Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Oral vaccination of broiler chickens against necrotic enteritis using a non-virulent NetB positive strain of *Clostridium perfringens* type A

Neha Mishra, Joan A. Smyth *

Department of Pathobiology & Veterinary Science, University of Connecticut, 61 North Eagleville Road, Storrs, CT 06269, USA

ARTICLE INFO

Article history:
Received 6 July 2017
Received in revised form 3 October 2017
Accepted 12 October 2017
Available online xxxx

Keywords:
Necrotic enteritis
Mucosal
Vaccine
NetB
Clostridium perfringens
Adjuvant

ABSTRACT

Necrotic enteritis (NE) is a severe disease of chickens and turkeys caused by some strains of *Clostridium perfringens* type A. The disease is well controlled by the use of in-feed antibiotic growth promoters (AGPs). However, due to worldwide public and regulatory pressure to reduce the use of AGPs *inter alia*, there is an urgent need to develop non-antibiotic based preventative measures. Vaccination would be a suitable control measure, but currently there is no commercial vaccine.

NetB (necrotic enteritis toxin B-like) is a pore-forming toxin produced by *C. perfringens* that has been reported as an important virulence factor in the pathogenesis of NE. The present study tests a non-virulent NetB producing strain of *C. perfringens* (nvNetB$^+$), with or without adjuvants, as an orally administered live vaccine. Adjuvants used were Gel 01™, Cholera toxin (CT), *Escherichia coli* wild type heat-labile holotoxin (LT) and mutant *E. coli* LT (dmLT) (R192G/L211A). Several vaccine administration regimes were tested.

All vaccination regimes elicited serum and mucosal antibody responses to alpha toxin and to secreted proteins of both nvNetB$^+$ and a very virulent NetB positive (vvNetB$^+$) strain ($p < 0.0001$ to $p < 0.05$). In some vaccinated groups, there was milder intestinal pathology upon disease challenge. 55% of birds vaccinated orally at days 2, 12 with nvNetB$^+$ adjuvanted with CT did not develop any lesions of NE by 6 days post challenge, compared to a 100% incidence of NE lesions in the unvaccinated disease challenged group.

© 2017 Published by Elsevier Ltd.

1. Introduction

Necrotic enteritis (NE) is a serious disease of chickens and turkeys, caused predominantly by *Clostridium perfringens* type A [1–3]. Affected birds may also develop cholangiohepatitis [2,4,5]. *C. perfringens* is an ubiquitous, gram-positive anaerobic bacterium, which is commonly found in the normal intestine [2,6,7].

NE can be well controlled by use of in-feed antibiotic growth promoters (AGPs) [8]. However, use of AGPs in poultry production is discouraged by the WHO and has been banned in many countries [9,10]. The incidence of NE increases when use of AGPs stops [11]. Therefore, there is a need to develop non-antibiotic based preventative measures. Vaccination is a possible alternative strategy, but currently there is no vaccine available against NE [12]. There have been several published studies looking at candidate vaccines, which were toxoids [13–16], culture supernatants [17,18] or subunit vaccines [14,19–22]. These vaccines either provided only partial protection or were impractical for commercial use as they required multiple vaccinations by injection [12,17,22–25].

The mechanism for virulence of *C. perfringens* in the context of NE is still under investigation [26–28]. Early work suggested that alpha toxin is important for virulence but later it was concluded that other toxins contribute to the pathogenesis of NE [9,29,30]. For example, a pore-forming toxin (NetB) has been shown to be important for the ability of *C. perfringens* to cause NE in chickens [27,31–35].

We have a unique *netB* positive strain of *C. perfringens* type A (α-toxin positive) which produces active NetB toxin (NetB$^+$), but which produces minimal intestinal pathology, and then only in a small percentage of challenged birds [32]. These attributes give this strain the potential to serve as a live oral vaccine, which could be administered in food, water or by spray, in the same way as

Abbreviations: AGPs, antibiotic growth promoters; CFU, colony-forming unit; CT, Cholera toxin; dmLT, mutant LT; EsoG, esophageal gavage; FTG, fluid thioglycollate medium; LT, *Escherichia coli* wild type heat-labile holotoxin; NE, necrotic enteritis; NetB, necrotic enteritis toxin B-like; nvNetB$^+$, non-virulent NetB and alpha toxin positive strain of *C. perfringens*; PFGE, pulsed field gel electrophoresis; SBA, sheep blood agar; vvNetB$^+$, very virulent NetB and alpha toxin positive strain of *C. perfringens*.

* Corresponding author.
E-mail addresses: neha.mishra@uconn.edu (N. Mishra), joan.smyth@uconn.edu (J.A. Smyth).

https://doi.org/10.1016/j.vaccine.2017.10.030
0264-410X/© 2017 Published by Elsevier Ltd.

Please cite this article in press as: Mishra N, Smyth JA. Oral vaccination of broiler chickens against necrotic enteritis using a non-virulent NetB positive strain of *Clostridium perfringens* type A. Vaccine (2017), https://doi.org/10.1016/j.vaccine.2017.10.030

other live broiler poultry vaccines e.g. coccidia vaccines. This approach would be practical for field use and has the potential advantage of presenting multiple *C. perfringens* type A antigens including NetB, in native format, directly to the target organ, the intestinal mucosa.

In this study we investigate the potential of this unique non-virulent NetB positive strain (nvNetB$^+$) as a live oral vaccine.

2. Material and methods

2.1. Bacterial strains

A non-virulent *netB* positive strain of *C. perfringens* type A (Strain 11 [32]) was used as the test vaccine strain (nvNetB$^+$). A very virulent *netB* positive strain of *C. perfringens* type A (vvNetB$^+$) (Strain 1 [32]) was used both as a disease challenge organism, and as a positive control "vaccine" based on previously published work which showed that immunization with virulent *C. perfringens* protected approximately 90% of birds against NE disease challenge [9].

2.2. Vaccine preparation and immunization

Both nvNetB$^+$ and vvNetB$^+$ *C. perfringens* were first grown on sheep blood agar (SBA) then passed through broth cultures as follows. For vaccination by administration in feed, *C. perfringens* was grown in brain heart infusion broth (BHI) (2 passes) and administered in feed (3:4 {v:w}) as previously described [32]. For administration by esophageal gavage (EsoG) or for adjuvanted vaccines, C. *perfringens* was twice passed in each of cooked meat medium and fluid thioglycollate medium (FTG) as previously described [9]. *C. perfringens* broth culturs used were of the order of 10^8 CFU/ml, barring a few exceptions (10^9 CFU/ml).

All the adjuvanted vaccines were delivered by EsoG, once a day (0.5 ml at 2 day old, and 2 ml at other ages). Amounts of adjuvants to be incorporated in vaccines were calculated based on average body weight of birds at the time of vaccinations.

In Experiment 1 (Table 1), adjuvants Gel 01™ and Cholera toxin (CT) were tested. For preparation of Gel 01™ adjuvanted vaccine, MONTANIDE™ GEL 01 (Seppic) suspension was mixed with *C. perfringens* broth culture at a ratio of 1:9 by vortexing for 5 min. CT adjuvanted vaccine was prepared by mixing CT (List Biological), reconstituted according to manufacturer's instructions, with *C. perfringens* broth, by vortexing for 2 min, to provide 0.025 μg CT/g body weight.

In Experiment 2 (Table 2), three adjuvants i.e. CT, *E. coli* labile toxin (LT) and *E. coli* dmLT (R192G/L211A) were used at a dose of 0.2–0.5 μg/g body weight of bird (Supplement 1a). Adjuvanted vaccines were prepared by mixing the respective adjuvants with concentrated FTG culture (Supplement 1b) containing 10^{12} to 10^{13} CFU/ml (Supplement 4, Table 4d), by vortexing for 5 min.

C. perfringens CFU were determined before and after addition and mixing-in of the adjuvants.

2.3. Birds and accommodation

Day-old male Ross X Ross broiler chickens (n = 282) were obtained from a commercial hatchery and raised in a BSL2 facility as described previously [32]. Birds were weighed on the day before vaccination commenced, on the day after vaccination was completed, before disease challenge and at necropsy.

2.4. Experimental design

There were two experiments.

Experiment 1: There were 6 groups of 20 birds each (Table 1). Group 1 was unvaccinated and later disease challenged, thus serving as both the unvaccinated control for the pre-disease challenge period, and then as the positive disease control for the disease challenge system. Groups 2–6 were vaccinated with the test vaccines for 5 consecutive days starting at 8 days of age. Group 2, was vaccinated with non-adjuvanted nvNetB$^+$ by EsoG and in feed. Groups 3 and 4 were vaccinated by EsoG with nvNetB$^+$ containing adjuvants Gel 01™ (Group 3) or CT (Group 4) respectively, and also with non-adjuvanted nvNetB$^+$ in feed. Group 5, the positive "vaccine" control, was "vaccinated" with vvNetB$^+$ for 5 days in feed, then treated with antibiotic for 6 days in order to eliminate the virulent strain from these birds. Group 6, was vaccinated with nvNetB$^+$ in feed and later challenged with coccidia alone, thus serving as a vaccinated, non-NE disease challenged, negative control, to test safety of nvNetB$^+$ strain.

Table 1
Experimental design, Experiment 1. There were 6 groups of 20 birds each. Groups 2–6 were vaccinated for 5 consecutive days (see below). Pre-disease challenge examinations were done at two time points, i.e. one day after the 5 day vaccination regime (day 13), and the day before challenge (day 22). Four chickens were examined per group at these time points (except for Group 6 from which 3 chickens were examined on the day pre-challenge). For assessment of colonization of the small intestine of birds by the respective vaccine strains, swabs from standardized sites in the intestine were streaked on to sheep blood agar, incubated anaerobically, and a plate grade score [32] for *C. perfringens* was assigned. Selected *C. perfringens* isolates were examined by pulsed field gel electrophoresis (PFGE) [32] and compared to the vaccinal PFGE types to determine the colonization percentage. At day 23, all groups (10 birds/group) were disease challenged, except Group 6 (negative control) which was challenged with coccidia alone.

	Groups	Mucosal adjuvants	Vaccine strains	Vaccination at days-of-age 8, 9, 10, 11 & 12		Birds colonized with the respective vaccinated strains[†]		Disease challenge (day 23)
				EsoG	Feed	Post vaccination, (day 13) n = 4	Pre challenge (day 22) n = 4	
1	Negative vaccine/Positive disease control	–	–	–	–	0%	0%	√
2	Vaccinate	–	nvNetB$^+$	√	√	75%	25%	√
3	Vaccinate	Gel 01TM	nvNetB$^+$	√	√*	25%	25%	√
4	Vaccinate	CT (0.025μg/g B.wt)	nvNetB$^+$	√	√*	100%	75%$^{\Delta}$	√
5	Positive "vaccine" control	–	vvNetB$^+$	–	√	100%	100%	√
6	Non disease challenged negative control	–	nvNetB$^+$	–	√	50%$^{\Delta}$	0%$^{\Delta\Delta}$	Only coccidia

CT, Cholera toxin.
EsoG, Esophageal gavage (adjuvanted vaccine).
nvNetB+, non-virulent necrotic enteritis toxin B-like positive strain of *C. perfringens*.
vvNetB+, very-virulent necrotic enteritis toxin B-like positive strain of *C. perfringens*.
* Non-adjuvanted nvNetB+ in feed.
$^{\Delta}$ Only one C. perfringens colony was recovered from one bird from this group and it was not examined by PFGE.
$^{\Delta\Delta}$ Only 3 birds were examined in this group due to insufficient numbers.
[†] Depicts percentage of birds positively confirmed by PFGE; details of C. perfringens grade for individual birds in Supplement 4 (Table 4a).

Please cite this article in press as: Mishra N, Smyth JA. Oral vaccination of broiler chickens against necrotic enteritis using a non-virulent NetB positive strain of *Clostridium perfringens* type A. Vaccine (2017), https://doi.org/10.1016/j.vaccine.2017.10.030

Download English Version:

https://daneshyari.com/en/article/8486552

Download Persian Version:

https://daneshyari.com/article/8486552

Daneshyari.com