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 (Verherstraeten et al. 2013)
- Sudden death → ~~treatment~~ → preventive measures needed, such as **vaccination**
- Lesions of **hemorrhagic enteritis** in the small intestine
- Veal calves
  - produce **less anitbodies** against *C. perfringens* toxins than beef calves
  - **more vulnerable** to enterotoxaemia than beef calves (Valgaeren et al. 2015)

Objectives

- Can antibodies against *C. perfringens* toxins protect agianst the develoment 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 perfingolysin O measured by ELISA

Vaccine	Alpha toxin titer	Perfringolysin O titer
Native toxins	64.44 ± 0.2227	25600 ± 0
L-lysine/formaldehyde toxoid	24.26 ± 2.960	16000 ± 9600
Commercial formaldehyde inactivated vaccine	45.14 ± 20.42	4800 ± 1600

Toxin-neutralizing antibodies protect against *C. perfringens*-induced necrotic lesions

The potential of the antisera to inhibit *C. perfringens*-induced necrosis, was evaluated by neutralizing the development of necrotic lesions using the antisera in an intestinal loop assay (Figure 1).

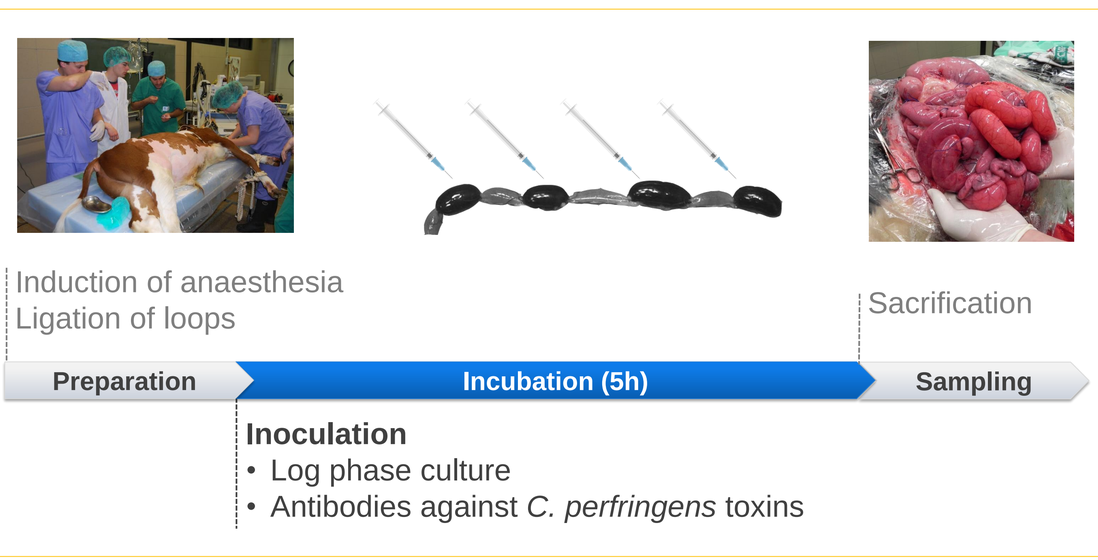
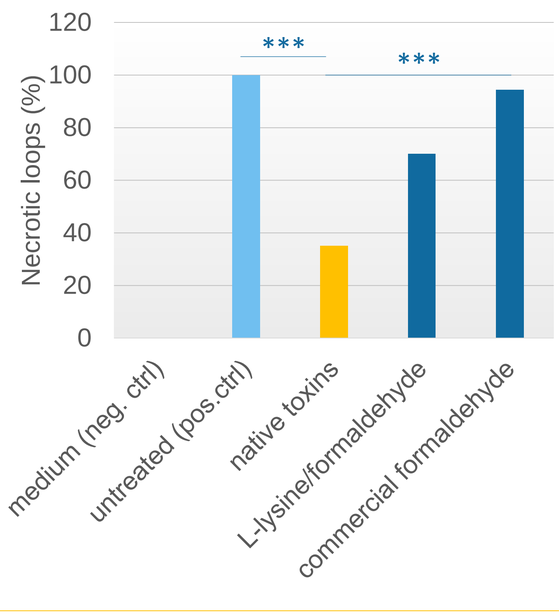


Figure 1: experimental set up
The antisera were tested in 4 calves, with a total of 20 loops for each vaccine (5 loops per animal)



All control loops inoculated with *C. perfringens* alone (untreated) developed necrosis.

Injection of *C. perfringens* together with antisera from calves vaccinated with native toxins resulted in significantly fewer necrotic loops.

Antisera from calves vaccinated with formaldehyde inactivated toxins (either L-lysine protected or the commercial inactivated vaccine) were unable to neutralize the lesion induction.

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)

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. perfingens* 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

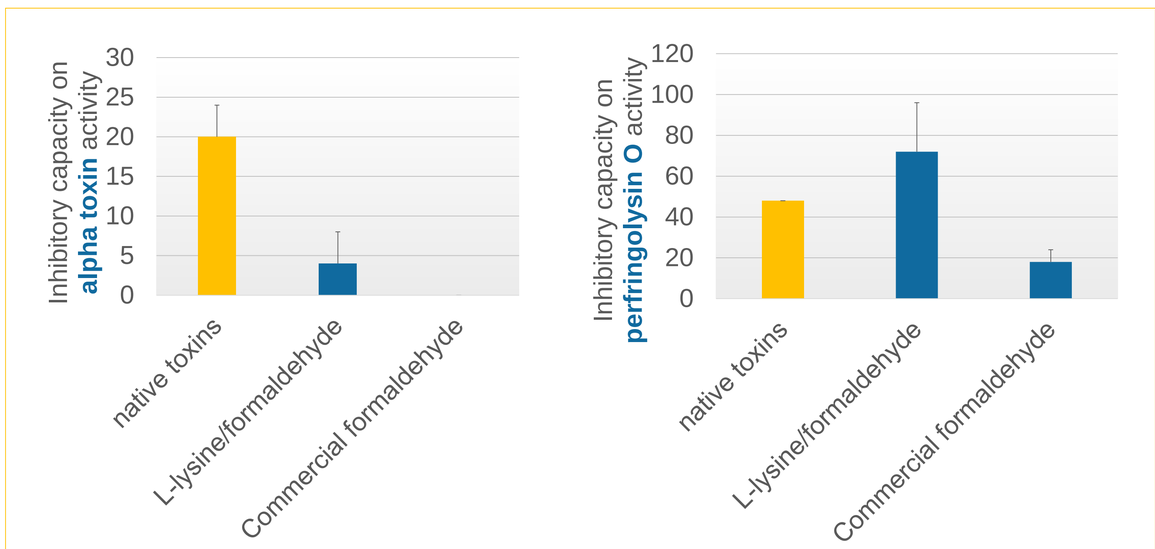
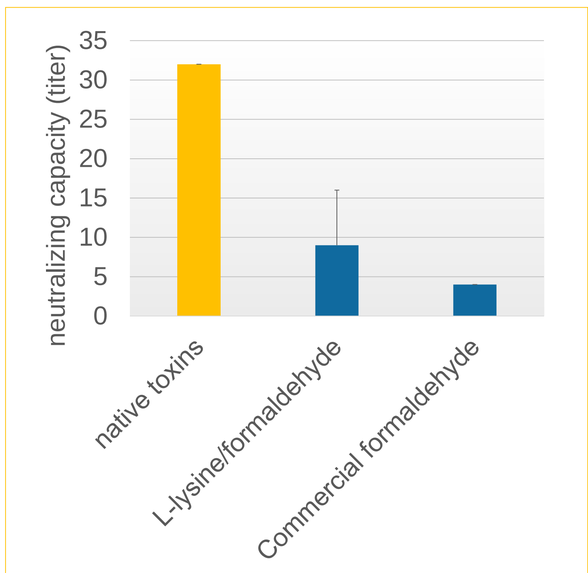


Figure 3: *in vitro* neutralization of biological activities of alpha toxin and perfringolsyin

Toxin-neutralizing antibodies neutralize the *C. perfringens*-induced cytotoxicity on bovine endothelial cells



Cytotoxicity assay:
Bovine Umbilical Vein Endothelial Cells (BUVEC) exposed to filter-sterilized supernatant of *C. perfringens*
Cytotoxicity quantified by a neutral red uptake (NRU) assay

Neutralization of cytotoxicity by pre-incubation with the antibodies.

Antibodies from calves vaccinated with native *C. perfingens* toxins were able to neutralize the cytotoxicity of *C. perfringens* on bovine endothelial cells.

Figure 4: neutralization of *C. perfringens* cytotoxicity

Conclusion

- **Toxin-neutralizing antibodies protected** against *C. perfringens* challenge
- **Prevention of endothelial damage** may be the mechanisme underlying this protective effect

- Immunization of both native and formaldehyde inactivated *C. perfringnes* toxins resulted in a strong immune respons 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

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
Valgaeren B, Pardon B, Goossens E, et al. Veal Calves Produce Less Antibodies against *C. Perfringens* Alpha Toxin Compared to Beef Calves. Toxins. 2015;7:2586-97