Enterotoxaemia and *Clostridium* in calves: an overview

Jacques Mainil, DVM, MS, PhD
Annick Linden, DVM, PhD
Maude Lebrun, DVM, MS

University of Liège, Veterinary Faculty
Department of Infectious and Parasitic Diseases, Bacteriology
Sart Tilman, Bât B43a, Liège, B4000, BELGIUM

http://www.genusclostridium.net
Enterotoxaemia and *Clostridium* in calves: an overview

1. Bovine enterotoxaemia yesterday
2. *Clostridium* and enterotoxaemia
3. Bovine enterotoxaemia today
4. And the perfringens $\beta_2$ toxin ?
5. Bovine enterotoxaemia tomorrow
From « dyssenterie » and « coliques rouges » to enterotoxaemia:

- 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

- 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

Enteritis

Necro-haemorrhagic enteritis

Sudden death
## 2. *Clostridium* and enterotoxaemia

<table>
<thead>
<tr>
<th><em>Clostridium</em></th>
<th>α toxin</th>
<th>β toxin</th>
<th>ε toxin</th>
<th>τ toxin</th>
<th>enterotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type A</strong></td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td><strong>Type B</strong></td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Type C</strong></td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Type D</strong></td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>(+)</td>
</tr>
<tr>
<td><strong>Type E</strong></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>Clostridium sordellii</em></td>
<td>Lethal toxin (LT)</td>
<td>Haemorrhagic toxin (HT)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium difficile</em> (?)</td>
<td>Cytotoxin (ToxB or TcdB)</td>
<td>Enterotoxin (ToxA or TcdA)</td>
<td>Binary toxin (CDT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium chauvoei</em></td>
<td>α toxin</td>
<td>β toxin</td>
<td>γ toxin</td>
<td>δ toxin (chauveolysin)</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium septicum</em></td>
<td>α toxin</td>
<td>β toxin</td>
<td>γ toxin</td>
<td>δ toxin (septicolysin)</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium tertium</em></td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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?

- Population: 90% suckling beef calves, 2-4 months of age (78 calves)

- Clinical signs: very rare (except sudden death)

- Lesions: generalised (sometimes localised) necro-haemorrhagic enteritis of the small intestine (rarely of the colon)

- Recent (<24 hours) stress-causing circumstances, diets with too much energy and not enough fibers, over-eating, under-drinking, ...


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 ($\alpha$ toxin)
  - *C. sordellii*: non-toxigenic

- **Toxinology:** ELISA or immunochromatography for the $\alpha$ toxin

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 (?)
- **Main clinical sign:** sudden death !
- **Main lesion:** (necro-)haemorrhagic enteritis
- **Circumstances:**
  - 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 antioprophylaxis)
  - 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 $\beta 2$ toxin?

- **Description of the $\beta 2$ 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

- $\beta 2^{con}$ toxin is ten times more cytotoxic than $\beta 2^{aty}$ 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\text{\textsuperscript{con}}-positive (100%)
  - 18/28 cpb2\text{\textsuperscript{con}}-positive isolates expressed the β2\text{\textsuperscript{con}} toxin (64%)

- Typing of 40 cpb2-positive isolates from 14 control calves
  - 27/40 cpb2-positive isolates were cpb2\text{\textsuperscript{con}}-positive (67%)
  - 6/27 cpb2\text{\textsuperscript{con}}-positive isolates expressed the β2\text{\textsuperscript{con}} toxin (22%)
  - 13/40 cpb2-positive isolates were cpb2\text{\textsuperscript{aty}}-positive (33%)
  - 9/13 cpb2\text{\textsuperscript{aty}}-positive isolates expressed a β2\text{\textsuperscript{aty}} toxin (69%)
6. Bovine enterotoxaemia tomorrow

- Future research in suckling calves:
  - *in vivo* expression of the «bovine» cpb2con 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