What is the best way to deliver *Clostridium perfringens* alpha toxin to chickens to yield protection against avian necrotic enteritis?

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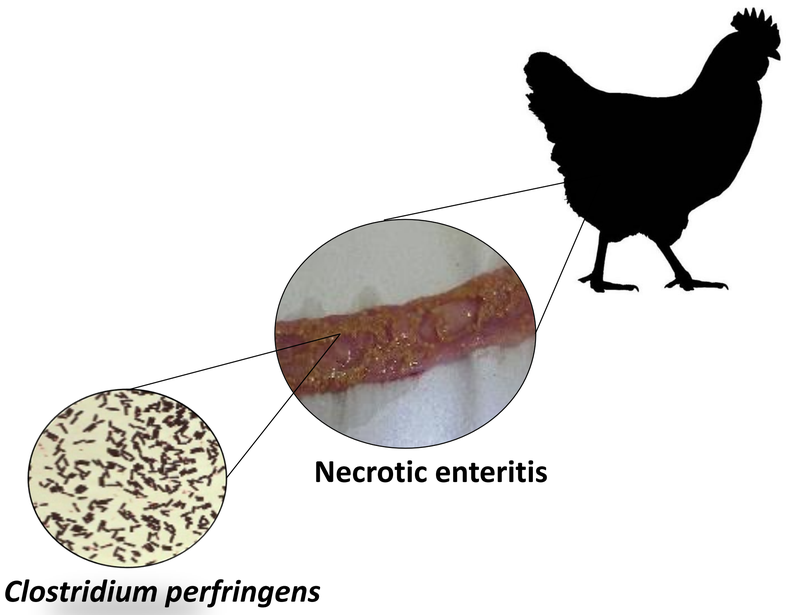
Necrotic enteritis in broiler chickens is caused by *Clostridium perfringens*, and there is currently no effective vaccine for this disease. Previous research identified promising vaccination candidates like the essential toxin NetB and alpha toxin. Vaccination with either NetB or alpha toxin results in similar levels of protection against necrotic enteritis. Previous research concerned the subcutaneous vaccination with either or both antigens.



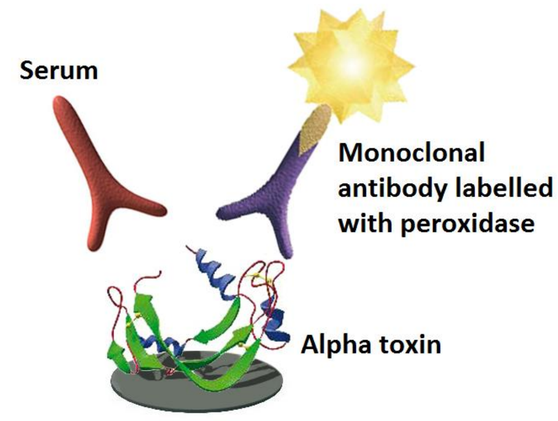
Concerning the feasibility in the field, an oral vaccination method would be preferred. In this study the immune development in broiler chickens after oral vaccination with live recombinant *Bacillus* spores expressing alpha toxoid on the spore coat was compared to the immune response after subcutaneous vaccination with the alpha toxoid. Oral vaccination of the birds resulted in systemic antibodies. Future *C. perfringens* challenge studies are planned to elucidate whether this immunization protocol can provide superior protection against experimental disease.

Methodology

Broiler chickens were divided into groups of six. Four groups were given the recombinant *Bacillus* spores expressing the alpha toxoid once, two-, three- or ten times at different days. The spores were given orally. One group received a parenteral administration of the alpha toxoid, $CPA_{247-370}$, by injection. Negative control groups were included and were given the native PY79 *Bacillus* spores or were injected with PBS.

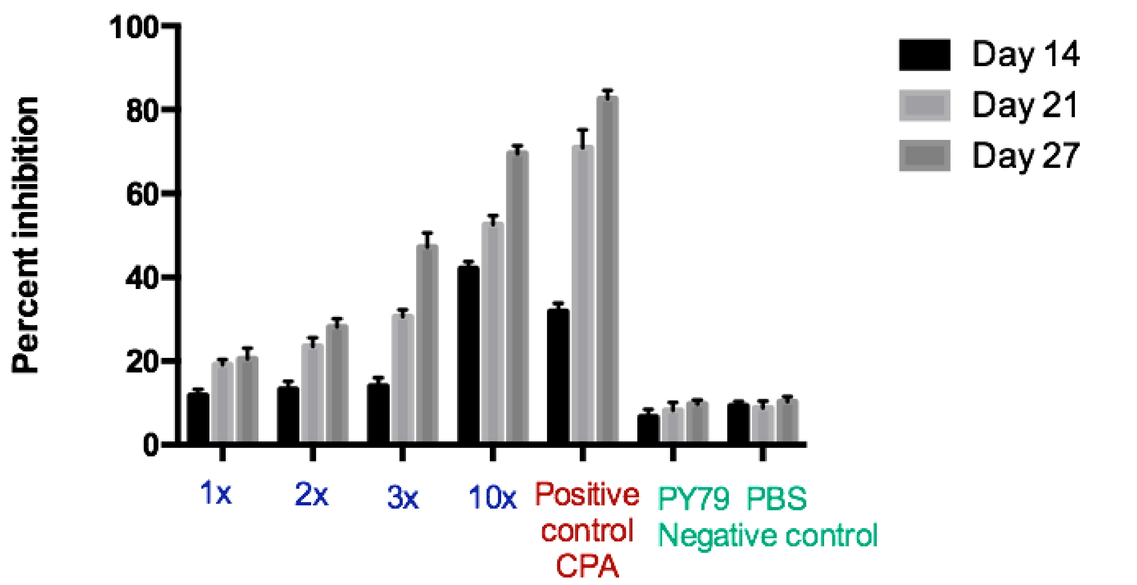
Group	Animals /group	Inoculation product	Inoculation dose	Inoculation day(s)	Serum collecting days (all groups)	Litter collecting days (all groups)
1	6	HT 251	$5x10^{10}$ spores/ml	3	1-14 21-27	0-27
2	6	HT 251	$5x10^{10}$ spores/ml	3, 12		
3	6	HT 251	$5x10^{10}$ spores/ml	3, 12, 21		
4	6	HT 251	$5x10^{10}$ spores/ml	3, 5, 7, 10,12, 14, 17, 19, 21, 24		
5	5	CPA 247-370	70µg	3, 12		
6	6	PY79	$5x10^{10}$ spores/ml	3, 12, 21		
7	6	PBS	1 ml	3, 12, 21		

The serum was collected at the end of the trial and was analysed using a serum inhibition ELISA (BioX-laboratories, Belgium) to quantify the antibody response in the serum.



Results

The percentage of inhibition was measured within the different serum samples. An increasing frequency of oral administration of the *Bacillus* spores expressing the alpha toxoid resulted in an increasing amount of antibodies against the alpha toxin in the serum. A tenfold administration of the oral vaccine resulted in a similar antibody response as the parenteral administration of the alpha toxoid.



Conclusion

Oral administration of a vaccine against necrotic enteritis caused by *Clostridium perfringens* would be more applicable in the field. Our study shows the similarity in antibody response of the oral vaccine as compared to the response after parenteral administration. Further research will focus on the effect of this vaccination strategy on the prevalence of necrotic enteritis in broiler chickens.

Multiple live vectors like *Bacillus subtilis* or *Eimeria tenella* can be used to administer an antigen to the host. Possibly this vector, which expresses the antigen, can be mixed in the feed or water of the animal or can be administered by spray application. The use of a live vector gives us a bright prospective for future research regarding vaccination strategies.