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Sandwich ELISA detection of *Clostridium perfringens* cells and α-toxin from field cases of necrotic enteritis of poultry

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Abstract

Sandwich ELISAs (sELISAs) for the detection of *Clostridium perfringens* cells and α-toxin were developed and used to screen intestinal samples from normal broiler chickens and from clinical cases of necrotic enteritis. The assays clearly distinguished between the two sets of samples. The sELISA absorbance values from samples obtained from the majority of healthy birds were low and those from the majority of necrotic enteritis cases were high. Together, the assays provide a suitable test for the rapid screening for the diagnosis of necrotic enteritis in poultry.

Keywords: Necrotic enteritis; *Clostridium perfringens*; Sandwich ELISA; α-Toxin

1. Introduction

Clostridium perfringens is a common environmental bacterium and is readily isolated from the intestine of birds and mammals (Hofshagen and Stenwig, 1992). The species is divided into five types (A, B, C, D and E) on the basis of production of major lethal exotoxins. All types produce α-toxin, a phospholipase C that is considered to be a major contributory factor towards development of intestinal mucosal necrosis, the characteristic lesion of necrotic enteritis in poultry (Al-Sheikhly and Truscott, 1977; Wages and Opengart, 2003). Both *C. perfringens* types A and C are associated with necrotic enteritis in poultry, a disease of considerable economic significance to the poultry industry due to increased mortality and reduced weight gain. Several predisposing factors such as feed components, litter and coccidia can lead to a proliferation of *C. perfringens* in the gut of the chicken and increased production of α-toxin (Al-Sheikhly and Truscott, 1977; Branton et al., 1987; Kaldhusdal and Hofshagen, 1992).

In the present study, the application of two sandwich ELISAs (sELISAs), developed for the detection of *C. perfringens* whole cell and α-toxin, for screening poultry intestinal contents for the diagnosis of necrotic enteritis, was examined.

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2. Materials and methods

2.1. Antiserum

Polyclonal rabbit antiserum was raised against *C. perfringens* type A whole cell antigen (8237; National Collection of Type Cultures, Collindale, London). Colonies cultured overnight on blood agar were washed off the plate with Ringer's solution containing 0.3% formaldehyde. The rabbit was inoculated subcutaneously with formalised antigen, mixed with Freund's complete adjuvant, and boosted intravenously with antigen alone 1 month later. The antiserum showed a high positive titre specific to *C. perfringens* when tested against the immunising antigen and similarly prepared antigens from other *Clostridium* spp. by slide agglutination. Monoclonal antibody 4F2 and equine polyclonal antibody to *C. perfringens* α-toxin were kindly supplied by Mr. Tim Webber, DERA, Porton Down. The immunoglobulins were purified by caprylic acid precipitation (McKinney and Parkinson, 1986) and some of them were biotinylated for use in the sELISAs (Hofman et al., 1982).

2.2. Sandwich ELISA

Two sELISAs were developed, one for the detection of *C. perfringens* cells and the other for the detection of *C. perfringens* α-toxin. In the cell sELISA, purified polyclonal IgG was used as the capture antibody, with biotinylated polyclonal IgG as detection antibody. In the *C. perfringens* α-toxin sELISA, equine polyclonal antitoxin to *C. perfringens* α-toxin was used as capture antibody, and biotinylated 4F2 monoclonal as detection antibody.

The two sELISAs were performed as described by Ball et al. (1993). Briefly, microtitre plates using 100 μl volumes of each reagent per well were used. The optimum dilution of each reagent was determined by titration. Capture antibody in 0.05 M carbonate buffer, pH 9.5 was used to coat the plates at 4 °C overnight or at 37 °C for 1 h. All further incubation stages were at 37 °C, and after each incubation stage, the wells were washed four times with wash buffer (0.01 M PBS, pH 7.2 containing 0.05% (v/v) Tween 20). Antibody reagent dilutions were carried out in PTN (0.01 M PBS with 0.04% Tween 80 and an additional 2% NaCl added). The stages after coating were as follows: test sample for 90 min, biotinylated reagents (polyclonal IgG or 3A4F2 monoclonal) for 1 h, strepavidin-peroxidase (Sigma) for 1 h, peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) for 10 min. The reaction was stopped by the addition of 50 μl of 2.5 M H_2SO_4 per well. The optical density (OD) was read at 450 nm with an ELISA plate reader (Titretek, Multiscan).

C. perfringens type A cultured in Brain Heart Infusion broth (BHI; Oxoid) at an approximate titre of 10^7 cfu/ml) and phospholipase C (P4039; Sigma) were used as respective positive controls in the *C. perfringens* cell and α-toxin ELISAs, and PTN as negative control in both.

C. perfringens types A (NCTC 8237), B (NCTC 3110), C (NCTC 3180), D (NCTC 8346), E (NCTC 9179) and other *Clostridial* spp. clinical isolates (*C. chauveoi*, *C. sordelli*, *C. septicum* and *C. novyi*) were cultured overnight in cooked meat broth and used to test the *C. perfringens* sELISA for specificity. Equal volumes of the culture and PTN were used as test sample. In addition, colonies from a collection of clinical aerobic bacterial field isolates from poultry, cultured on bovine blood agar, were re-suspended in PTN for application as the test sample.

The sensitivity of the cell sELISA was determined from serial 10-fold dilutions of *C. perfringens* type A cultured in BHI broth incubated anaerobically at 37 °C for 24 h. Colony counts were determined on bovine blood agar after incubation at 37 °C in an anaerobic incubator. The sensitivity of this assay was also examined on serial 10-fold dilutions of samples from six field cases of necrotic enteritis. Colony counts were similarly culturally determined. The sensitivity of the α-toxin sELISA was determined by titration of partially purified phospholipase C (P4039, Type XIV; Sigma) containing 150–350 units/mg.

2.3. Test samples

Two longitudinal studies were carried out with birds from commercial chicken houses, one starting in October 2001 for 6 weeks, and the second starting in August 2002 for 5 weeks. All birds were clinically normal throughout this study. Ten birds from a single house in the first study and 20 from each of two houses in the second were randomly selected and removed at