Pak J Sci Ind Res 2001 44(5) 308-311

PRODUCTION OF HIGH TITRE ANTISERA IN RABBITS AGAINST *CLOSTRIDIUM PERFRINGENS* BETA AND EPSILON TOXOIDS

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(Received 30 November 1998; accepted 29 May 2001)

To investigate the validity of production of high titre antisera against *Clostridium perfringens* beta and epsilon toxoids in rabbit, the immune response of these toxoid inoculated rabbits were measured. The highest neutralizing antibody titres from the beta and epsilon toxoids were equally $\log_{10}1.50 \pm 0.042$ on 21st day post-inoculation of toxoid. The precipitating antibodies were detected by agar gel immunodiffusion test on the above mentioned period. Statistically significant ($P<0.01$) rise of antibody titre was observed from 14th day to 21st day post-inoculation of toxoid. Antibody production did not vary significantly due to interaction between day of inoculation and toxoid. It suggested the utility of rabbit for excellent production of high titre antisera against *Clostridium perfringens* beta and epsilon toxoids.

Key words: High titre antisera, *Clostridium perfringens,* Antibody, Toxoid.

Introduction

Enterotoxaemia, an important disease of cattle in Bangladesh, is an acute toxaemia of ruminants caused by proliferation of *Clostridium perfringens* in the intestine (Rahman *et al* 1998a). The organism produces several toxins of which beta and epsilon have the most lethal effects (El-Idrissi and Ward 1992). Antisera is the only method of treatment of enterotoxaemia caused by *Clostridium perfringens* (Radostits *et al* 1994). For diagnosis, treatment, prevention and control of enterotoxaemia high titre antisera against *Clostridium perfringens* is essential. But these are not available locally and need to be imported. Therefore, an attempt has been made to prepare standard high titre antisera in rabbits using *Clostridium perfringens* beta and epsilon toxoids.

Materials and Methods

Reconstitution of toxoid. Reference preparations of *Clostridium perfringens* beta and epsilon toxoids obtained from International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England were reconstituted in 1.0 ml of distilled water. The entire contents of ampoule were completely resuspended in 18.0 ml of physiological saline containing 0.01% concentration of thiomersalate and 5.0 ml of sterile aluminium hydroxide gel (2%). These were thoroughly mixed and the reconstituted reference toxoid was transferred to the 23.0 ml of diluted aluminium hydroxide, making a total volume of 25.0 ml. The trace of toxoid remaining in the ampoule was washed out by transferring volume of the mixture to and from the ampoule several times using a Pasteur pipette and mixing thoroughly after each transfer. The toxoid was allowed to absorb at room temperature for three days, shaking at intervals to ensure a homogenous suspension. The reference toxoid was further diluted in a diluent consisting of 1 part of aluminium hydroxide gel (2%) and 4 parts of physiological saline. Dilutions were prepared in five-fold steps for example 1/5, 1/25, 1/125. This diluent was used as a constant proportion of adjuvant. The reconstituted toxoid was tested for safety and sterility as per standard procedure (Anon 1992).

Experimental design. A total of 24 healthy, weighing 1.4-2 kg, 3-5 months old disease free New Zealand white male rabbits were used in this experiment. The animals were maintained in separate cages in a well ventilated room for at least one month prior to inoculation. During this period they were given normal laboratory diet and fresh drinking water. The rabbits were randomly divided into four groups of six animals each for inoculation of beta, epsilon, beta and

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epsilon (1:1) toxoid and control. An amount of 2 ml of each toxoid solution was inoculated subcutaneously.

Collection of antisera. Blood was collected from the ear veins of each rabbit. The animals were bled on 0, 14th and 21st day of inoculation of toxoids. The sera were separated as per standard procedure (Ahad 1995).

Serological tests. (a) Neutralization test: The antibody titre of immunized serum were tested in mice by the toxinantitoxin neutralization test as described by (Rahman *et al* 1998b).

(b) Agar gel immunodiffusion test: The procedure of Kida and Yanagawa (1981) was followed for the agar gel immunodiffusion test. The test was performed on 1% agar gel containing 8.5% sodium chloride and 0.08% sodium azide. Both beta and epsilon toxoids were used as antigen and the antisera prepared in rabbits were used as antibody.

Statistical analysis. Analysis of variance was computed according to Gupta (1983) to determine the difference among the intervals of days, toxoids and groups.

Results and Discussion

Neutralizing antibody titres produced in rabbits by beta and epsilon toxoids of *Clostridium perfringens* are presented in Table 1. It revealed that the neutralizing antibody titre against beta toxoid on the 14th and the 21st day post inoculation were $\log_{10}1.20$ and $\log_{10}1.50$ respectively. Similar responses were observed against epsilon toxoid. However against the beta and epsilon combined toxoid the neutralizing antibody titres were $\log_{10}0.90$ and $\log_{10}1.20$ on days 14th and 21st post inoculation, respectively. Statistically significant ($P<0.01$) rise of antibody production from 14th day to 21st day post inoculation of toxoid was observed and the highest titre was noted at the 21st day post inoculation. It is evident that the antibody titre of beta and epsilon toxoid are statistically similar when inoculated seperately while the titres were significantly lower when the combined toxoid was inocula-ted. The antibody production did not vary significantly due to interaction between day of inoculation and toxoid used. Detectable antibody titre in preimmunizing sera collected on day '0' and in controlled rabbits were not observed. The toxoids independently induced antibody responses in rabbits on the 21st day post-inoculation (Fig 1).

Table 1
Neutralizing antibody titres in rabbit antisera produced by beta and epsilon toxoids of *Clostridium perfringens* (n= 6).

Parameter	Antibody titre in $\log_{10}$		±SE
	Mean	Range	
14th day	1.100	0.800-1.300	±0.024
21st day	1.400	1.100-1.600	±0.024
Significant level	*0.01*		
Beta	1.350	1.100-1.600	±0.029
Epsilon	1.350	1.100-1.600	±0.029
Beta + Epsilon	1.050	0.800-1.300	±0.029
Significant level	*0.01*		
Beta at 14th day	1.200	1.100-1.300	± 0.042
Epsilon at 14th day	1.200	1.100-1.300	± 0.042
Beta + Epsilon at 14th day	0.900	0.800-1.000	± 0.042
Beta at 21st day	1.500	1.400-1.600	± 0.042
Epsilon at 21st day	1.500	1.400-1.600	± 0.042
Beta + Epsilon at 21st day	1.200	1.100-1.300	± 0.042
Significant level	*NS*		

n, No. of rabbits used in each toxoid inoculated group; NS, Not significant; SE, Standard error.

Verma (1986) injected subcutaneously aluminium hydroxide gel absorbed bacterin of *Clostridium perfringens* into albino rabbit for production of immunity. Correa and Taylor (1989) also used *Clostridium perfringens* bacterin vaccine subcutaneously to New Zealand white rabbit for the production of antisera and observed the antisera level by immunodiffusion test on 21st day of post inoculation. In this study, we used the beta and epsilon toxoids of *Clostridium perfringens* to investigate the validity of production of high titre antisera in rabbit by evaluating the immune response to the inoculated rabbit through agar gel immunodiffusion and mouse neutralization test. The results of the present study revealed that single dose of beta or epsilon toxoids induced

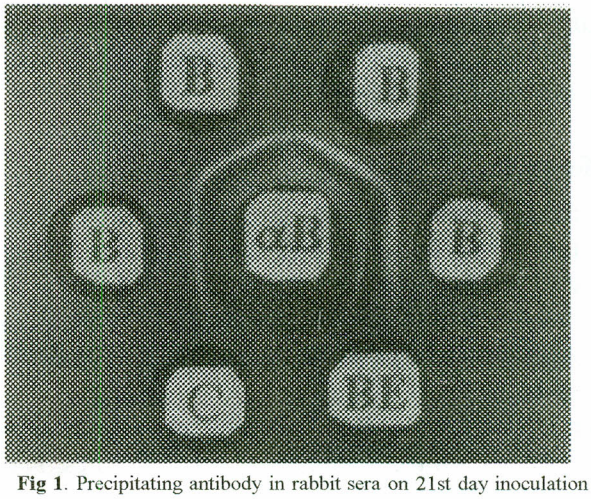

Fig 1. Precipitating antibody in rabbit sera on 21st day inoculation of toxoid by agar gel immunodiffusion test, where antisera against beta toxoid (αB), epsilon toxoid (B), beta and epsilon toxoid (BE) and control sera (C).

satisfactory ($P<0.01$) level of immune responses in rabbit on 14th day and 21st day post inoculation while the level of antibody was not satisfactory when inoculated in combination. This might be due to the antigenic competition of the two toxoids. Ripley (1983) used a neutralization test for detecting antibody titres against the *Clostridium perfringens* beta toxoid. Ebert *et al* (1999) also used mouse neutralization test to estimate immune response against the *Clostridium perfringens* beta and epsilon toxoids. The immune response against *Clostridium perfringens* is a controversial subject and different groups of workers have different claims (Ryskulov 1983; Udovicic *et al* 1983; Nilo and Clio 1985) besides the fact that the inactivated cultures and the toxoids possess weak immunogenic properties (Ispolatovskaya 1971). Verma (1987) also used *Clostridium perfringens* bacterin vaccine for the production of immune serum and determined primarily the immunoglobulin level in vaccinated rabbits at different intervals. He observed satisfactory level of immunoglobulin response in the 4th week. The difference in the results might be due to different factors like composition, and pH of the medium, time of incubation, patho-genicity of the organism, intervals of immunizing doses etc. (William and Chase 1967; Macario and Macario 1975; Gonzalez 1984). In agar gel immunodiffusion test, it was not possible to detect the antibody before the 21st day of inoculation of toxoids. Our result here regarding the immune responses in rabbit suggests the utility of this laboratory animal for production of high titre antisera against *Clostridium perfringens* beta and epsilon toxoids.

Acknowledgement

The authors are grateful to International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England, for the supplying of beta and epsilon toxoids of *Clostridium perfringens*.

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