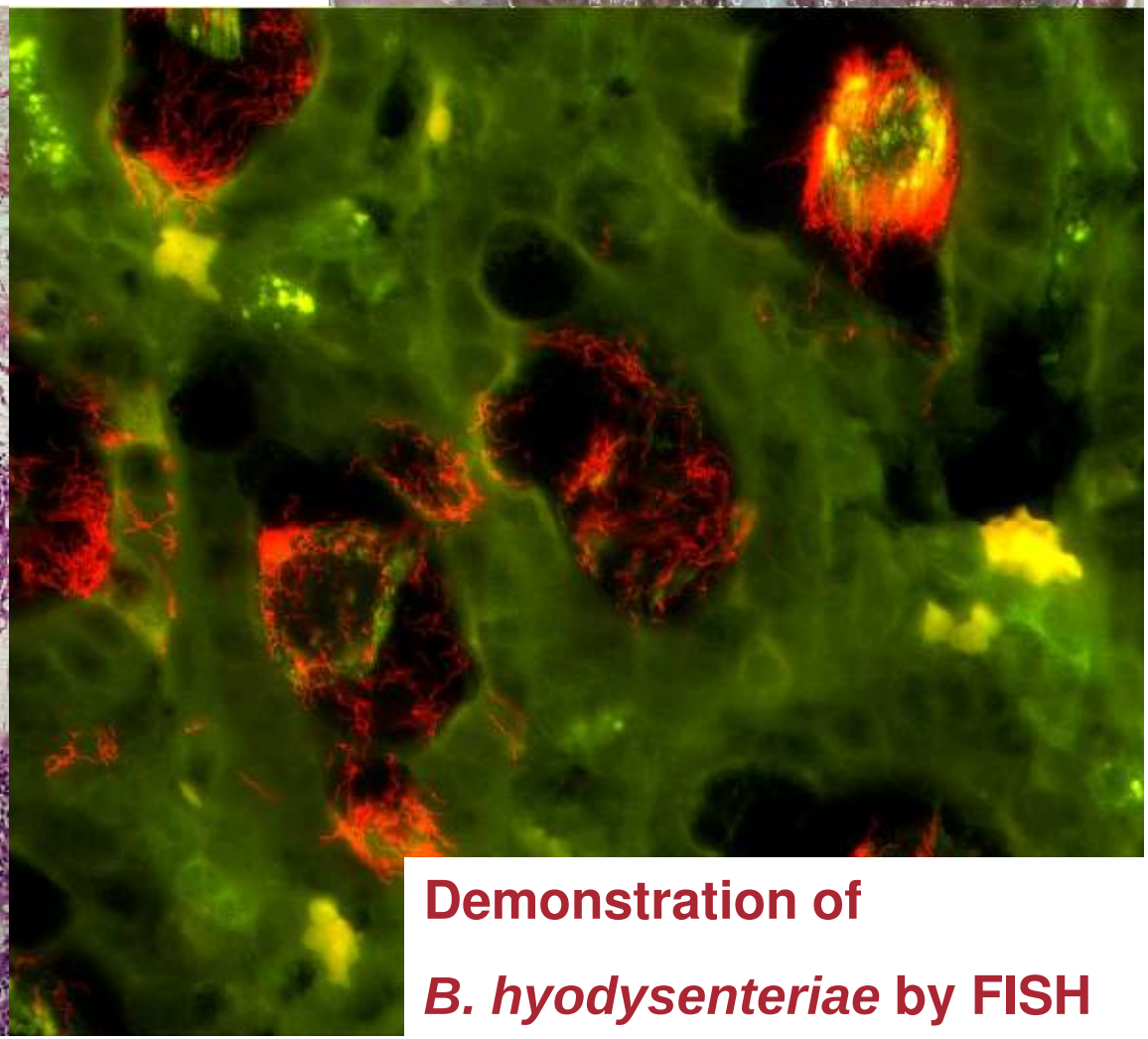
Demonstration of Salmonella by FISH

Intestinal diseases and differential diagnostic problems



Swine dysentery
B. hyohysenteriae

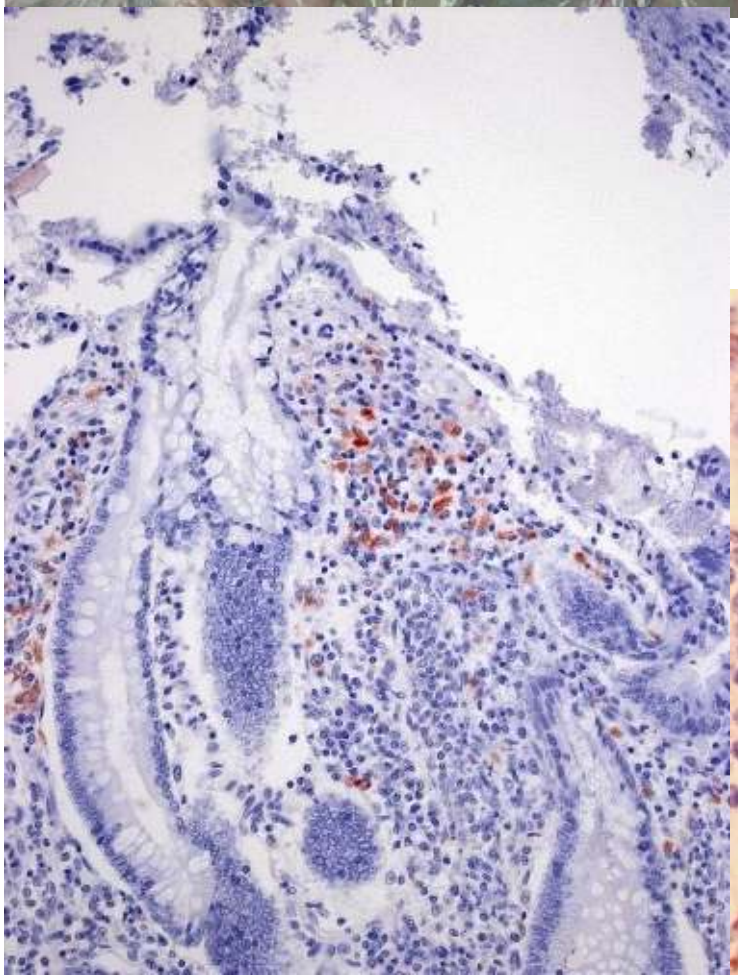




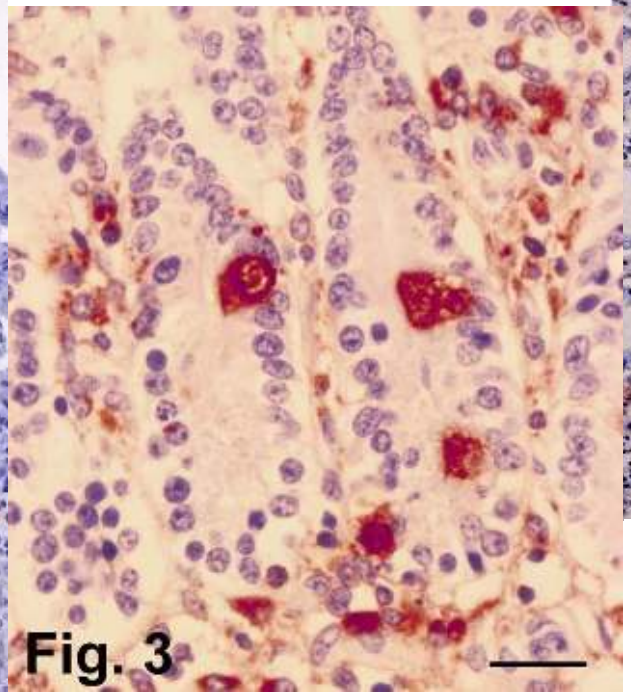
Demonstration of

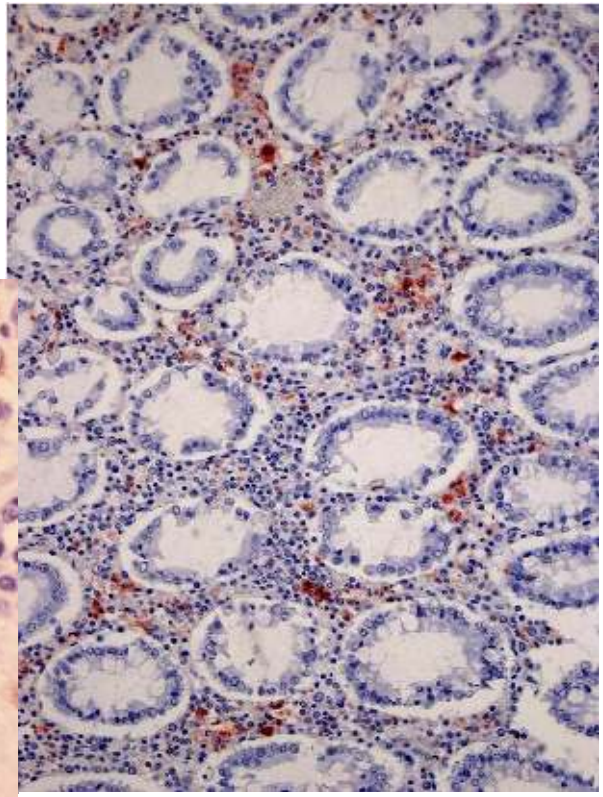
***B. hyodysenteriae* by FISH**

Intestinal diseases and differential diagnostic problems



PCV2 enteritis





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

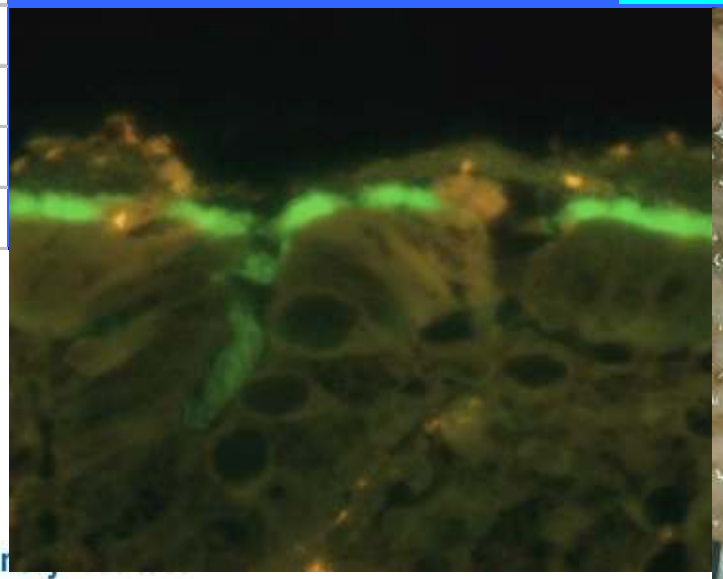
	L.intra	*Bhy+Bpil*	*B.hyo*	*B.pilo*	*B.inter*	B. inn	B. mur	PCV2
L.intra	**28**	**2**	**7**	**1**	**1**	**2**	**3**	**5**
B.hyo			**22**	**4**		**2**		
B.pilo				**3**		**1**		**2**
B.inter					**2**	**1**		
B. inn						**3**		**2**
B. mur							**3**	
PCV2								**14**

23 pigs were negative for all agents

In situ detection of large intestinal pathogens in pigs

Demonstration of mono- and polyinfections in pigs

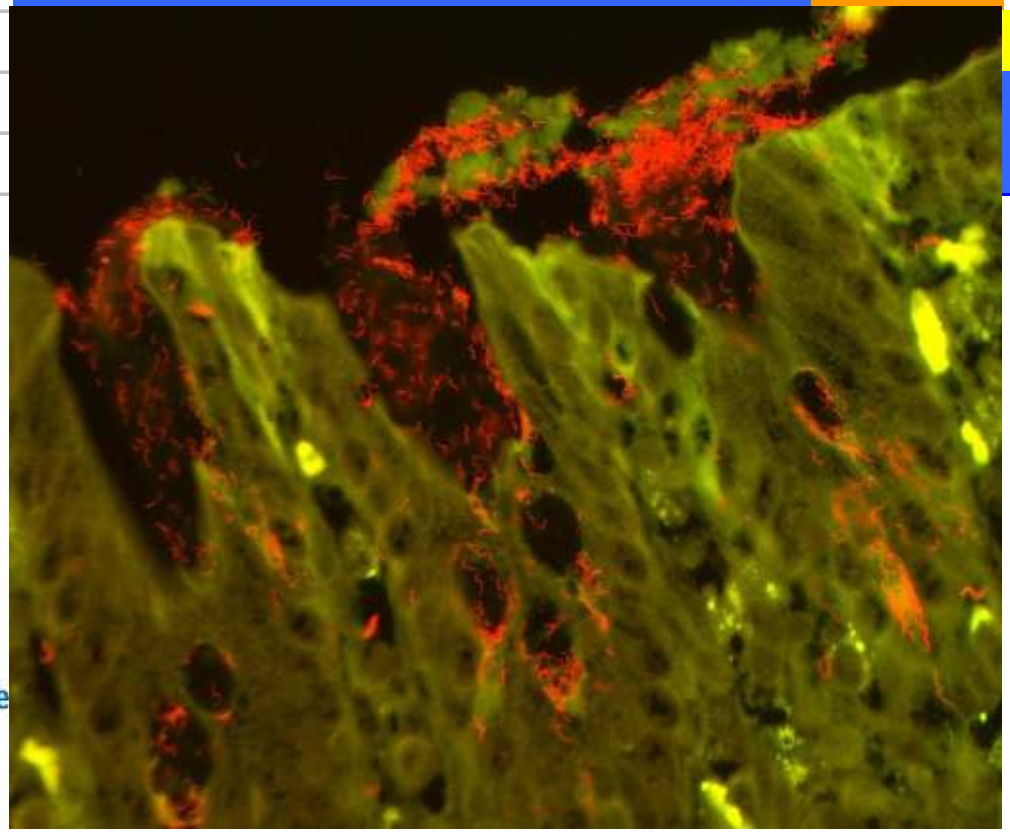
	L.intra	*Bhy+Bpil*	*B.hyo*	*B.pilo*	*B.inter*	B. inn	B. mur	PCV2
L.intra	28	2	7	1	1	2	3	5
B.hyo			22	4		2		
B.pilo				3		1		2
B.inter								
B. inn								
B. mur								
PCV2								



In situ detection of large intestinal pathogens in pigs

Demonstration of mono- and polyinfections in pigs

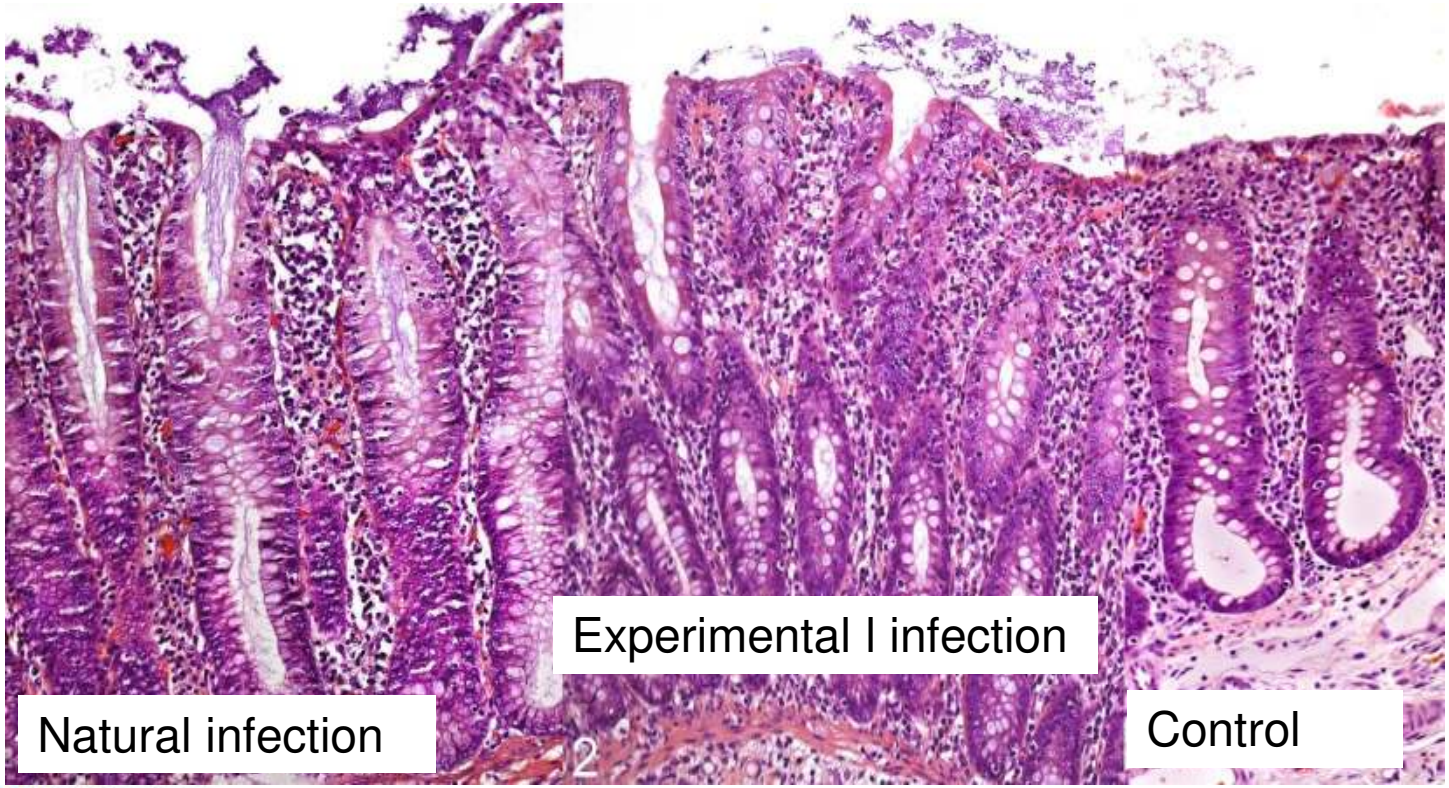
	L.intra	*Bhy+Bpil*	*B.hyo*	*B.pilo*	*B.inter*	B. inn	B. mur	PCV2
L.intra	28	2	7	1	1	2	3	5
B.hyo			22	4		2		
B.pilo				3		1		2
B.inter					2	1		
B. inn						3		2
B. mur							3	
PCV2								14



Experimental *B. murdochii* colitis

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

Diarrhoea was not observed.



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Jensen et al., Vet. Pathol. 2010

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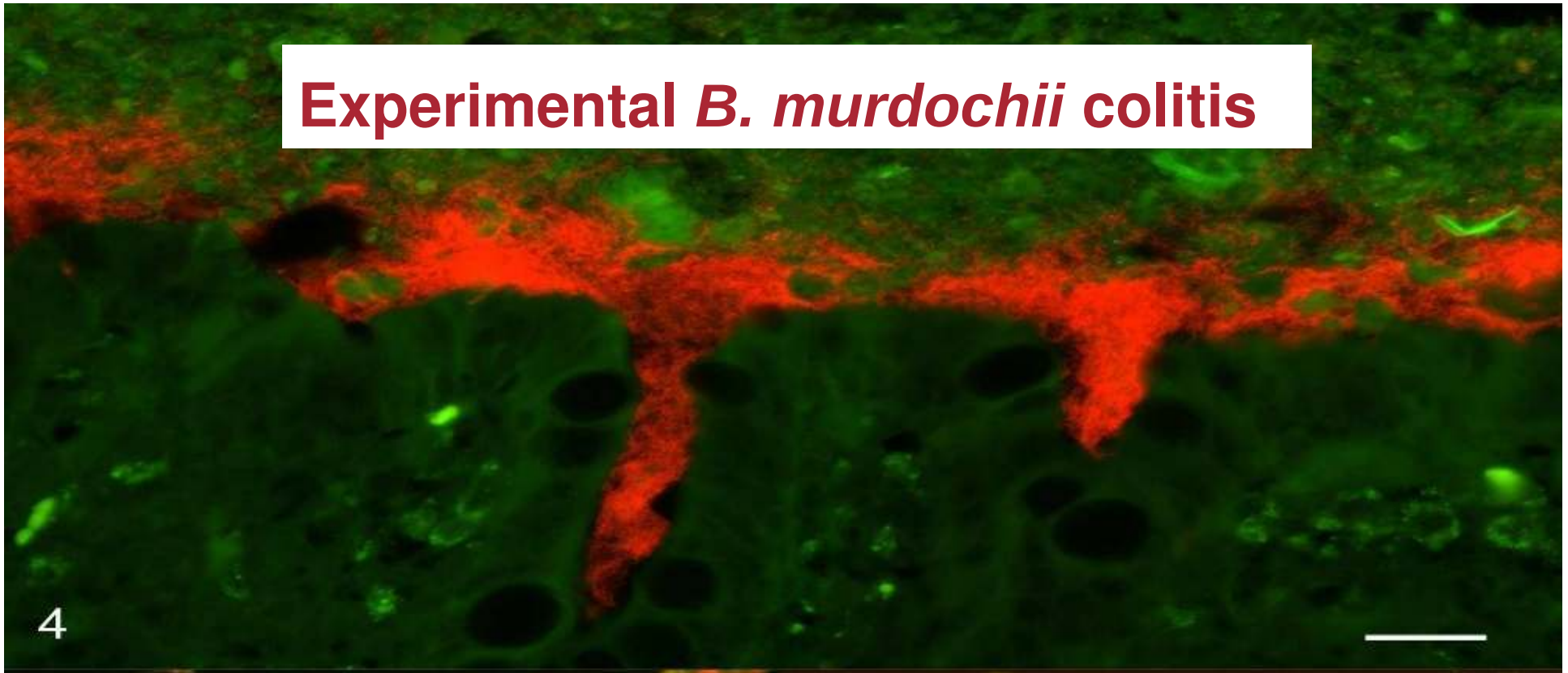
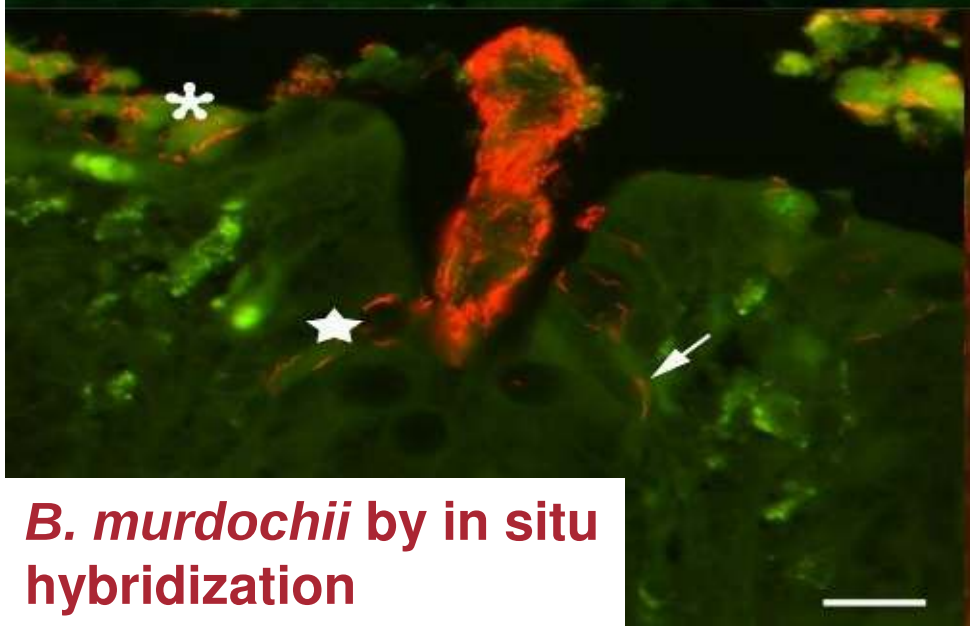
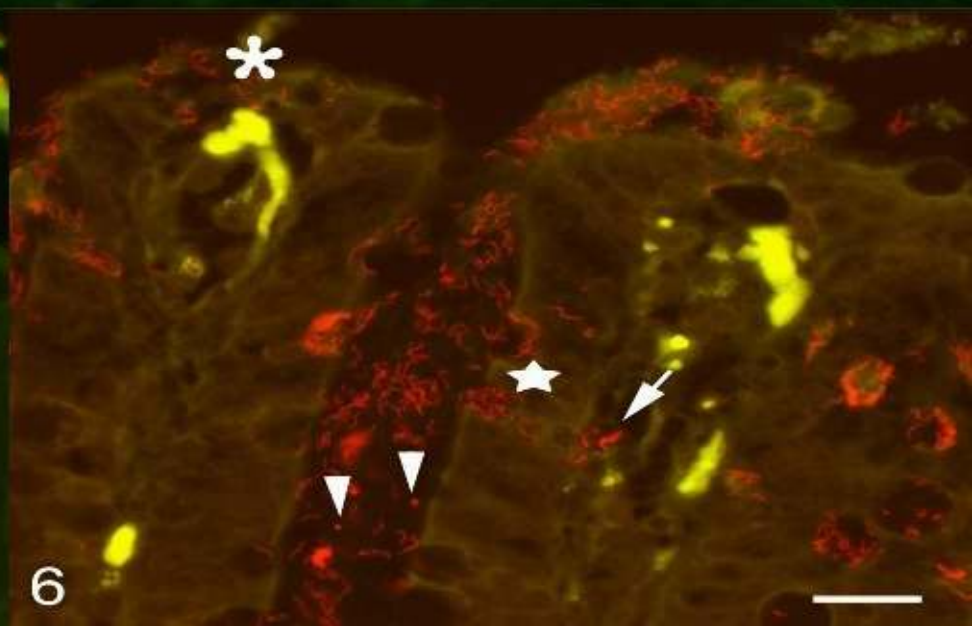
Experimental *B. murdochii* colitis







***B. murdochii* by in situ hybridization**

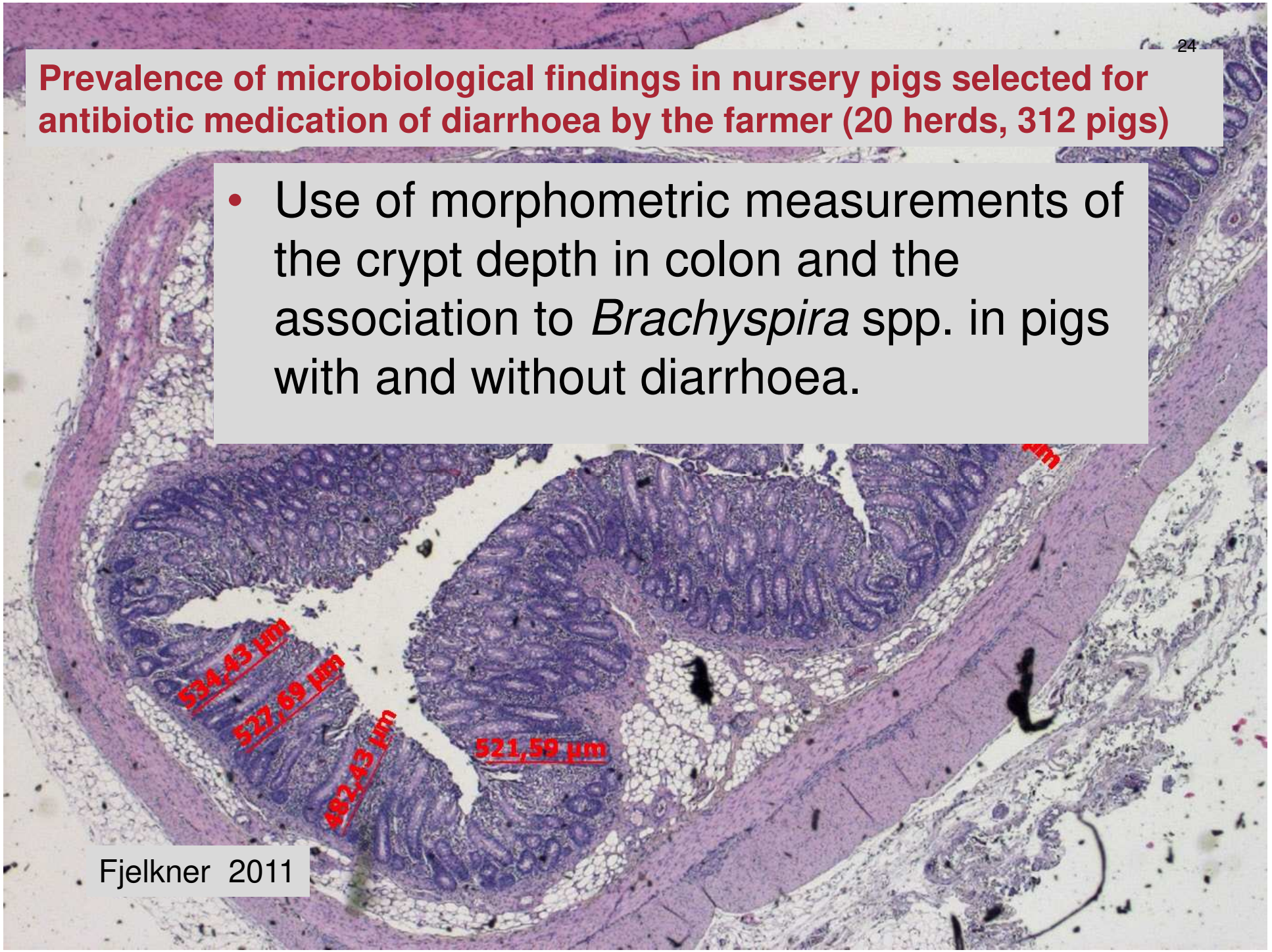
Prevalence of microbiological findings in nursery pigs selected for antibiotic medication of diarrhoea by the farmer (20 herds, 312 pigs)

Pig prevalences (%)

• *L. intracellularis*	19
• PCV2	4
• *Brachyspira spp*	40
B. pilosicoli	7
B. intermedia	10
B. innocens	16
B. murdochiii	21
• Hemolytic *E. coli*	15

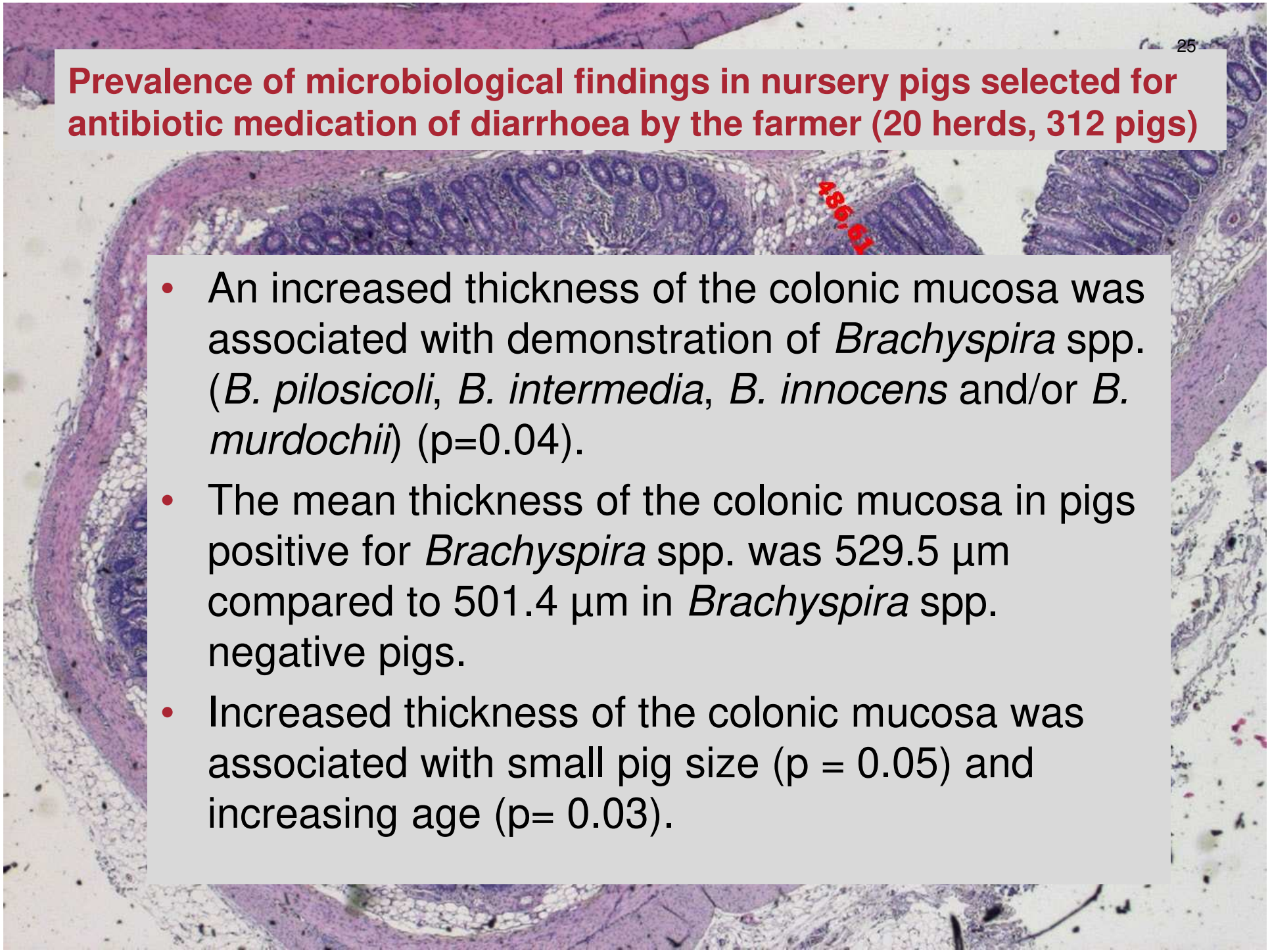
Prevalence of microbiological findings in nursery pigs selected for antibiotic medication of diarrhoea by the farmer (20 herds, 312 pigs)

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



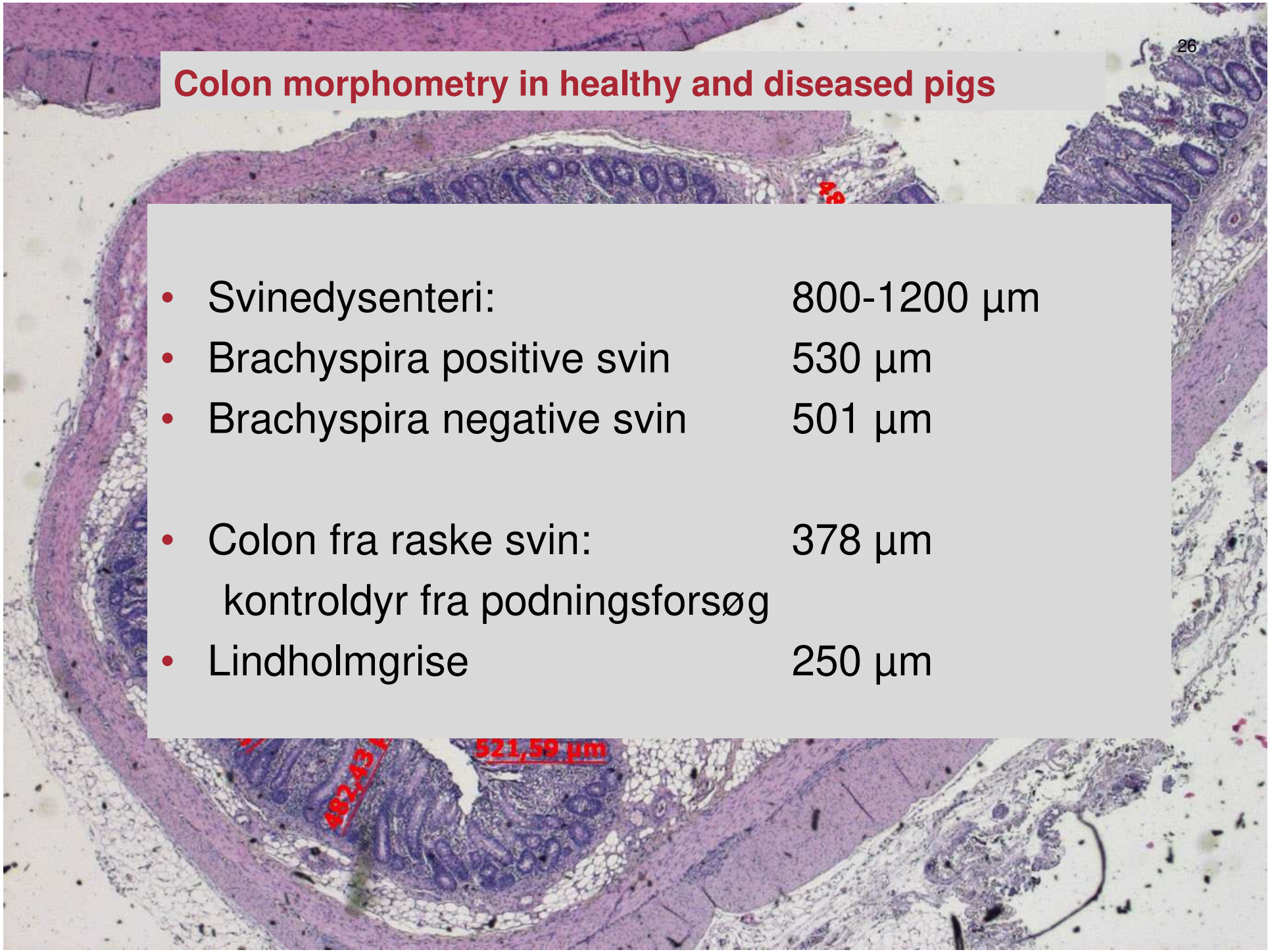
Fjelkner 2011

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 µm compared to 501.4 µm 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

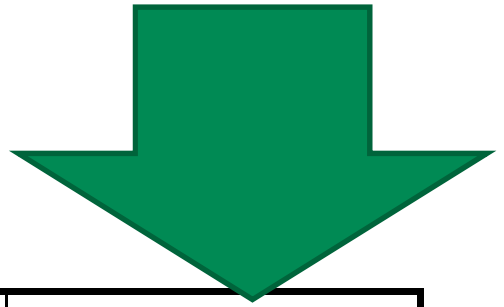


- Svinedysenteri: 800-1200 µm
- Brachyspira positive svin 530 µm
- Brachyspira negative svin 501 µm

- Colon fra raske svin: 378 µm
  kontroldyr fra podningsforsøg
- 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)
Uncomplicated PE (15) Necrotizing enterocolitis (10) PHE (3)	Uncomplicated PE (3) Necrotizing enterocolitis (3)	Necrotizing enterocolitis (12) Other lesions (11)	Necrotizing enterocolitis (6) Other lesions (1)

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Jensen et al. J. Comp. Pathol. 2006.

DTU

Porcine necrotizing enterocolitis

- Necropsy

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

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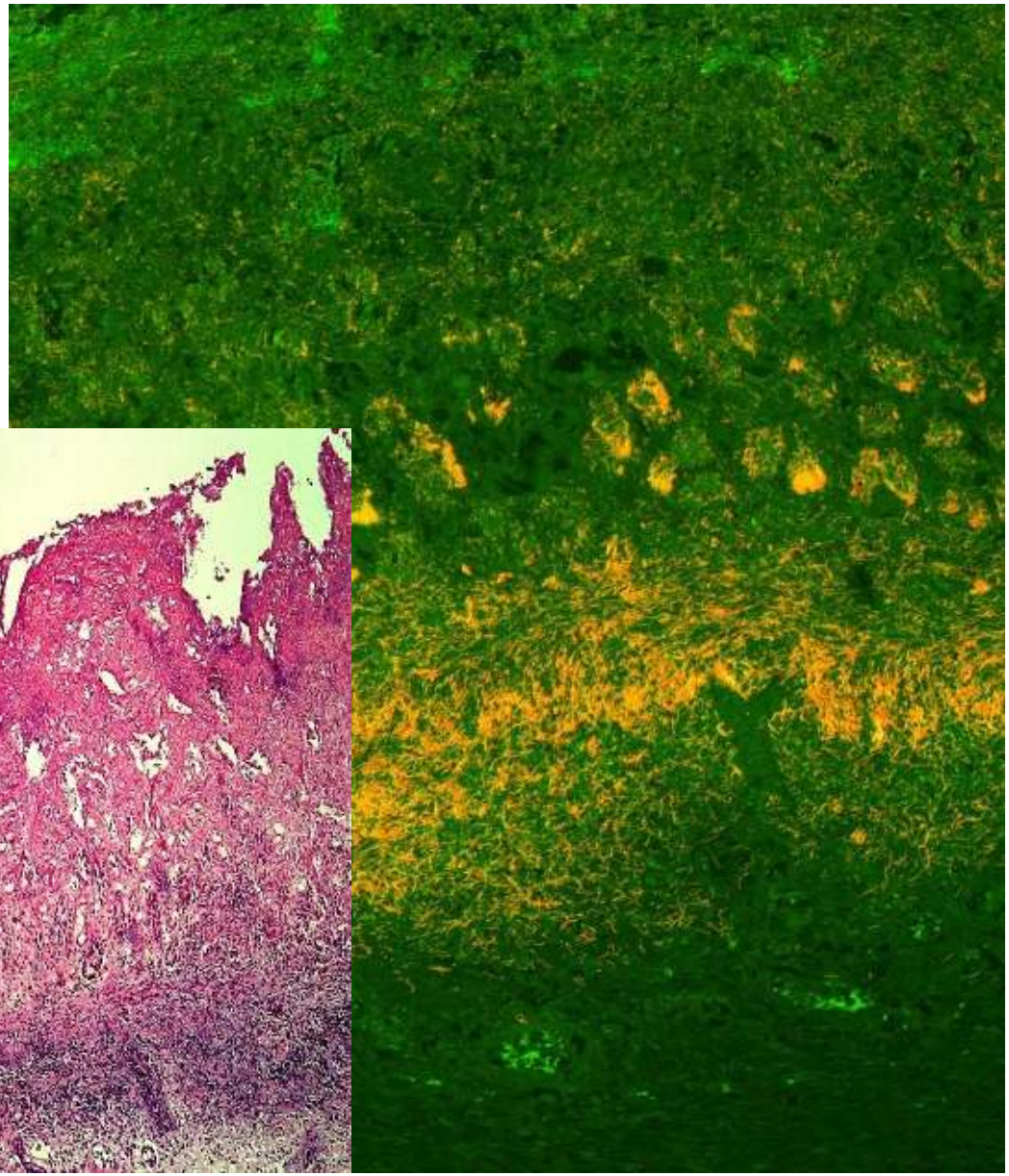
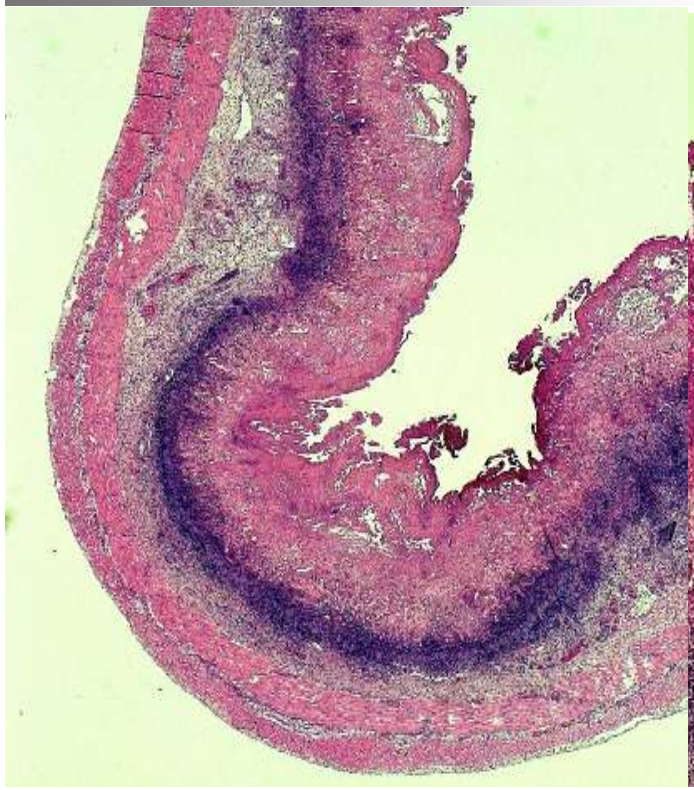
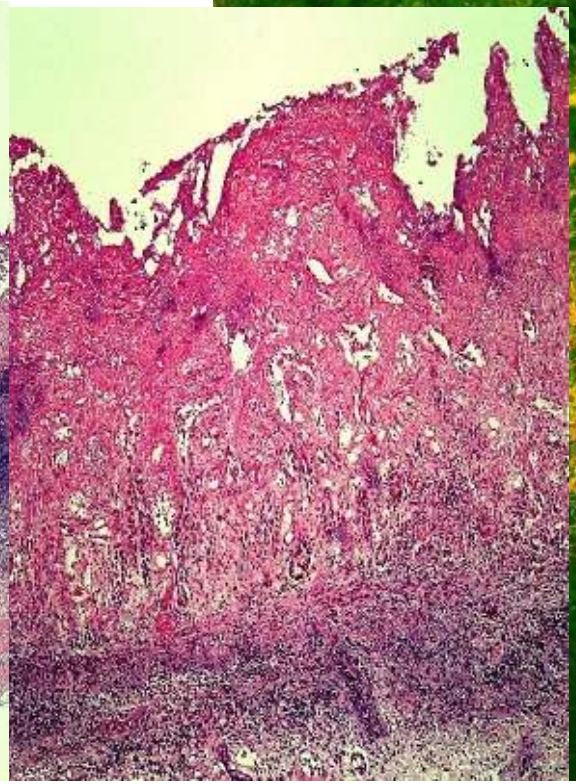
Porcine *Fusobacterium necrophorum* necrotizing enterocolitis

Histopathology:

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





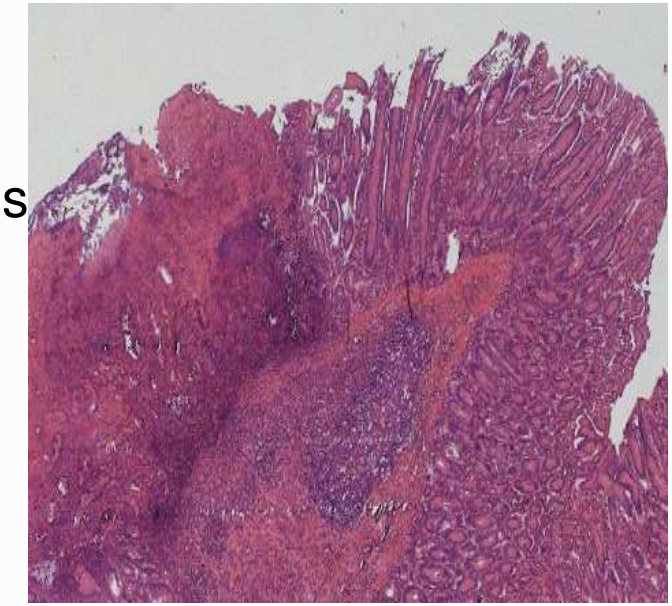


Porcine *Fusobacterium necrophorum* necrotizing enterocolitis

Retrospective FISH study on 84 intestines from pigs with suspicion of *L. inttracellularis* 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

Wolf-Jäckle: Manuscript in preparation

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
Lars Mølbak
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