Antibody responses in buffalos immunized with *Clostridium perfringens* beta and epsilon toxoids

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ABSTRACT: The antibody responses to toxoids were measured to investigate whether *Clostridium perfringens* beta and epsilon toxoids induced protective humoral immune responses in buffalos. Total of 24 buffalos were divided into 4 groups ($n = 6$), beta toxoid, epsilon toxoid, combination and control groups. These buffalo groups were administered each of the designated toxoids. Immunizations in the beta and epsilon toxoid groups induced strong antibody responses. The neutralizing antibody titres from the beta and epsilon toxoid groups were equally $\log_{10}1.2$ on day 21 after inoculation whereas there was no antibody titre detected from the control group. A statistically significant ($P < 0.01$) increase in antibody titre was observed from day 0 to day 14 and 21 after inoculation. The antibody production did not vary significantly due to day of inoculation and toxoid interactions.

Keywords: *Clostridium perfringens*; antibody titre; toxoid; buffalo

Clostridium perfringens is considered to be one of the most widespread pathogenic organisms in animals (Hatheway, 1990). This organism causes acute enterotoxaemia in ruminants such as cattle, sheep, goats and buffalos by proliferation in the intestine and production of several toxins such as alpha, beta, epsilon and tau (Chakrabarty *et al*., 1980; Al-Mashat and Taylor, 1983; Popoff, 1984; Worral *et al*., 1987; Radostits *et al*., 1994; Secasiu *et al*., 1997). Among the toxins, beta and epsilon exert the most lethal effects (El-Idrissi and Ward, 1992). Clostridial vaccines are used for immunization against enterotoxaemia in cattle, sheep and goats. Nevertheless, there are no either currently available vaccines or information on immunization for buffalos. In the present paper, to obtain information on validity in the use of clostridial beta and epsilon toxoids for immunization of buffalos we studied whether these toxoids induced protective humoral immune responses by measuring antibody responses to potent toxoids.

The beta and epsilon toxoids of *Clostridium perfringens* were recently developed by International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England. The weights of used materials were approximately 70 mg for the beta toxoid and 115 mg for the epsilon toxoid. Each material was reconstituted in 1 ml of distilled water. The entire content was resuspended in 18 ml of physiological saline containing 0.01% thiomersalate and 0.5% ml of sterile aluminium hydroxide gel. These were thoroughly mixed and transferred to 25 ml of 2% aluminium hydroxide. The toxoids were allowed to dissolve at room temperature for three days with shaking at intervals to ensure a homogeneous suspension. These suspensions were further diluted at 1:5 (1 part of suspension : 4 parts of diluent) in a diluent containing 1 part of 2% aluminium hydroxide gel and 4 parts of physiological saline. The final concentrations of these reconstituted toxoids were roughly 0.32 mg per ml for beta and 0.52mg/ml for epsilon and 0.42 mg per ml for beta-epsilon combination.

A total of 24 apparently healthy, one-year-old, clostridial disease-free Monipuri buffalo calves were used in this experiment. The buffalos were divided into four groups. Six animals were randomly allocated to each group. These four groups were designated to each inoculation of beta, epsilon, beta-epsilon combination (1 : 1) and control. Three ml of each toxoid solution was inoculated subcutaneously into each of all six buffalos of the designated groups. The animals of control group were administered 3 ml of the diluent. All these animals were maintained on a clean buffalo farm. Blood samples were collected on days 0, 14 and 21 after inoculation. The collected sera were stored at –25°C until used.

The antibody titres of immunized sera were determined by the toxin-antitoxin neutralization test using mice as described by Rahman and Rahman (1999). Briefly, toxins purified from *Clostridium perfringens* were used as antigens. Immunized sera prepared against each toxoid were used as antibodies. Immunized sera were incubated at 56°C for 30 min before used for the test. One day

old suckling mice were used as an indicator host. Serum sample was diluted in PBS, pH 7.0 ranging from 1 : 2 to 1 : 256. 0.1 ml of each toxin (0.1mg/ml) was mixed with 0.1 ml of diluted individual serum sample. The mixture was incubated at room temperature for 1 hour. 0.2 ml of toxin-serum mixture was inoculated intraperitoneally into each of 5 suckling mice. To determine the neutralizing titre the highest dilution of sera protecting more than 50% of inoculated mice was recorded (Pal *et al*., 1990)

Analysis of variance was computed according to the method of Gupta (1983) to determine the difference between the particular groups receiving each toxoid separately and combined, and at different intervals after inoculation.

The neutralizing antibody titres in buffalos against the beta, epsilon toxoids, and the combination of *Clostridium perfringens* are shown in Table 1. It revealed that the neutralizing antibody levels against the beta toxoid on days 14 and 21 after inoculation were $\log_{10}$ 0.9 and $\log_{10}$ 1.2, respectively. Similar responses were observed against the epsilon toxoid. A statistically significant ($P < 0.01$) increase in antibody titre from days 0 to day 14 and 21 after inoculation was determined. The antibody levels against the beta-epsilon combination toxoid were $\log_{10}$ 0.6 and $\log_{10}$ 0.9, respectively, on days 14 and 21 after inoculation. It is evident that the antibody titres of the beta and epsilon toxoids are statistically similar when inoculated separately while the titre was significantly lower when inoculated as a combination. The antibody production did not vary significantly due to the day of inoculation and toxoid interaction. The antibody titre was not detected in the pre-inoculation sera collected on day 0 and in the control buffalos.

Table 1. Buffalo antibody titres of clostridial beta and epsilon toxoids in mouse neutralization test

Group	Antibody titre in log 10 ± SE ($P < 0.01$) on days after inoculation		
($n = 6$)	0	14	21
Beta	0	0.9 ± 0.048	1.2 ± 0.048
Epsilon	0	0.9 ± 0.048	1.2 ± 0.048
Combination	0	0.6 ± 0.048	0.9 ± 0.048
Control	0	0	0

n = number of buffalos used in toxoid inoculated and control group
SE = standard error

Prevention of clostridial diseases with vaccines has been practised for many years in cattle, sheep and goats (Jansen, 1967; Blackwell *et al*., 1983; Stokka *et al*., 1994). Most of the vaccines for clostridial diseases in cattle are bacterins incorporating several species of *Clostridium* into a single vaccine. Recently, the toxoid vaccines have been developed for sheep and goats (Uzal and Kelly, 1998; Uzal *et al*., 1999).

Ripley (1983) used a neutralization test for detecting antibody titres against the beta toxoid of *Clostridium perfringens*. Ebert *et al*. (1999) also used mouse neutralization test to estimate an immune response to the epsilon toxoid of *Clostridium perfringens*. In this study, using the mouse neutralization test to obtain information on validity in the use of the beta and epsilon toxoids of *Clostridium perfringens* for protection of buffalos from clostridial enterotoxaemia we determined whether these toxoids induced humoral immune responses in those vaccinated buffalos. The results showed that a single dose of the beta or epsilon toxoid induced a satisfactory level of protective immune responses in buffalos on days 14 and 21 after inoculation, based on the collective data indicating that these ranges of antibody titres were considered protective against enterotoxemia in animals (Smith and Marsh, 1953; Bullen and Batty, 1957; Jansen, 1960). A recent experiment in guinea-pigs also indicated that the production of a similar antibody level induced by the toxoids protected the animals from clostridial enterotoxaemia (Rahman and Rahman, 1999).

The level of antibody was relatively lower when these toxoids were inoculated in combination. This might be due to the antigenic competition of the two toxoids.

The vaccinated buffalos have been currently undergoing challenges with *Clostridium perfringens* for protection tests against clostridial enterotoxaemia.

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