Opportunities and problems in vaccination against opportunistic pathogens: the example of *Clostridium perfringens* in calves and chickens

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### ANIMAL PRODUCTION

**AIM**

Maximal productive performance of the animal

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### Past

- Antimicrobial growth promoters (AGP) in feed
- Mostly against Gram positive bacteria
- Increased performance

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### Today

- AGP prohibited
- Curative antibiotics!

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### Future

- Probiotics
- Prebiotics
- Bacteriocins
- …
- **Vaccination**
CLOSTRIDIUM PERFRINGENS
AN IMPORTANT OPPORTUNISTIC PATHOGEN

- Predisposing gut damage
- Wide array of toxins → Disease
- Member of normal intestinal microbiota

- Anaerobic
- Gram-positive
- Spore-forming
- Auxotrophic for 13 AA

- Widely distributed in the environment
## Clostridium Perfringens

### Toxinotypes

<table>
<thead>
<tr>
<th>Type</th>
<th>Alpha</th>
<th>Beta</th>
<th>Epsilon</th>
<th>Iota</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>++</td>
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<td>-</td>
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<td>B</td>
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- **Universal**
- Enterotoxin
- NetB toxin
- Perfringolysin O
- Enzymes (collagenases, proteases, hyaluronidases, ...)
- Novel toxins to be identified and explored
<table>
<thead>
<tr>
<th>Necrotic enteritis in broilers</th>
<th>Necro-haemorrhagic enteritis in calves</th>
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</thead>
<tbody>
<tr>
<td>• Clinical and subclinical</td>
<td>• Necro-haemorrhagic lesions in the gut</td>
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<tr>
<td>• Necrosis in the gut</td>
<td>• End of fattening period</td>
</tr>
<tr>
<td>• Performance problems</td>
<td>• Economically important</td>
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<tr>
<td>• Economically important</td>
<td>• Economically important</td>
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</tbody>
</table>

![Necrotic enteritis in broilers](image1.png)

![Necro-haemorrhagic enteritis in calves](image2.png)
CLOSTRIDIUM PERFRINGENS
VACCINATION STRATEGY

Vaccine

Oral immunization

Administration route

Secreted toxins (and/or proteins)

Safe

toxoid

Parenteral immunization

Single dose

Single dose

Single dose
NECROTIC ENTERITIS
PROBLEMS IN VACCINATION

Vaccination supernatants, sc d3, d12

Loss of protection when producing toxoid

Mot et al. 2013
NECROTIC ENTERITIS
PROBLEMS IN VACCINATION

Mot et al. 2013

Loss of protection at single dose at day-old
NECROTIC ENTERITIS
PROBLEMS IN VACCINATION

Oral immunization
- Live vectors
  - *Bacillus* spores
  - Eimeria
  - ...

Administration route
- Parenteral immunization
  - Single dose
  - Safe
  - Formaldehyde
  - Genetic toxoid

Vaccine
- Secreted toxins (and/or proteins)
- Genetic toxoid
- Safe
Variable protection against experimental broiler necrotic enteritis after immunization with the C-terminal fragment of *Clostridium perfringens* alpha-toxin and a non-toxic NetB variant

Sérgio P. Fernandes da Costa, Dorien Mot, Sofie Geeraerts, Monika Bokori-Brown, Filip Van Immerseel and Richard W. Titball

NetB is essential to cause disease
vaccination against NetB not sufficient to provide full protection
SAME PROBLEMS
IN VACCINATION AGAINST
NECRO-HAEMORRHAGIC ENTERITIS IN CALVES
NECRO-HAEMORRHAGIC ENTERITIS IN CALVES

PROBLEMS IN VACCINATION

Loss of protection when producing toxoid

Goossens et al. 2016
Alpha toxin is essential to cause intestinal necrosis, but antibodies against alpha toxin are not sufficient to protect against intestinal necrosis.

Goossens et al. 2016
THE PROBLEM?

Vaccination against essential toxins not sufficient to provide full protection

- Other immunogenic proteins?
- Parenteral vaccination impossible in chickens
- Mucosal or systemic immunity?
- Difficult to eradicate bacteria in commensal state
  - reduce colonization?
OPPORTUNITIES

Other immunogenic proteins?

- multiple antigens identified in serum form chickens immune to necrotic enteritis
  → no full protection after vaccination
  - Kulkarni et al. 2006, 2007

- Other proteins present in protective antisera from cattle
  → further investigation needed

Exudation of serum IgG across the inflamed intestinal epithelium possible (Kulkarni et al. 2007)

What if we would could **reduce intestinal colonization**?
Mucosal antibodies needed?
Which antigens are detected by intestinal antibodies?
VACCINATION AGAINST OPPORTUNISTIC PATHOGENS

- Commensal → colonize normal mucosal surfaces
- Problem arises when the mucosal surface is damaged
- Subclinical infections → reduced animal performance

What if we could reduce intestinal colonization?
CnaA: a novel vaccine antigen?

- CnaA = putative collagen adhesion
- mutant strains has **reduced ability to colonise the chicken intestine**
- CnaA is **involved in virulence**

Wade et al. 2016
EFFECTIVE VACCINATION AGAINST OPPORTUNISTIC PATHOGENS

• Future research should focus on intestinal colonization and live vectors inducing both mucosal and systemic immunity
  → combined approach: reduce colonization and neutralize toxins

• Improved animal performance

• Reduced antibiotic use
  – prophylactic
  – therapeutic
THANK YOU FOR YOUR ATTENTION