Enterotoxaemia and *Clostridium* in calves: an overview

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Enterotoxaemia and *Clostridium* in calves: an overview

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1. Bovine enterotoxaemia yesterday

From « dyssenterie » and « coliques rouges » to enterotoxaemia:

(Manteca and Kaeckenbeeck, Ann. Méd. Vét., 2000, 144, 405-408)

- **young idle, but not lean, calves are affected; relation to nutritional problems (Vitet, 1771)**

- **acute to peracute evolution; presence of blood in the intestinal content (Baron, 1862)**

- **pulpy kidney disease in sheep in Australia caused by *Bacillus ovitoxicus* (= *Clostridium perfringens* toxintype D) (Bennets, 1932)**

- **bovine enterotoxaemia in cattle in Australia caused by *Clostridium perfringens* toxintype A (Rose and Edger, 1932)**

2. *Clostridium* and enterotoxaemia

Untersuchungen

über die

Entwickelungsgeschichte und Fermentwirkung

einiger

Bacterien-Arten.

1880

Inaugural-Dissertation

zur

Erlangung der philosophischen Doctorwürde

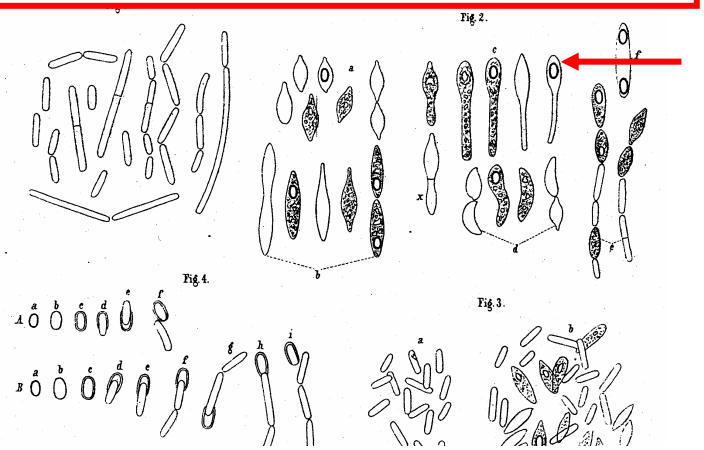
an der

Universität Leipzig

vorgelegt

von

Adam Prażmowski.



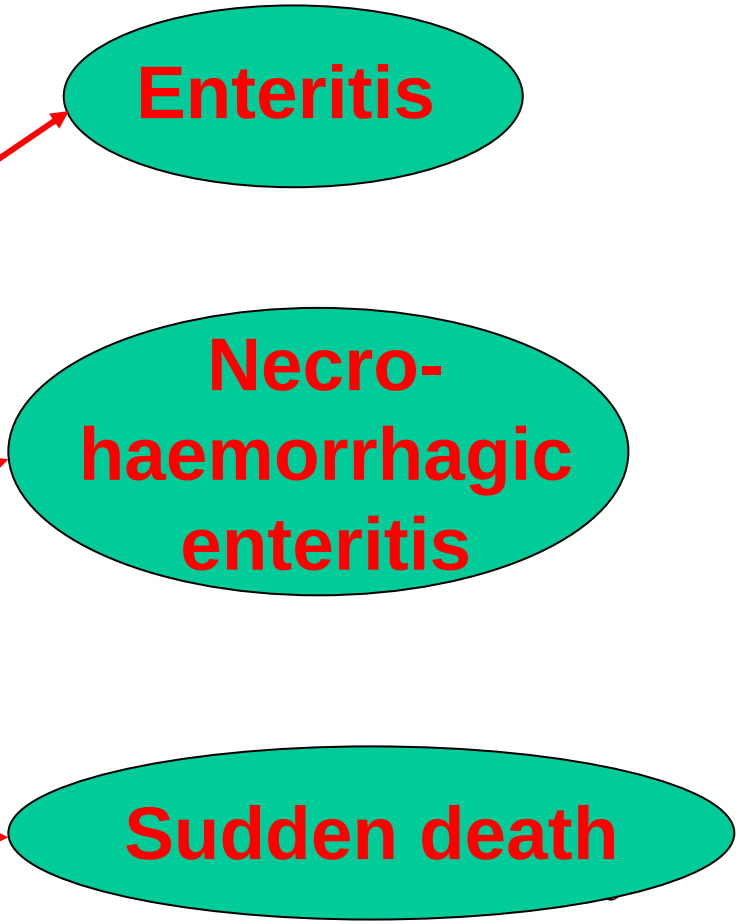
Clostridium butyricum

- **Sporulating, strictly to facultative anaerobic, Gram positive rods**
- **Most pathogenic clostridia produce several toxins acting on the host cells and tissues**

2. *Clostridium* and enterotoxaemia

PATHOGENESIS OF CLOSTRIDIAL ENTERITIS/ENTEROTOXAEMIA

- **Contamination of small intestine by spores <caecum or colon**
- **Anaerobiosis/ low oxidation-reduction potential**
- **Spore germination**
- **Bacteria multiplication**
- **Toxin production**
- **Toxin activity**
- **Toxin absorption and resorption**
- **(Entero)toxaemia**



2. *Clostridium* and enterotoxaemia

C. perfringens	α toxin	β toxin	ε toxin	ι toxin	enterotoxin
Type A	++	-	-	-	(++)
Type B	+	++	+	-	-
Type C	+	++	-	-	-
Type D	+	-	++	-	(+)
Type E	+	-	-	++	-
C. sordellii	Lethal toxin (LT)		Haemorrhagic toxin (HT)		
C. difficile (?)	Cytotoxin (ToxB or TcdB)		Enterotoxin (ToxA or TcdA)		Binary toxin (CDT)
C. chauvoei	α toxin	β toxin	γ toxin	δ toxin (chauveolysin)	
C. septicum	α toxin	β toxin	γ toxin	δ toxin (septicolysin)	
C. tertium	?				

Manteca and Daube. Ann. Méd. Vét., 1994, 138, 155-164

3. Bovine enterotoxaemia today

- **Sudden deaths with haemorrhagic enteritis at necropsy**
  - **suckling calves, especially of the Belgian Blue breed**
  - **up to 10000 deaths/year in Wallonia, up to 5% deaths in one farm**
  - **some vaccinal success: enterotoxaemia and *Clostridium perfringens* ?**
- **<u>Population:</u> 90% suckling beef calves, 2-4 months of age (78 calves)**
- **<u>Clinical signs:</u> very rare (except sudden death)**
- **<u>Lesions:</u> generalised (sometimes localised) necro-haemorrhagic enteritis of the small intestine (rarely of the colon)**
- **<u>Recent (<24 hours) stress-causing circumstances, diets with too much energy and not enough fibers, over-eating, under-drinking, …</u>**

Manteca et al., Ann. Méd. Vét., 2000, 144, 75-82

BREED AND INDIVIDUAL GENETIC BACKGROUND

3. Bovine enterotoxaemia today

- **Bacteriology: growth and isolation of**
  - ***C. perfringens* (80% of the cases vs 19% of the controls) and *C. sordellii* (20% of the cases)**
  - **higher numbers of *C. perfringens* in cases than controls: mean values of 10^{7-8} CFU versus 10^{4-5} CFU**
- **Molecular typing: with gene probes/PCR**
  - ***C. perfringens*: non-enterotoxigenic type A (α toxin)**
  - ***C. sordellii*: non-toxigenic**
- **Toxinology: ELISA or immunochromatography for the α toxin**

Manteca et al., Vet. Microbiol., 2001, 81, 21-32

3. Bovine enterotoxaemia today

- **Also present in veal calves especially of beef, less of dairy, breeds**
- **Raise of number of cases after bans on Zn-bacitracine and of individual pens (?)**
- **<u>Main clinical sign:</u> sudden death !**
- **<u>Main lesion:</u> (necro-)haemorrhagic enteritis**
- **<u>Circumstances:</u>**
  - **existence of a «stress» within 24h prior to death**
  - **uneven distribution of the milk**
  - **problem with the milk replacer preparation**
- **Bacteriology, toxinology: ?**

3. Bovine enterotoxaemia today

- **Diagnostic criteria**
  - **The most performant young beef calves**
  - **Existence of a «stress» within 24 hours prior to sudden death**
  - **Generalized (sometimes localised) necro-haemorrhagic enteritis of the small intestine (rarely of the colon)**
  - **>10^7 CFU of *Clostridium perfringens* per ml of intestinal content: within 12 hours of death after transportation at 4°C**
  - **α toxin in intestinal content and peritoneal or pericardic effusion (immunochromatography and ELISA): also within 12 hours of death**

3. Bovine enterotoxaemia today

- **Prophylaxis**
  - **General management of suckling and veal calf farming/industry**
  - **Avoid «stress»: handling, oestrus of the dam, heat, change of pasture, …**
  - **Beware rapid change of diet, high energy diet, frozen food, over-eating, under-drinking, milk uneven distribution, milk replacer preparation, …**
  - **Add fibers to the diet (spelt) to stimulate intestinal motility, probiotics to keep balanced the intestinal commensal flora (ban on antibioprophylaxis)**
  - **Protect against clostridial toxins by vaccination or with colostrum/ hyperimmune serum (Manteca et al., Ann. Méd. Vét., 2004, 148, 147-152)**

4. And the perfringens β2 toxin ?

- **Description of the β2 toxin (Gibert et al., Gene, 1997, 203, 65-73)**
  - **associated neonatal haemorrhagic enteritis in piglets**
  - **coded by a plasmid-located gene (*cpb2*)**
- **Two *cpb2* gene variants (Jost et al., Infect. Immun., 2005, 73, 652-656)**
  - **the typical or consensus variant: *cpb2*^{con} gene**
  - **the atypical variant: *cpb2*^{aty} gene**
- **β2^{con} toxin is ten times more cytotoxic than β2^{aty} toxin**
- **In piglets**
  - ***cpb2* gene in isolates from diarrheic, but not from healthy, piglets**
  - **>90% of the positive isolates harbour the *cpb2*^{con} gene**
  - **the level of expression of the *cpb2*^{con} gene is >90%**
- **In horses: cases of typhlocolitis with production *in vivo***
  - **detection of the *cpb2*^{con} and *cpb2*^{aty} genes**
  - **the level of expression of either *cpb2* gene is ~50% !**
- **And in calves with enterotoxaemia ?**

4. And the perfringens β2 toxin ?

- By colony hybridization for the β2 toxin-encoding gene (*cpb2*)
  - *C. perfringens* from 80% of the case and 20% of the control calves
  - 1/3 of the isolates from case and from control calves were *cpb2*-positive
  - 2/3 of the *C. perfringens*-positive case and control calves harboured *cpb2*-positive isolate(s)
  - the proportion of *cpb2*-positive isolates was higher in case calves

- Necro-haemorrhagic lesions in the intestinal ligated loop assay with one *cpb2*-positive, but not with 3 *cpb2*-negative, *C. perfringens*

- Typing of 28 *cpb2*-positive isolates from 8 case calves
  - 28/28 *cpb2*-positive isolates were *cpb2*^{con}-positive (100%)
  - 18/28 *cpb2*^{con}-positive isolates expressed the β2^{con} toxin (64%)

- Typing of 40 *cpb2*-positive isolates from 14 control calves
  - 27/40 *cpb2*-positive isolates were *cpb2*^{con}-positive (67%)
  - 6/27 *cpb2*^{con}-positive isolates expressed the β2^{con} toxin (22%)
  - 13/40 *cpb2*-positive isolates were *cpb2*^{aty}-positive (33%)
  - 9/13 *cpb2*^{aty}-positive isolates expressed a β2^{aty} toxin (69%)

Manteca et al., Vet. Microbiol., 2002, 86, 191-202
Lebrun et al., Vet. Microbiol., 2006, in press

6. Bovine enterotoxaemia tomorrow

- **Future research in suckling calves:**
  - ***in vivo* expression of the «bovine» *cpb2*^{con} gene**
  - ***in vivo* model of the disease**
  - **testing of mutants**
  - **evaluation of the protection by antibodies**

- **Future research in the veal industry:**
  - **General: survey of sudden death causes**
  - **Ecology: inventory of «stress»-causing circumstances**
  - **Necropsy: description of intestinal macroscopic lesions**
  - **Histology: description of intestinal microscopic lesions**
  - **Bacteriology: on the small and large intestinal flora**
  - **Toxinology: identification in the intestinal contents**