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Efficacy of an anticoccidial live vaccine in prevention of necrotic enteritis in chickens

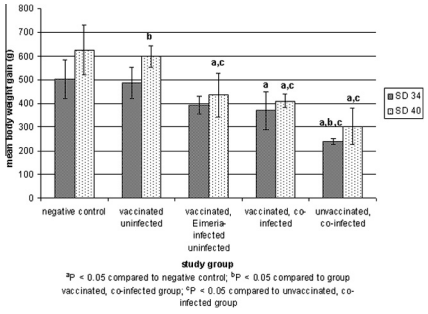
Berit Bangoura [a,*], Alaa Aldin Alnassan [a], Matthias Lendner [a], Awad Ali Shehata [b], Monika Krüger [b], Arwid Daugschies [a]

[a] Institute of Parasitology, Faculty of Veterinary Medicine, Centre for Infectious Diseases, University of Leipzig, An den Tierkliniken 35, 04103 Leipzig, Germany
[b] Institute for Bacteriology and Mycology, Faculty of Veterinary Medicine, Centre for Infectious Diseases, University of Leipzig, An den Tierkliniken 29, 04103 Leipzig, Germany

HIGHLIGHTS

- A necrotic enteritis (NE) model with *C. perfringens* and *Eimeria* spp. was used.
- Anticoccidial vaccine decreased clinical signs and weight depression due to NE.
- Cytokine expression profile was altered during clinically relevant coinfections.
- Vaccine prevented severe NE and is a promising tool for field control measures.

GRAPHICAL ABSTRACT



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ABSTRACT

Necrotic enteritis (NE) is an important disease in poultry caused by *Clostridium perfringens* combined with predisposing factors, mainly eimeriosis. In the present study, we investigated the protective effect of a commercial attenuated anticoccidial live vaccine against NE in a clinical infection model using 60 day-old chicks. Vaccination was performed on study day (SD) 1 with natural booster-infections for 4 weeks from *Eimeria* spp. oocysts present in litter. On SD 28, five groups were formed ($n = 12$): group V+/C−E− (vaccinated, uninfected), group V+/C−E+ (vaccinated, infected with *Eimeria* spp.), group V+/C+E+ (vaccinated, infected with clostridia and *Eimeria* spp.), group V−/C+E+ (unvaccinated, infected with clostridia and *Eimeria* spp.), and group NC (negative control). Efficacy was measured by clinical parameters, pathogen multiplication, and pathological parameters assessed during two necropsies on SD 34 and SD 40, respectively. Additionally, cytokine expression was measured in gut and spleen tissues at necropsy. Clinical signs of NE were observed only in the coinfected groups, mainly in group V−/C+E+. Accordingly, lowest body weight gain was observed in group V−/C+E+ (301.8 g from SD 28 to SD 40; group NC: 626.2 g). Oocyst excretion varied significantly ($P < 0.01$) between all *Eimeria* spp. infected groups and was highest in group V−/C+E+, followed by V+/C+E+, and lowest in group V+/C−E+. NE typical intestinal lesions showed only in groups V+/C+E+ and V−/C+E+. The intestinal mucosa featured partly severe lesions in the jejunum, *C. perfringens* colonization was histologically visible. Upregulation of IFN-γ, was observed in the jejunal tissue of group V−/C+E+ ($P < 0.01$ (SD 34) or $P < 0.05$ (SD 40) compared to all other groups). IL-10 and IL-12 were upregulated in group V−/C+E+, IL-10 also in group V+/C+E+ (SD 40) while IL-2 expression remained unaltered.

In conclusion, vaccination against coccidiosis was effective in preventing NE in a mixed infection comparable to field situations.

* Corresponding author. Fax: +49 341 9738095.
E-mail address: bangoura@vetmed.uni-leipzig.de (B. Bangoura).

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1. Introduction

Necrotic enteritis (NE) is an important disease in different animal species, especially in poultry, and is of high relevance in regard of animal health and farm economy (Van der Sluis, 2000; Lovland and Kaldhusdal, 2001). The main pathogen is *Clostridium perfringens* type A (Si et al., 2007) which secretes several toxins, e.g., alpha, beta 2, TpeL and necrotic enteritis B-like toxin (NetB). Currently it is known that expression of last-mentioned NetB predisposes for NE development in chickens (Keyburn et al., 2008) by forming pores in the target cell membranes (Savva et al., 2013). If clostridiosis coincides with predisposing factors such as stress, inadequate diets or coccidial infections, NE occurs (Williams et al., 2003; Van Immerseel et al., 2009). For experimental infection models, coinfections with coccidia and clostridia have been used before to mimic natural conditions (Williams et al., 2003; Williams, 2005; Alnassan et al., 2013). Coccidiosis may lead to severe enteritis, reduced weight gain, and a varying mortality (Williams, 1996) according to the different *Eimeria* spp. in chickens. *Eimeria* spp. infections promote *C. perfringens* growth and thus toxin production and NE by causing intestinal damage (Williams, 2005) and enhanced mucus secretion (Al-Sheikhly and Al-Saieg, 1980; Van Immerseel et al., 2004; Collier et al., 2008). For a long time, NE prophylaxis has been achieved by routine administration of antimicrobial feed additives in broilers (Elwinger et al., 1998; Lanckriet et al., 2010) but this option has expired in many countries due to legal restrictions or clostridial resistances (Anon, 2003; Castanon, 2007; Silva et al., 2009). Other control strategies have been tested, e.g. anticoccidial treatment (Lanckriet et al., 2010; Alnassan et al., 2013) or an experimental vaccination with a *C. perfringens* toxoid vaccine (Mot et al., 2013). Recently, Tsiouris et al. (2013) showed the prophylactic efficacy of an anticoccidial live vaccine against experimentally induced subclinical NE due to infection with *C. perfringens* and *Eimeria maxima*. In the present study, we investigated the protective effect of an attenuated live vaccine on clinical NE which often occurs in the field and was in this trial induced by coinfections with *C. perfringens*, *Eimeria tenella* and *Eimeria brunetti*.

2. Materials and methods

2.1. Chickens and experimental design

In total, 60 commercial broiler chickens were purchased as one-day-old chicks (Cobb 500®, Cobb-Germany Avimex GmbH, Wiedemar, Germany) and reared in floor pens. They were fed a diet without antibiotics and anticoccidials and had water access *ad libitum*. On the day of housing in, they were divided in two groups reared in two different pens one of which was vaccinated ($n = 36$) and the other one remained unvaccinated ($n = 24$). On SD 28, five groups were formed ($n = 12$): one group was vaccinated and remained uninfected (group V+/C−E−) a second group was vaccinated and infected with *Eimeria* spp. (group V+/C−E+), a third group received vaccination and infection with clostridia and *Eimeria* spp. (group V+/C+E+), a fourth group was not vaccinated but infected with clostridia and *Eimeria* spp. (group V−/C+E+), and a fifth group remained unvaccinated and uninfected as negative control (group NC). For study design see also Table 1. Starting with the infections (SD 28), chickens were moved to wire cages to enable monitoring of the feed consumption.

Two necropsies were carried out in 34- and 40-day-old chickens for evaluation of coccidiosis and NE lesions. Samples for histological examination were collected from 2 cm of each jejunum, ileum and cecum in 4% phosphate buffered formalin. Blood samples were collected after slaughter, centrifuged by 2000*g* for 10 min. The serum was stored in −20 °C until use.

Table 1
Experimental study design.

Study group	n[a]	Vaccination (SD 1, 7, 14, and 21)	*Eimeria* spp. (SD 28)[b]	*C. perfringens* (SD 32)[c]
NC	12	−	−	−
V+/C−E−	12	+	−	−
V+/C−E+	12	+	+	−
V+/C+E+	12	+	+	+
V−/C+E+	12	−	+	+

SD: study day.
[a] Each study group was divided in two subgroups ($n = 6$) slaughtered on SD 34 or SD 40, respectively.
[b] Infection with 25,000 oocysts of *E. tenella* and 75,000 oocysts of *E. brunetti*.
[c] Infection with 10^9 cfu *C. perfringens*.

2.2. Eimeria infections

The Houghton strain of *E. tenella* (kindly provided by Dr. D.P. Blake, Royal Veterinary College, University of London, Hatfield, UK) and an *E. brunetti* field isolate (Institute of Parasitology, Faculty of Veterinary Medicine, Leipzig University) were used less than 6 months after passage as previously described (Alnassan et al., 2013). Oocysts were quantified using a modified McMaster method. The purity of the used *Eimeria* spp. strains was confirmed by a conventional multiplex polymerase chain reaction (PCR) as described by Su et al. (2003) using species specific primers (Alnassan et al., 2013). For oral infection, respective birds received 25,000 oocysts of *E. tenella* and 75,000 oocysts of *E. brunetti* each once on SD 28. This infection dose was determined as suitable to induce clinical NE in preliminary trials (data not shown).

2.3. C. perfringens infection

In this study, a NetB toxin positive *C. perfringens* strain (lab No. 2-288, kindly provided by RIPAC-LABOR (Potsdam-Golm, Germany) was used as previously described (Alnassan et al., 2013). The presence of the NetB toxin gene was verified by conventional PCR as described by Baums et al. (2004). The *C. perfringens* strain was cultured anaerobically *in vitro*. Counting was performed by a spread-plating method (Sanders, 2012). Chickens of groups V+/C+E+ and V−/C+E+ were inoculated with 10^9 colony forming units (cfu) of *C. perfringens* in 1 ml water once on SD 32.

2.4. Vaccination

Vaccination was performed in the respective study groups with a commercial attenuated live vaccine against chicken coccidia (Paracox®-8, Intervet Deutschland GmbH, Unterschleissheim, Germany) containing attenuated strains of *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *Eimeria mitis*, *Eimeria necatrix*, *Eimeria praecox*, and *E. tenella*. Chickens were inoculated individually with 0.1 mL directly into the crop using a plastic pipette. In study groups V+/C−E−, V+/C−E+, and V+/C+E+ vaccination took place on study day 1 (SD 1, first day of life). Natural boostering occurred because chickens were raised on deep litter (according to the recommendation of the manufacturer) and confirmed by oocyst excretion monitoring in fecal samples.

2.5. Assessment of efficacy

2.5.1. Clinical signs, mortality, weight gain feed, and conversion ration

Clinical observation was carried out daily for all birds throughout the experiment including the recording of clinical signs and mortality. Feed was weighed in daily and the residual feed was weighed out daily. Body weight was assessed on SD 14, SD 27,