Toxin-neutralizing antibodies protect against *Clostridium perfringens* challenge in an intestinal loop model for bovine enterotoxaemia

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### Introduction

**Bovine enterotoxaemia**

- Caused by *Clostridium perfringens*
  - Alpha toxin and perfringolysin O involved in the pathogenesis
  - Sudden death → treatment → preventive measures needed, such as vaccination
  - Lesions of hemorrhagic enteritis in the small intestine
  - Veal calves → more susceptible to enterotoxaemia than beef calves

**Ducatelle et al. (2013)**

### Objectives

- Can antibodies against *C. perfringens* toxins protect against the development of necrotic lesions in the intestine?
- Can we remove the undesired toxin activity, but conserve the immune-protective potential, from the toxin preparations?

### Vaccination with *C. perfringens* toxins resulted in strong antibody responses

Two calves were immunized for each antigen
- Native *C. perfringens* toxins
- L-lysine protected, formaldehyde inactivated *C. perfringens* toxins
- Commercial formaldehyde inactivated clostridial vaccine

In all calves a strong antibody response against both alpha toxin and perfringolysin O was detected.

**Table 1:** antibody response towards alpha toxin and perfringolysin O measured by ELISA

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Alpha toxin titer</th>
<th>Perfringolysin O titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native toxins</td>
<td>64.44 ± 0.2227</td>
<td>25600 ± 0</td>
</tr>
<tr>
<td>L-lysine/formaldehyde toxoid</td>
<td>24.26 ± 2.960</td>
<td>16000 ± 9600</td>
</tr>
<tr>
<td>Commercial formaldehyde</td>
<td>45.14 ± 20.42</td>
<td>4800 ± 1600</td>
</tr>
<tr>
<td>inactivated vaccine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### In vitro neutralization of alpha toxin and perfringolysin O

**Alpha toxin activity** → lecithinase effect on egg yolk lipoproteins.

Perfringolysin activity → hemolysis of horse erythrocytes.

**Toxin neutralization by pre-incubation of toxins with a dilution series of the antibodies**

- Antibodies from calves vaccinated with native *C. perfringens* toxins were able to neutralize both toxin activities
- Antibodies from calves vaccinated with L-lysine protected, formaldehyde inactivated toxins were able to inhibit the perfringolysin O activity, but had less effect on the alpha toxin activity
- Antibodies from calves vaccinated with a commercial formaldehyde inactivated vaccine were less capable to inhibit both toxin activities

**Table 2:** Cytotoxicity of *C. perfringens*-induced lesions measured by an ELISA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine/formaldehyde toxoid</td>
<td>30</td>
</tr>
<tr>
<td>Commercial formaldehyde</td>
<td>20</td>
</tr>
<tr>
<td>native B, S, P</td>
<td>5</td>
</tr>
<tr>
<td>inactivated vaccine</td>
<td>0</td>
</tr>
<tr>
<td>native B, S, P</td>
<td>0</td>
</tr>
<tr>
<td>L-lysine/formaldehyde toxoid</td>
<td>0</td>
</tr>
<tr>
<td>Commercial formaldehyde</td>
<td>0</td>
</tr>
<tr>
<td>inactivated vaccine</td>
<td>0</td>
</tr>
</tbody>
</table>

### Conclusions

- **Toxin-neutralizing antibodies protected against *C. perfringens* challenge**
- Prevention of endothelial damage may be the mechanism underlying this protective effect
- Immunization of both native and formaldehyde inactivated *C. perfringens* toxins resulted in a strong immune response against alpha toxin and perfringolysin O
- Only antibodies raised against native toxins were protective

At least for alpha toxin and perfringolysin O mediated diseases, antibody titers detected by ELISA are not a guarantee for protection even if protection against the disease is antibody mediated.

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**Figure 1:** experimental set up

The antisera were tested in 4 calves, with a total of 20 loops for each vaccine (5 loops per animal)

![Figure 1](image1)

**Figure 2:** neutralization of the lesion-inducing potential of *C. perfringens*.

*** p < 0.001 (Kruskall-Wallis analysis, followed by a Dunn’s multiple comparison test)

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**Figure 3:** in vitro neutralization of biological activities of alpha toxin and perfringolysin O

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**Figure 4:** neutralization of *C. perfringens* cytotoxicity

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**Verherstraeten S, Goossens E, Valgaeren B, et al.** The synergistic necrohemorrhagic action of *Clostridium perfringens* perfringolysin and alpha toxin in the bovine intestine and against bovine endothelial cells. Veterinary research. 2013;44:45