HIPRA

Diagnosis of enteric diseases in swine.

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Diagnos Manager - HQ

Contents

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Causes of diarrhoea

Causes of diarrhoea

INFECTIOUS

Bacteria

E. Coli (ETEC, EPEC, VTEC)
C. Perfringens (tipo A y C)
C. Difficile (A y B)
Enterococcus
Salmonella
Lawsonia intracellularis
Brachyspira spp.

Viruses

Rotavirus (A, C)
Coronavirus (PED, GET)

Parasites

Cystoisospora suis

Causes of diarrhoea

NON-INFECTIOUS

Feeding

- Formulation.
- Features (flour, granules...).
- Presence of contaminants (mycotoxins, fungi).
- Excessive milk consumption.

Drinking water quality

- Sanitizing treatments.
- Microbiological char.
- Physicochemical char.

Environment

- Temperature, humidity, ventilation, currents.
- Density of animals.
- Difficulty of access to water or food.
- Dirty environment.

Improper handling

- Biosecurity
- Stress

Inmunity

- Insufficient colostrum intake
- Mother not immunized

MULTIFACTORIAL

Causes of diarrhoea

Agents	N° positive (%)		OR[a]	p
	Case litters (n = 147)	Controls litters (n = 129)		
Coccidia	9 (6.1)	11 (8.5)	0.9	0.88
Cryptosporidium spp.	2 (1.4)	6 (4.6)	0.2	0.16
Clostridium perfringens type A	13 (8.8)	6 (4.6)	1.4	0.47
Rotavirus	11 (7.5)	5 (3.9)	1.6	0.43
Clostridium difficile[b]	7 (10.6)	11 (16.6)	0.5	0.33

[a] Odds Ratio; [b] n = 132 (66 cases and 66 controls).

Lippke, R et al, 2011 (Brazil)

Table 3 Comparative results on *E. hirae* culture and PCR detection of rotavirus A, *C. difficile* and *C. perfringens* type A carrying beta2 genes (CpA-cpb2) in case vs. control piglets

	Case piglets (n = 171)	Control piglets (n = 97)	P-value*
C. difficile	111 (65%)	55 (57%)	0.2
CpA cpb2	157 (96%)	90 (97%)	1
E. hirae present	76 (44%)	42 (43%)	0.9
Massive growth of *E. hirae*	28 (16%)	9 (9%)	0.1
Rotavirus A	42 (25%)	6 (6%)	< 0.001

*Two-sided Fisher's exact test

Kongsted et al. 2018 (Denmark)

Proportion and statistical values of enteric agents between diarrheic (n = 140) and healthy (n = 88) animals.

Agent	Proportion Cases % (n)	Proportion Controls % (n)	Pearson Chi-square	p-value	Fisher's exact test (p)
Viral agents					
RVA	61.4 (86)	31.8 (28)	18.95	0. 00013	–
RVB	12.1 (17)	4.9 (6)	1.69	0.19	–
RVC	33.6 (47)	36.4 (32)	0.18	0.67	–
PCoV	4.3 (6)	2.3 (2)	0.65	–	0.72
C. perfringens					
Cpα	73.5 (103)	79.5 (70)	1.05	0.30	–
Cpβ	2.8 (4)	1.1 (1)	0.75	–	0.65
Cpβ2	60.7 (85)	61.4 (54)	0.01	0.922	–
C. difficile					
TcdA	25.7 (36)	19.3 (17)	1.24	0.27	–
TcdB	27.1 (38)	29.5 (26)	0.15	0.69	–
E. coli adhesins					
F4	0	1.1 (1)	–	–	–
F5	0.7 (1)	0	–	–	–
F6	0	0	–	–	–
F18	0.7 (1)	0	–	–	–
F41	3.6 (5)	1.1 (1)	1.25	–	0.41
eae	13.6 (19)	14.8 (13)	0.065	0.8	–
E. coli toxins					
LT	0	0	–	–	–
Sta	1.4 (2)	0	–	–	–
Stb	5.7 (8)	3.4 (3)	0.63	–	0.54
EAST1	57.1 (80)	67 (59)	2.23	0.14	–
VT1	2.9 (4)	0	–	–	–
VT2	2.9 (4)	0	–	–	–

Vidal, A et al. 2019 (Spain)

PART OF THE NORMAL MICROBIOTA

Causes of diarrhoea

Distribution of farms positive to the different panel of enteric pathogens. RVA/B/C, Rotavirus A/B/C; *C. difficile*, toxigenic strains (TcdA, TcdB); *E. coli*, pathogenic *E.coli*; PCoV, porcine coronaviruses; Cp A/C, *C. perfringens* A/C.

Farms	N	RVA	RVB	RVC	PCoV	*C.difficile*	*Cp* A	*Cp* C	*E.coli*
Number of positive farms:	6	+	–	+	–	+	+	–	+
enteric pathogen	4	+	–	+	–	+	+	–	–
associations	3	+	–	–	–	+	+	–	+
	3	–	+	+	–	+	+	–	+
	2	+	–	+	+	+	+	–	+
	1	+	+	+	+	+	+	–	+
	1	+	–	–	+	+	+	–	+
	1	+	–	–	+	+	+	–	–
	1	+	–	+	–	+	+	+	+
	1	+	–	–	–	+	+	+	+
	1	+	+	+	–	+	+	–	–
	1	+	+	+	+	–	+	–	–
	1	+	–	+	–	–	+	–	+
	1	+	–	–	+	–	+	–	–
	1	+	–	–	–	–	+	–	–
	1	–	+	+	–	+	+	–	–
	1	–	–	+	–	+	+	–	+
	1	–	–	–	–	+	+	–	–
TOTAL FARMS	31	25	7	22	7	27	31	2	20

Vidal, A et al. 2019 (Spain)

COINFECTIONS


MULTIFACTORIAL
PART OF THE NORMAL MICROBIOTA
COINFECTIONS

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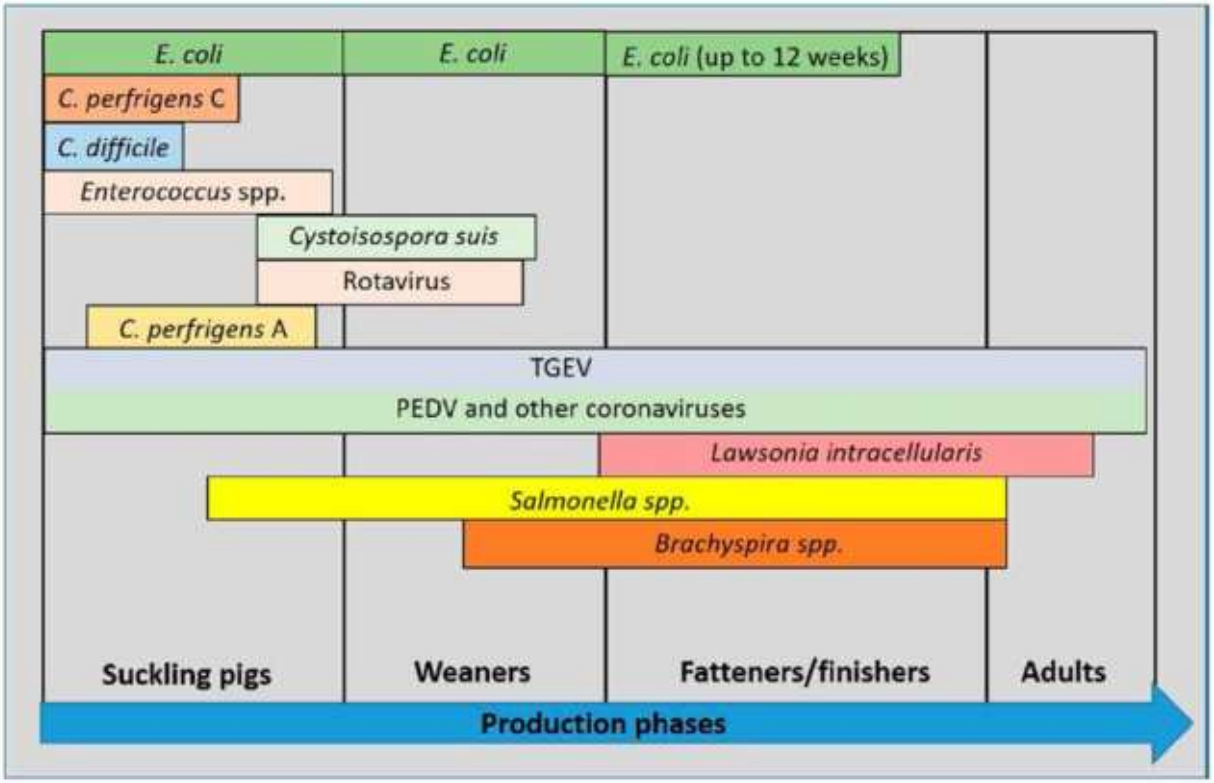
Essential for a good diagnosis

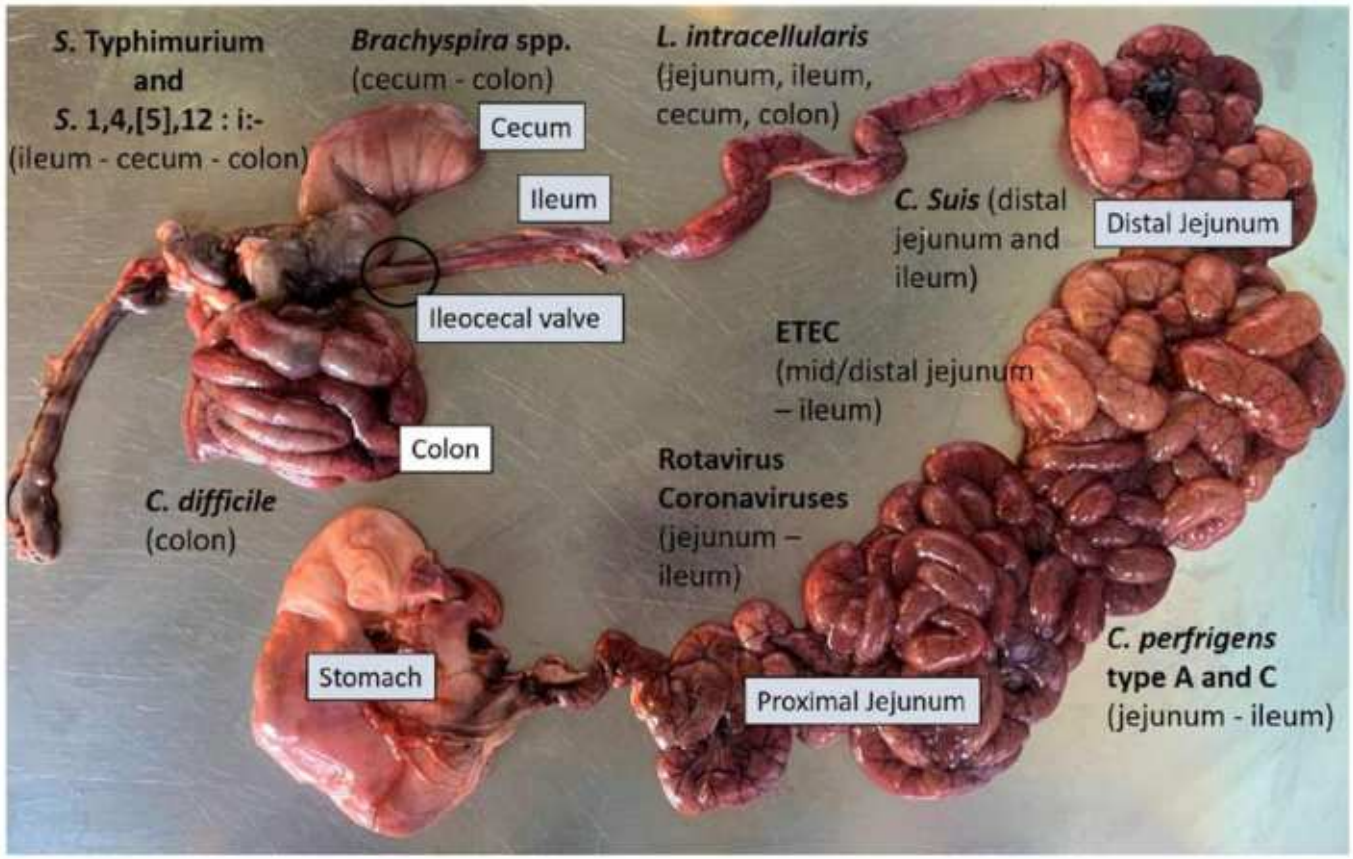
Essential for a good diagnosis

- **Farm history**
- **Age of the animals**
- **Clinical signs**
    - Diarrhoea appearence: changes in colour and consistency
    - Vomiting
    - Number of affected litters/piglets. Partiy of the sows, etc.
- **Gross lesions**
    - Type of enteritis (catarrhal, fibrinous, necrotic, etc.)
    - Localisation of the lesions (small and/or large intestine)
    - Distribution (focal, diffused, segmental, etc.)
- **Microscopic lesions (Histopathology)** (= gold standard)
- **Animal selection, sampling and shipment to the labo**
- **Combination different diagnostic techniques**

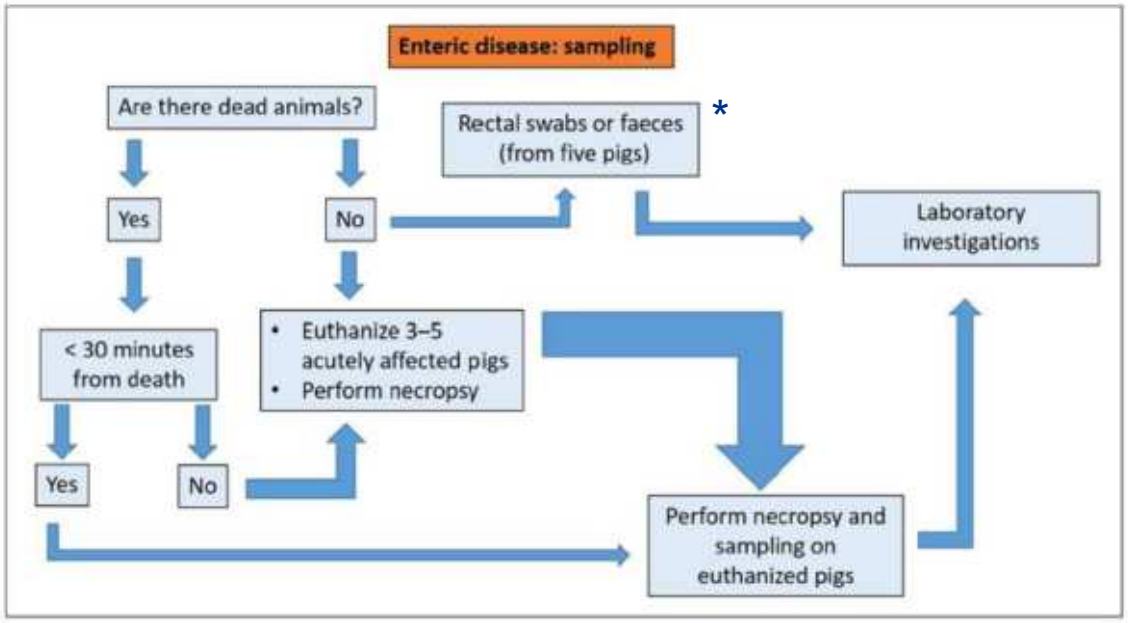
Incidence of pathogens in enteric disease in pigs related to age



Gross lesions



Sampling and shipment to the laboratory



- **3-5** pigs.
- **Acutely affected** (diarrhoea < 12-24h).
- **No antimicrobial** treatments.
- Parts of the **instestines** (jejunum, ileum, colon) not oppened.
- Containers separated from other samples to **avoid cross contaminations.**
- Keep at **4ºC**
- Submission **<24h**

Sampling and shipment

BACTEIAL ISOLATION:

- Asepsis (as much as posible)
- Aviod autolysis

PCR:

- Asepsis not crutial
- Avoid cross reactions
- Avoid autolysis

HISTOPATHOLOGY:

- Do not damage the sample

Feces/Stool: Collect directly from the rectum.

Swabbing: Rubbing gently to the walls, turning on itself.

Sampling and shipment

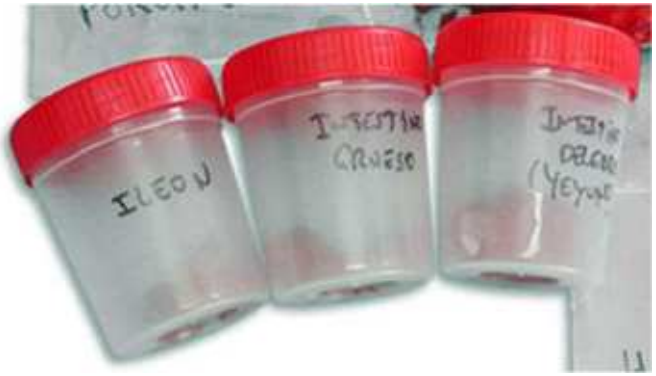
CRUTIAL
Refrigerated / 24h

VS

Room Temperature
Not so urgent*

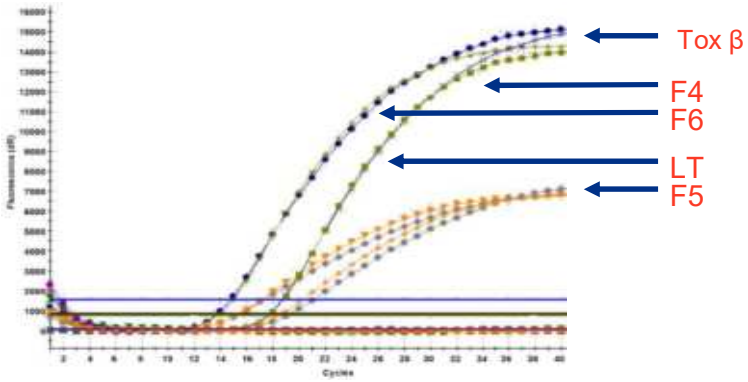
Sampling for histopathology

- 10% formalin.
- Representative fragments of different sections (ileum, jejunum, duodenum, colon, cecum, spiral colon*)
- 2 cm length each section.
- Not whole sections (spiral colon) because they do not fix well.
- Do not open lengthwise.
- Extremely care when manipulating the sample.
- Autolysis is very critical.
- Avoid dead animals. Better euthanized.

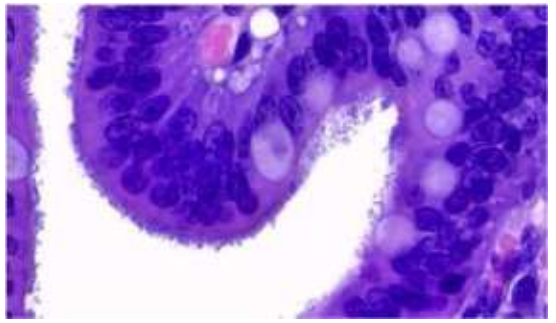


Laboratorial techniques

Bacterial isolation and counting



qPCR and virulenc genes



Histopathology*

Laboratory tests to be requested: depending on the clinical history and gross lesions.

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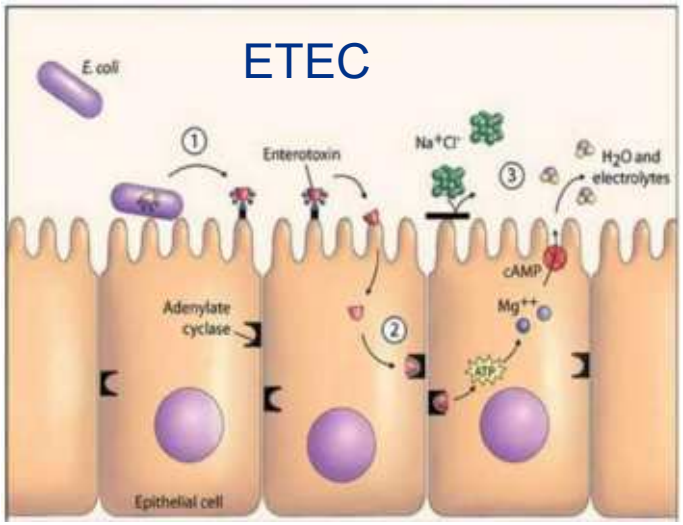
Diagnostic algorithm for each disease

Colibacillosis

Pathogenicity mechanisms

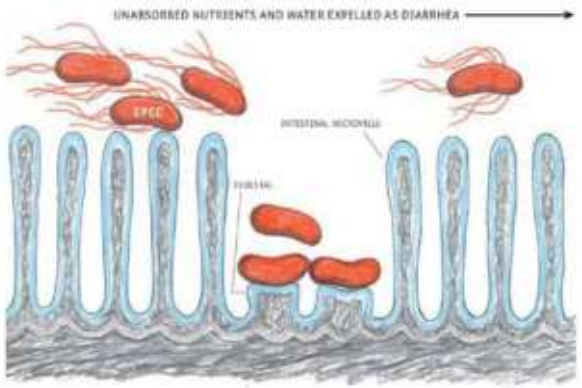
ETEC



F4, F5, F6, F18, F41,
STa, STb, LT, EAST1

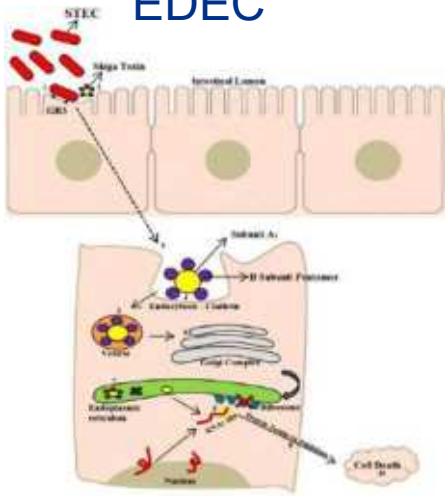
Hypersecretory diarrhea
(Alkaline and watery)

EPEC



Eae, intimina

EDEC



Vtx2e

Rota, corona

Damage and loss of epithelium functionality
Diarrhoea due to malabsorption or poor digestion
Osmotic diarrhea (acid and bulky)

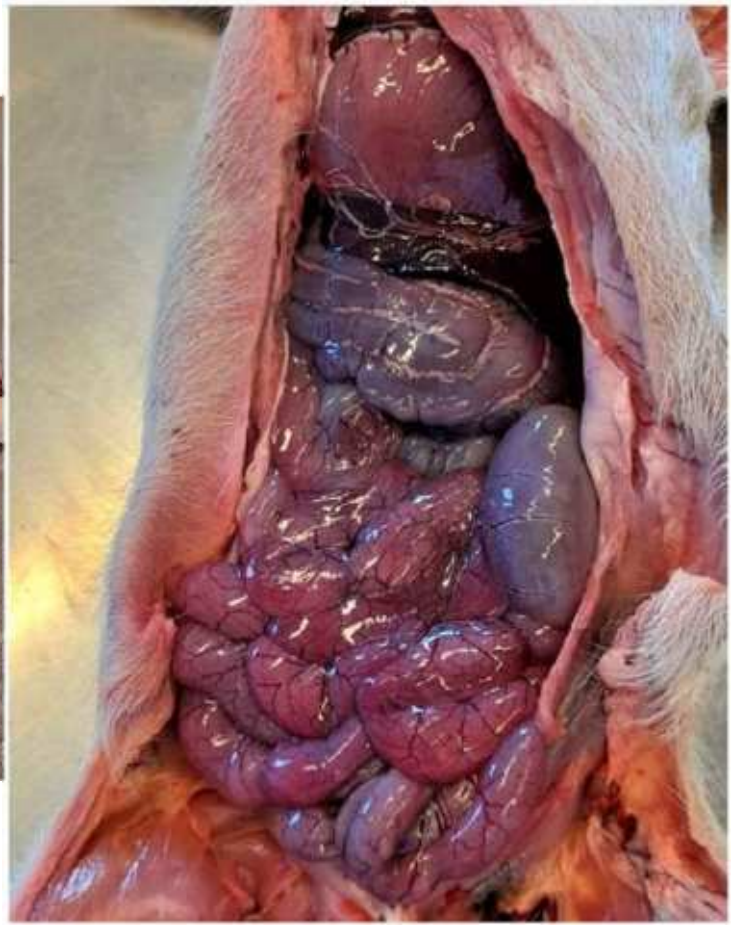
Colibacillosis

Reddened anus and perineum due to contact with alkaline stools (diarrhoea).

Hyperemia in the fundus of the stomach. Stomach dilated and full of milk.

Colibacillosis

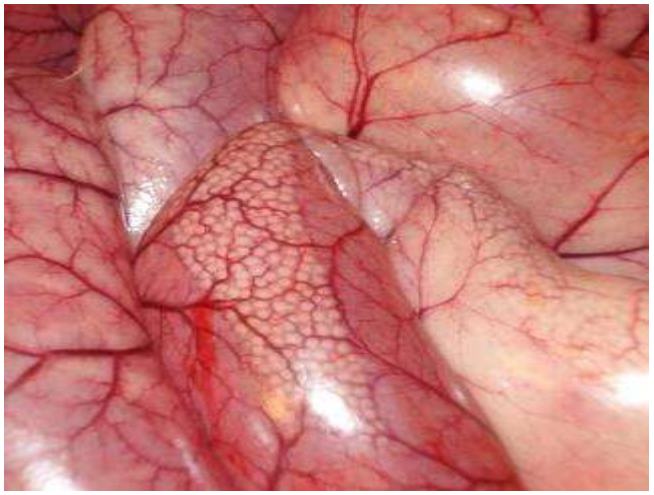
Dilated and hyperemic small intestine



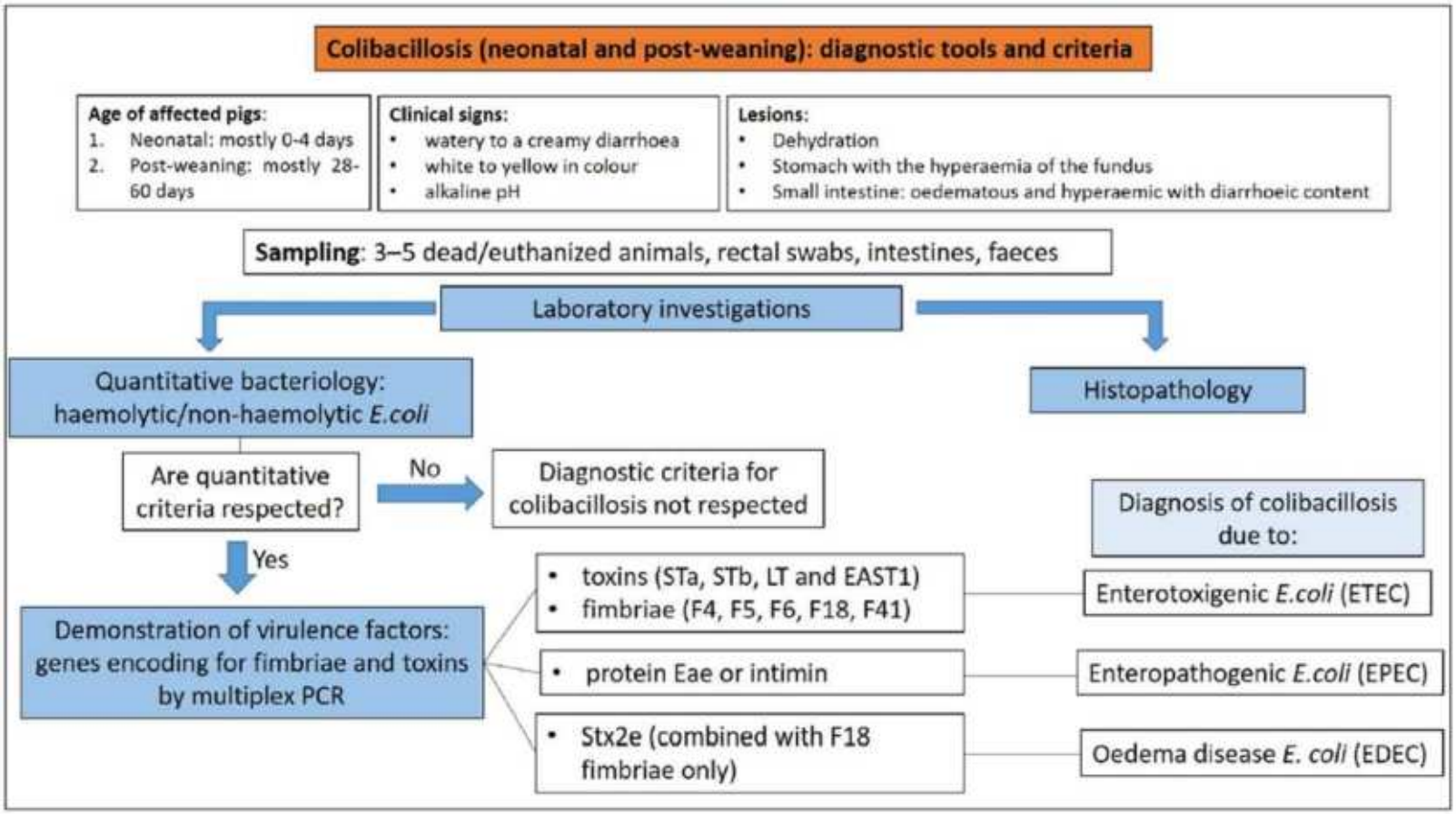
Colibacillosis

Weaning. Beta-hemolytic *E. coli*

Dilated and hyperemic small intestine



Colibacillosis

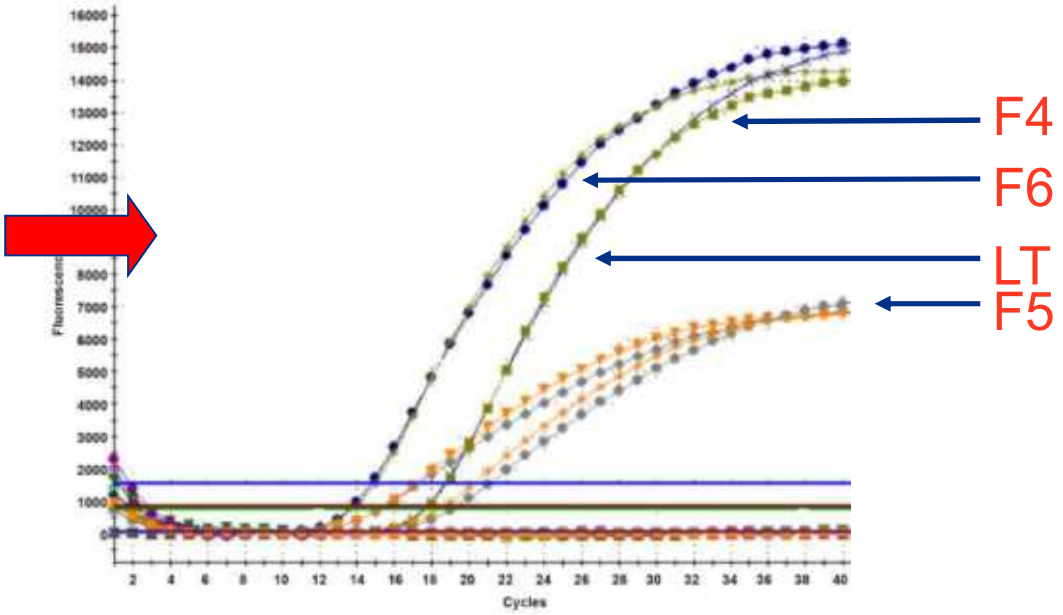


Colibacillosis

Stool/feces

Special swabs for FTA cards

Virulence factors detection by PCR



Clostridium perfringens

Toxinotypes of C. perfringens according to the production of the 4 main toxins (alpha, beta, epsilon, and iota).

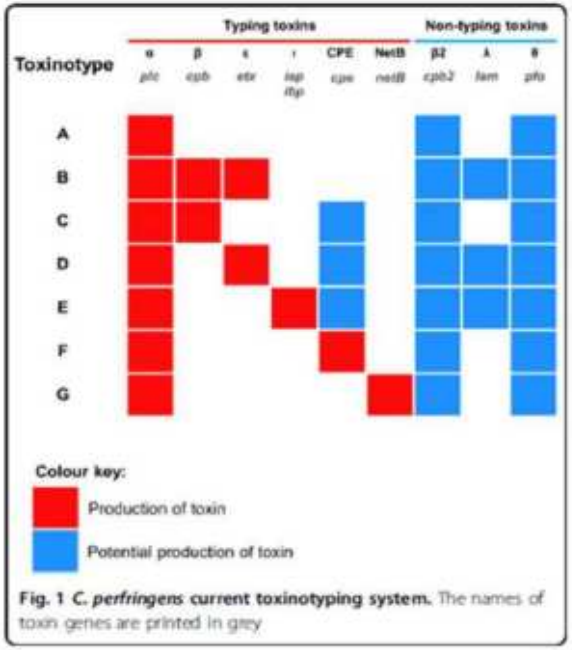


Fig. 1 *C. perfringens* current toxinotyping system. The names of toxin genes are printed in grey

Toxinotype	Major diseases
A	Gas gangrene in humans and animals, yellow lamb disease in sheep, necrotic enterocolitis in **neonatal pigs** (presumptive).
B	Lamb dysentery, hemorrhagic enteritis in cattle and possibly horses.
C	**Necrotic and/or hemorrhagic enteritis** in **neonatal pigs**, horses, cattle, sheep, and goats. Acute enterotoxemia (“struck”) in adult sheep.
D	Enterotoxemia in sheep, goats, and cattle.
E	Role in animal disease not fully determined.
F	Food poisoning in humans; role in animal disease not fully determined.
G	Necrotic enteritis of poultry.

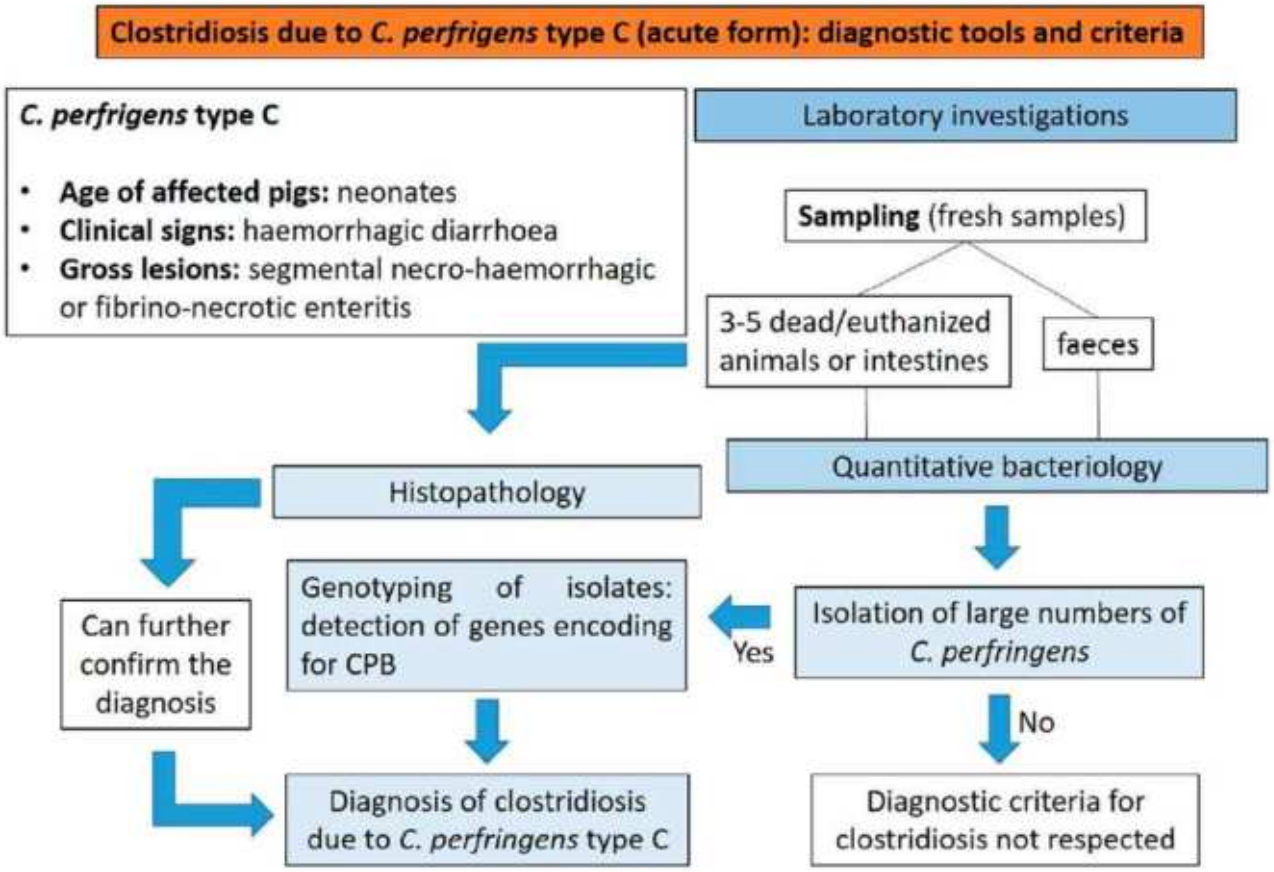
Clostridium perfringens. Type C

Frequent in 3-4 day old piglets, but can affect animals up to 3 weeks old.

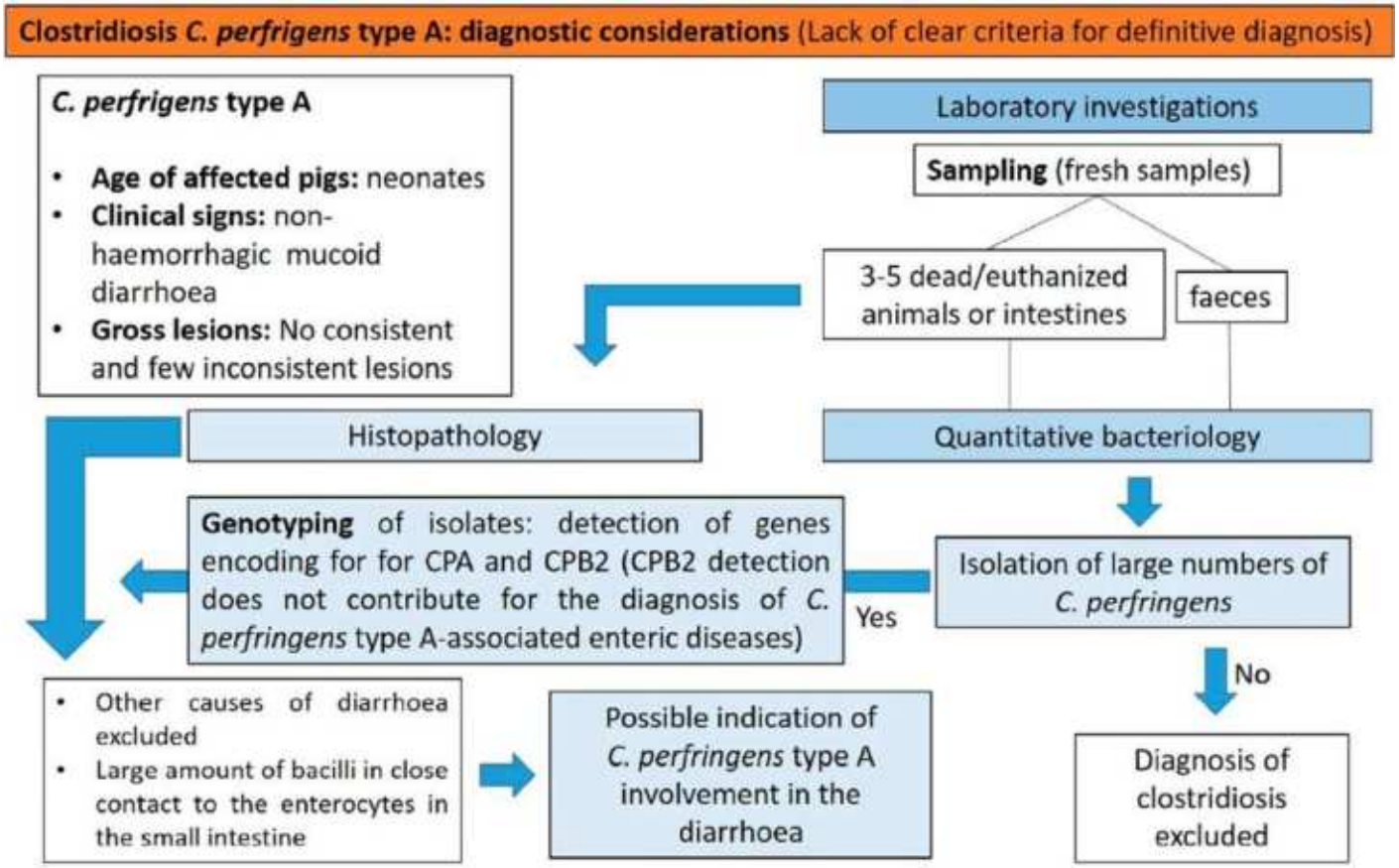
- Intestinal hemorrhage located in jejunum and ileum.

- Bloody intestinal contents
- Necro-hemorrhagic enteritis

Clostridium perfringens. Type C



Clostridium perfringens. Type A



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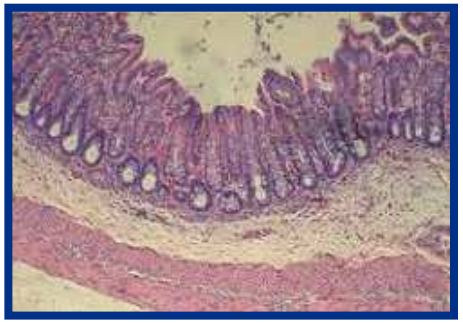
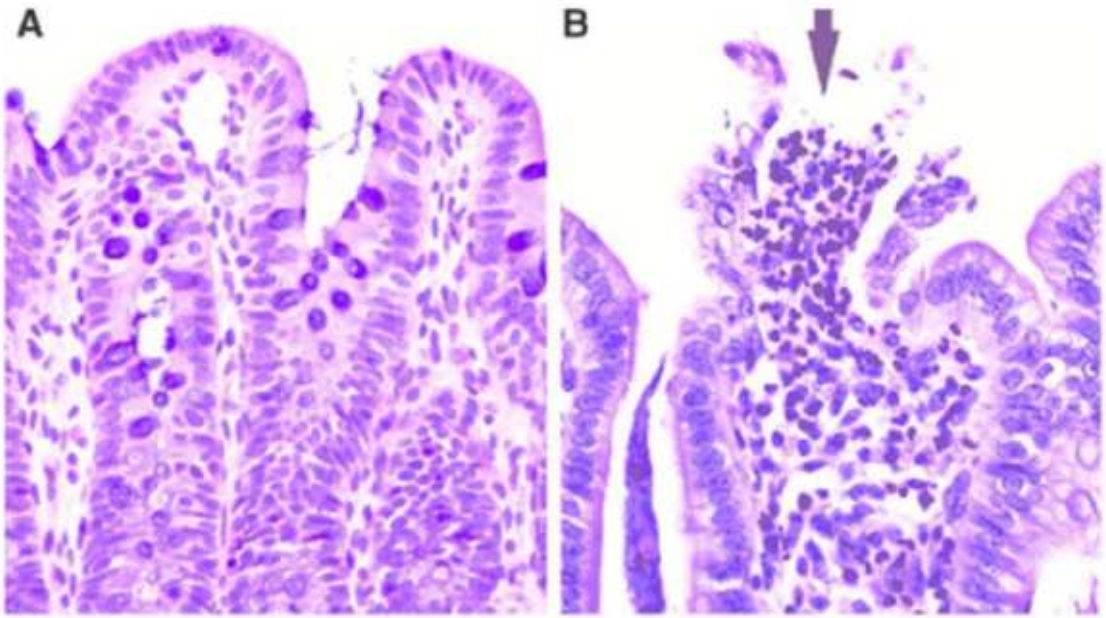
Clostridium difficile





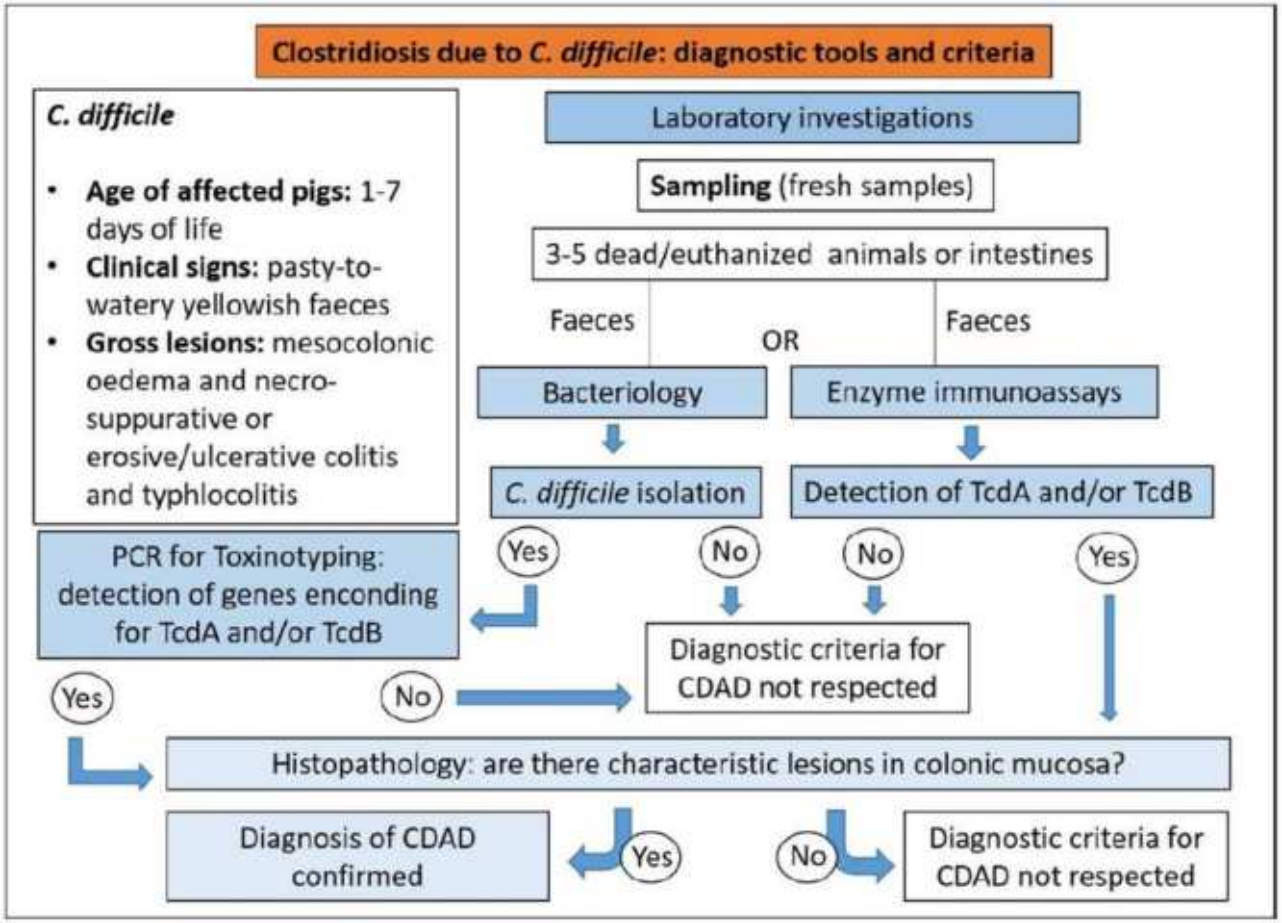




Clostridium difficile



Pig. Colon. a Normal colon lined by columnar epithelial cells. b Superficial, erosive colitis with infiltration of neutrophils into the lamina propria and effusion into the lumen – 'volcano lesion' (arrow) (Haematoxylin and eosin 20×)

Clostridium difficile



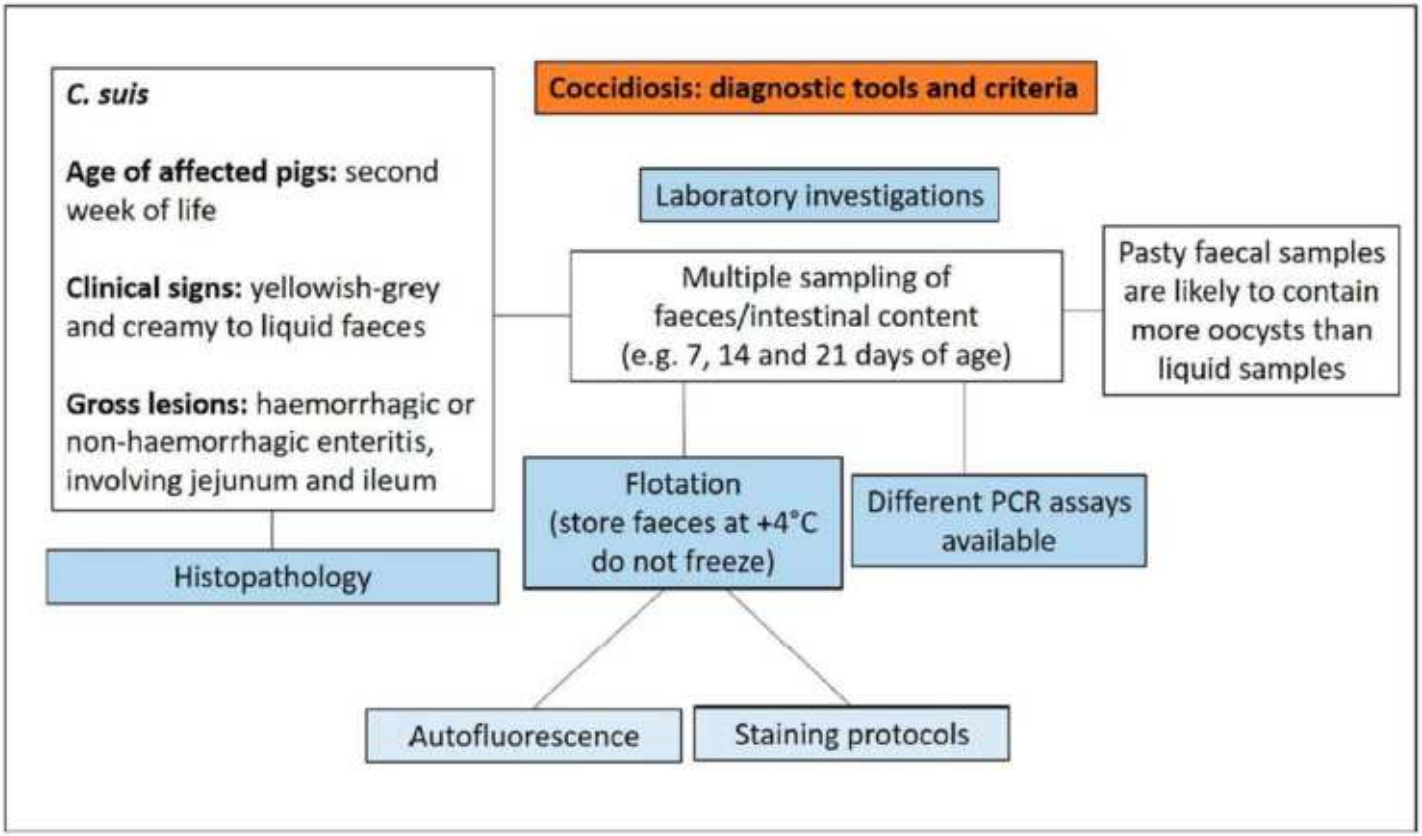
Coccidiosis

C. Suis -infected small intestine

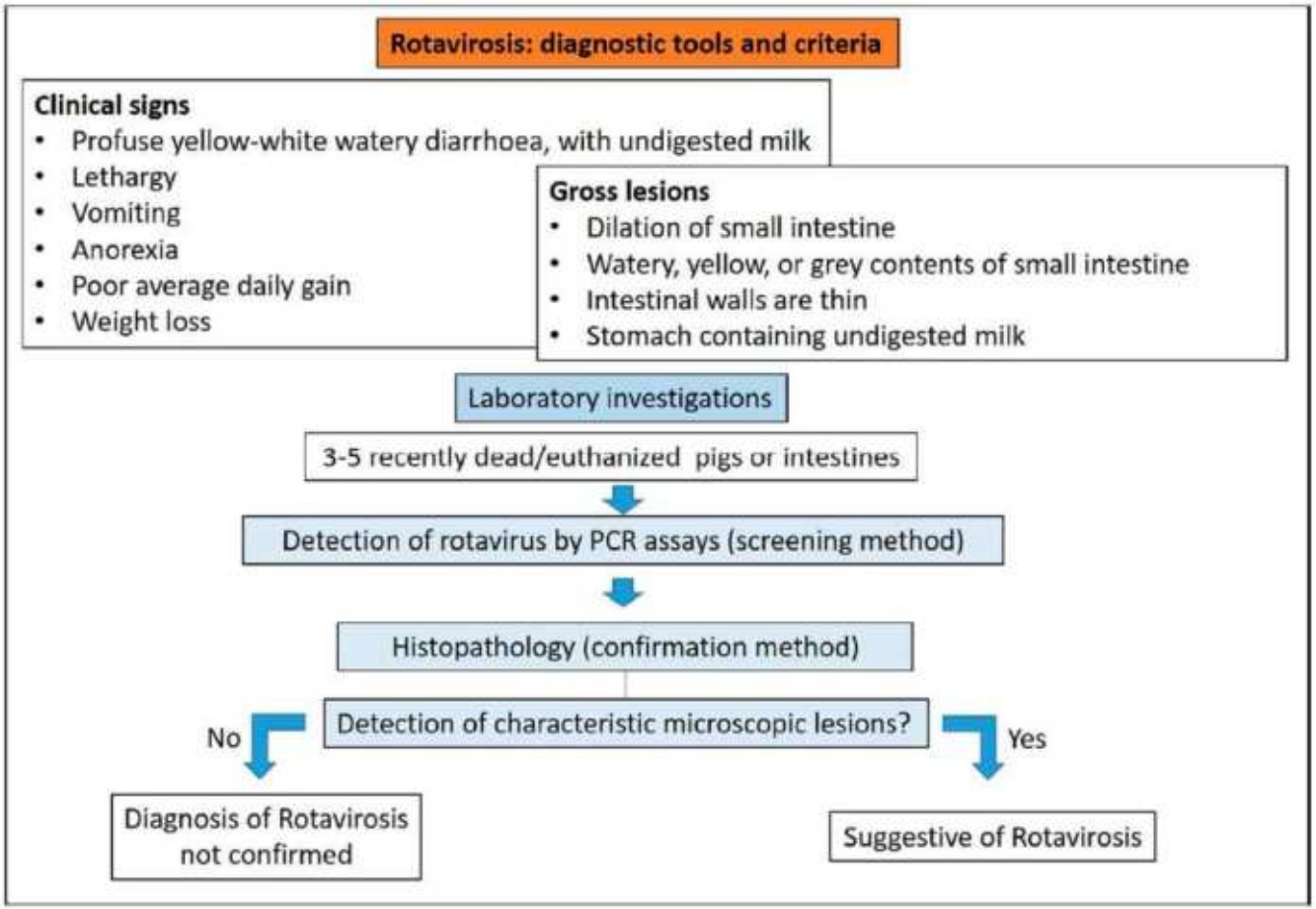
C. Suis oocysts. Flotation (feces). Sporulated oocyst

Oocysts of C. suis by UV fluorescence (feces). Sporulated oocyst

Coccidiosis



Rotavirus

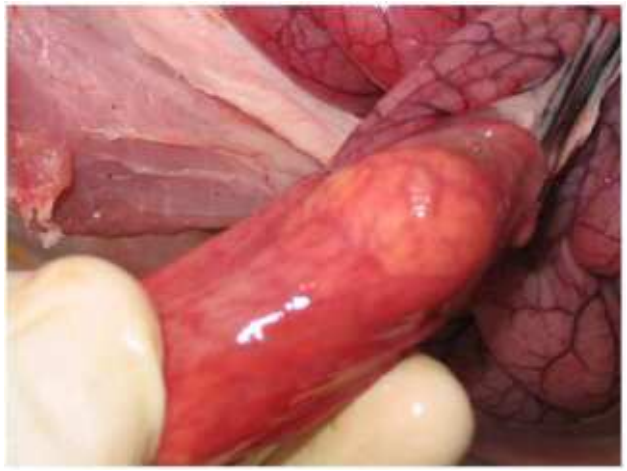

Rotavirosis: diagnostic tools and criteria
Clinical signs
• Profuse yellow-white watery diarrhoea, with undigested milk
• Lethargy
• Vomiting
• Anorexia
• Poor average daily gain
• Weight loss
Gross lesions
• Dilation of small intestine
• Watery, yellow, or grey contents of small intestine
• Intestinal walls are thin
• Stomach containing undigested milk
Laboratory investigations
3-5 recently dead/euthanized pigs or intestines
Detection of rotavirus by PCR assays (screening method)
Histopathology (confirmation method)
Detection of characteristic microscopic lesions?
No
Yes
Diagnosis of Rotavirosis not confirmed
Suggestive of Rotavirosis

Coronavirus

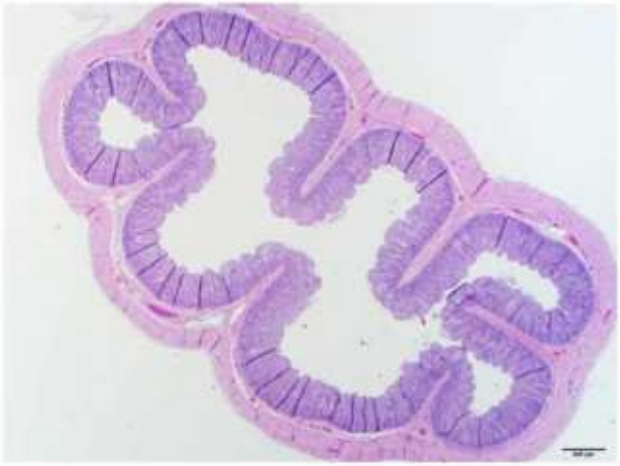
PED: Porcine Epidemic Diarrhea

TGE: Transmissible gastroenteritis (rare in Europe)

PDCoV: Porcine Deltacoronavirus (not described in Europe)



Thinning of intestinal mucosa (pig 45 days-PEDV)



Villous atrophy. Jejunum (Piglet 3 days PEDV infection). Madson, D.M., et al 2014.

DIAGNOSIS:

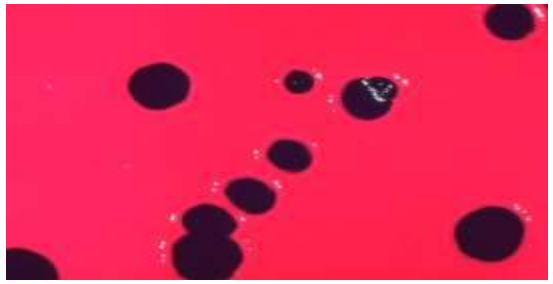
PCR (intestine/intestinal contents/stool).

Macroscopic lesions: mucosal thinning (jejunum/ileum)

Histopathology (small intestine: jejunum and ileum): villous atrophy

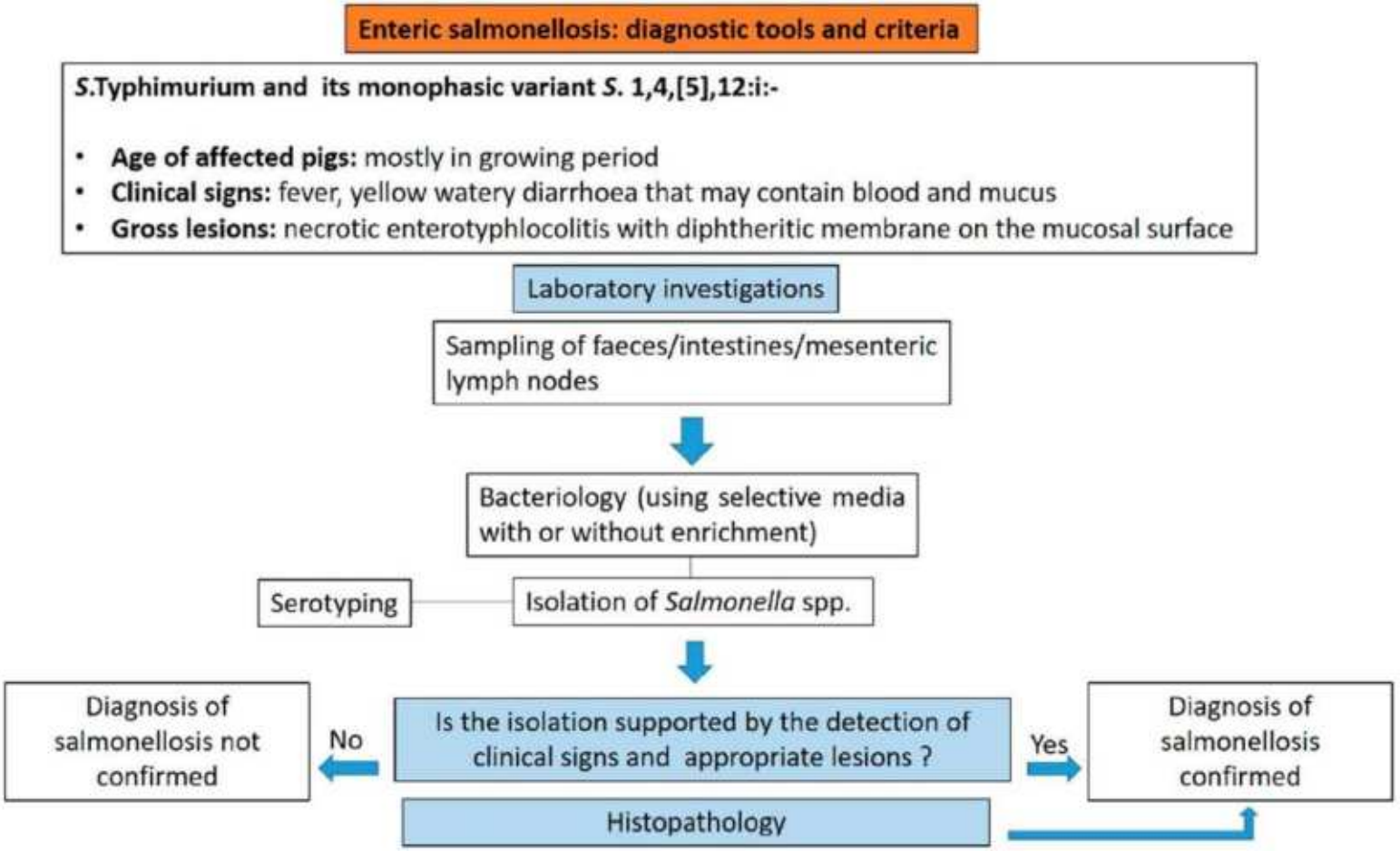
Salmonellosis

Fibrinous enteritis (colon and small intestine)



Isolation and culture on selective media

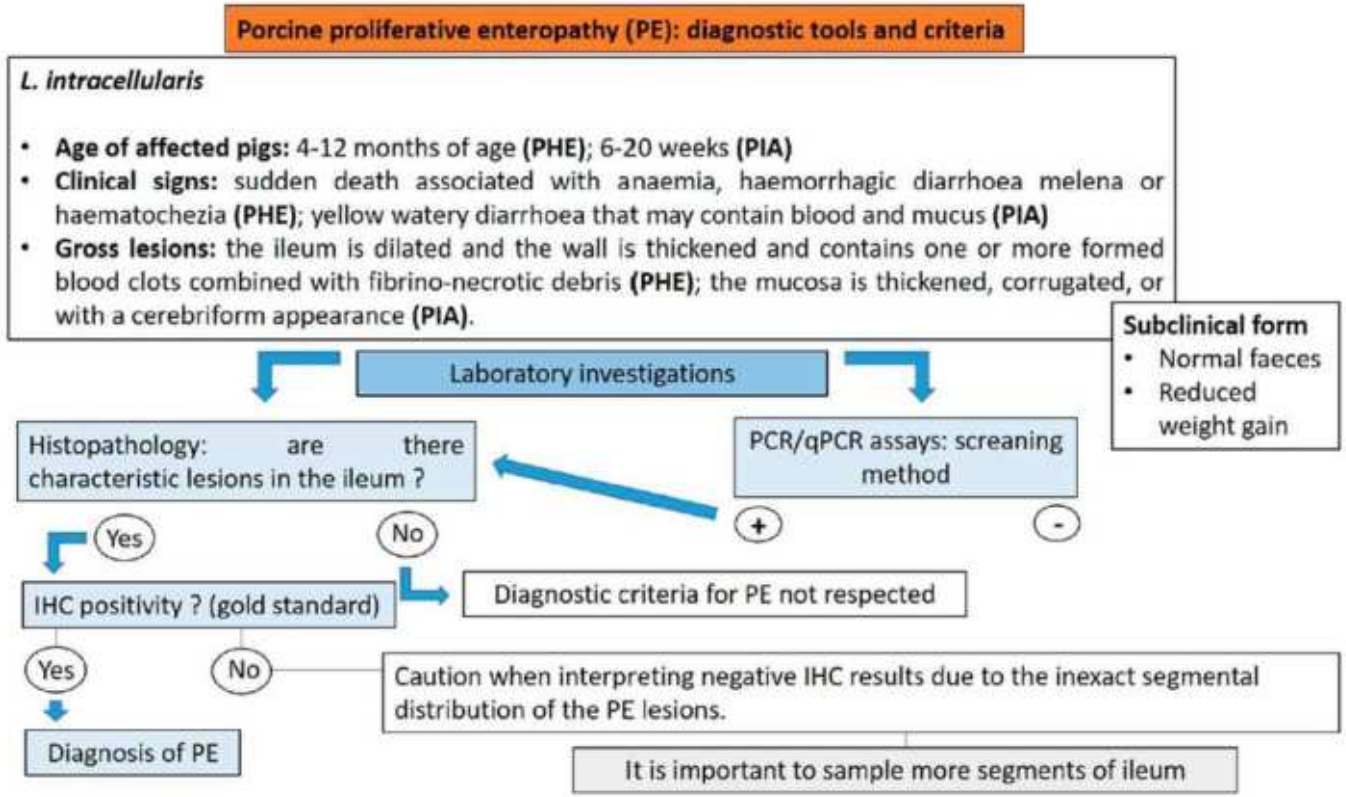
Salmonellosis



Porcine proliferative enteropathy (*L. intracellularis*)

Necrotic enteritis

Porcine proliferative enteropathy (*L. intracellularis*)

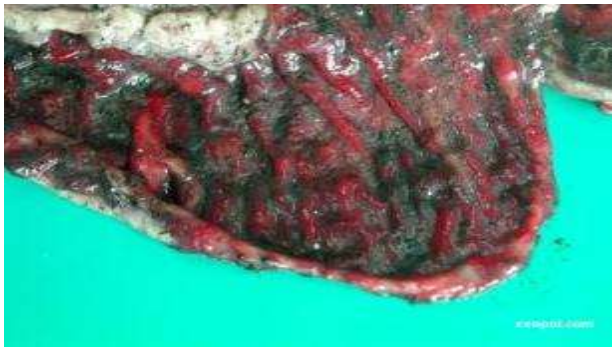


Swine dysentery (*Brachyspira spp*)

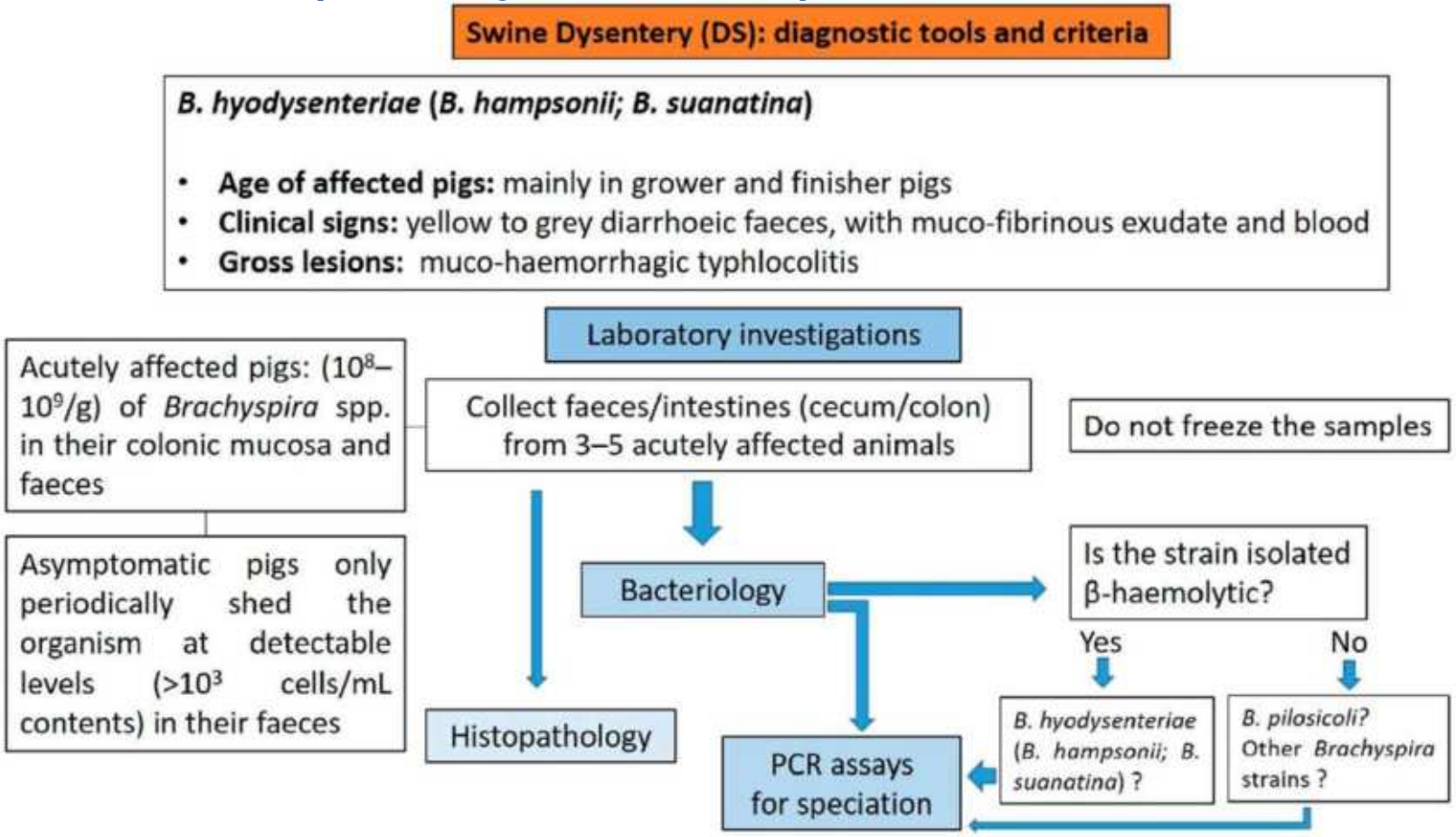
Mucous, bloody stools with muco-fibrinous exudates

Swine dysentery (*Brachyspira spp*)

Edema of the intestinal mucosa (colon). Mucous, fibrinous and hemorrhagic content.



Disentería (*Brachyspira spp*)



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Take home message

Essential for a good diagnosis

- **Farm history**
- **Age of the animals**
- **Clinical signs**
    - Diarrhoea appearence: changes in colour and consistency
    - Vomiting
    - Number of affected litters/piglets. Partiy of the sows, etc.
- **Gross lesions**
    - Type of enteritis (catarrhal, fibrinous, necrotic, etc.)
    - Localisation of the lesions (small and/or large intestine)
    - Distribution (focal, diffused, segmental, etc.)
- **Microscopic lesions (Histopathology)** (= gold standard)
- **Animal selection, sampling and shipment to the labo**
- **Combination different diagnostic techniques**

Definitive diagnosis and decision making (veterinarian)

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Building Immunity
for a Healthier World