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SEROLOGICAL STUDIES ON SOME CLOSTRIDIAL HYPERIMMUNE SERA

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Abstract

Hyperimmune sera against *Clostridium perfringens* types B and D were prepared in sheep. Sera were concentrated and refined by pepsin digestion method. Evaluation of sera by toxin neutralization test (TN) before and after refining revealed that there is great loss in the antitoxin titre during the process of purification. ELISA test may be used as alternative to the toxin neutralization assay for evaluation of clostridial antitoxins.

INTRODUCTION

Enterotoxaemia is a disease caused by *Clostridium perfringens* leading to a serious economic loss in cattle and sheep (Kennedy *et al.*, 1977). *C.perfringens* type B is responsible for lamb dysentery and the principle toxin produced is the beta toxin (Jansen, 1961). *C.perfringens* type D is the main cause of enterotoxaemia of sheep and lambs (pulpy kidney disease). Epsilon toxin is the main toxin, which is responsible for the disease (Worthington *et al.*, 1973). Active immunization against these diseases may be attained in older animals with toxoid of both types. Passive immunity can be induced in the newborn either by using the antitoxin or transferring the maternal antibody to the young. The antitoxin concentration in sera of vaccinated animals is measured by using toxin neutralization test in mice (British Pharmacopia, 1985). ELISA test is described as an alternative method to toxin neutralization assay (Wood, 1991). Hyperimmune serum is regarded as a short-term prophylactic treatment in animals, also used for different serological tests and evaluation of the vaccines. Since it is very difficult and more expensive to obtain these biological agents from the foreign countries, the purpose of this study is to prepare hyperimmune sera against *C.perfringens* types B and D in sheep, and evaluation of these sera by different serological methods to establish a line for production of these sera to be used as a standard sera in serological tests and titration of clostridial vaccines.

MATERIALS AND METHODS

Preparation of toxins

Beta and Epsilon toxins were prepared from culture filtrate of *C.perfringens* types B and D. The minimum lethal dose (MLD) and test dose of toxins were determined according to Gadalla *et al.* (1969).

Preparation of hyperimmune sera

Hyperimmune sera against beta and epsilon toxins of *C.perfringens* types B and D were prepared in sheep according to the method described by Odendaal *et al.* (1988). Two sheep were used for each type of toxin. Sheep were initially vaccinated with two doses of formalized alum precipitated toxoid of *C.perfringens* types B or D (obtained from Anaerobic Vaccine Department, Veterinary Serum and Vaccine Research Institute) 3 weeks apart. Four weeks later, they received another booster dose of the corresponding vaccine. Two weeks after boostering, four gradually increasing doses from 2 to 8 ml of beta and epsilon toxins, containing 4000 and 1000 MLD/ml respectively, were injected at 3 days intervals.

Collection of serum

Blood samples were taken after the 2nd and booster doses of vaccine and then after each toxin injection to measure the antitoxin level during the course of immunization. One week after the last dose of toxin injection, blood was collected from animals and serum was separated, pooled and stored at –20°C until used.

Concentration and purification of sera

C. perfringens ß and Σ antitoxic sera were refined by the pepsin digestion method described by Pope (1939). The refining process was kindly undertaken in the Egyptian Organization for Biological Products and Vaccines, Agouza, Giza.

Standardization of the antitoxic sera

Both ß and Σ antitoxins were assayed before and after refining by using toxin neutralization test according to the British Pharmacopia (1985) and ELISA test according to Wood (1991).

RESULTS AND DISCUSSION

Table 1. Beta and Epsilon antitoxin titers in sheep hyperimmune sera before and after refining.

Type of hyperimmune serum	Before refining		After refining	
	Serum volume	Titre	Serum volume	Titre
Beta	205 ml	2800 IU	74 ml	1800 IU
Epsilon	235 ml	190 IU	88 ml	98 IU

Table 2. Comparison between toxin neutralization (TN) test and ELISA assay for evaluation of beta and epsilon antitoxins in sheep hyperimmune sera.

Serum sample	Epsilon antitoxin titre IU/ml		Epsilon antitoxin titre IU/ml	
	TN	ELISA	TN	ELISA
After 2nd dose of vaccine	20	23.1	7	12.06
After booster dose of vaccine	96	115.4	35	55.44
After 1st dose of toxin	562	495.2	67	88.2
After 2nd dose of toxin	1125	1370.2	105	136.98
After 3rd dose of toxin	1920	2112.5	136	164.48
After the last dose of toxin	2800	3019.4	190	195.42

Figure 1. Results of evaluation of prepared beta hyperimmune serum by toxin neutralization test and ELISA assay

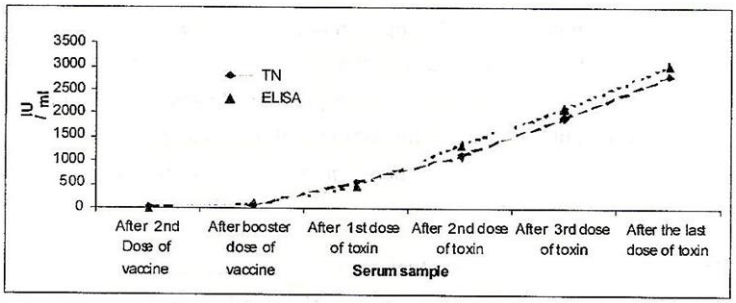
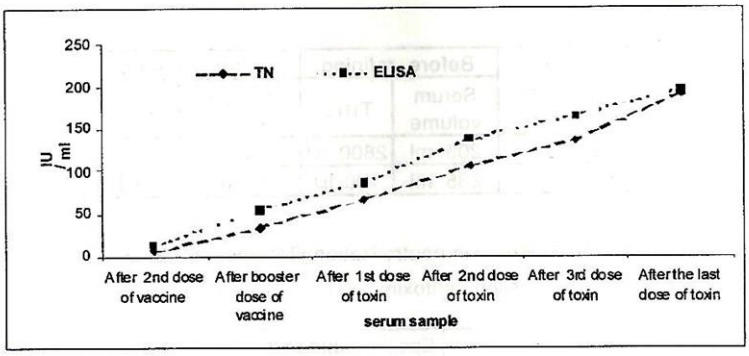


Figure 2. Results of evaluation of prepared epsilon hyperimmune serum by toxin neutralization test and ELISA assay.



The need of standard clostridial antisera, in department of Anaerobic Research and Vaccine production of Veterinary Serum and Vaccine Research Institute, for the application of different serological tests and for evaluating the potency of vaccine produced, together with its unavailability, pushed us to prepare hyperimmune sera against *C. perfringens* types B and D in sheep. Concentration and purification of the sera was made. The original volume of beta hyperimmune serum was 205 ml, and after the refining process it became 74 ml, while, the epsilon antiserum was 235 ml and became 88 ml after refining. The results of toxin neutralization test done on the sera before and after refining (Table 1) revealed that, although the volume of the sera was reduced by concentration, the beta antitoxin titre was decreased from 2800 IU/ml to 1800 IU/ml after the process of refining. Also, the epsilon antitoxin titre was reduced from 190 IU/ml to 98 IU/ml after refining. This loss of the antibody titre may be due to the action of the enzymatic activities during the refining process. These results were confirmed by Worthington and Mulders (1979) who stated that the usual procedures for the purification of gamma globulins against *C. perfringens* result in a great loss of antibody activity.

Correlation between results obtained by ELISA assay and toxin neutralization (TN) test was generally good for all assays (Table 2 and Figures 1&2). The benefits of replacement TN test by ELISA assay include the reduction in the use of great numbers of mice, which is of economic importance, and a reduction of time taken to achieve a potency result from 3-4 days to approximately 3 hours. These results agree with those obtained by Sojka *et al.* (1989), Wood (1991) and Makhareta *et al.* (1998) who re-

corded a good correlation between results obtained by both tests.

In conclusion, it is clear that, hyperimmune sera against *C.perfringens* types B and D can be prepared locally to be used as a prophylactic treatment and for different serological tests and evaluation of clostridial vaccines. Since the refining process by pepsin digestion method results in a great loss of antibody activity, it is necessary to investigate this problem and to use another method for purification of antitoxins. It is also clear that ELISA assay may be used as an alternative to the toxin neutralization test as an aid in evaluation of clostridial antitoxins.

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دراسات سيرولوجية على بعض الامصال المضادة للكلوستريديا

فتحية شافعي

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تم تحضير امصال عالية العيارية ضد الكلوستريديم برفرنجنز نوعي «ب» و«د» في الأغنام، وتم تركيز الامصال بإستخدام طريقة الهضم بإنزيم الببسين. وبمعايرة هذه الامصال بإستخدام إختبار السم المتعادل قبل وبعد عملية التكرير وجد ان هناك نقصاً كبيراً في معدل الأجسام المناعية أثناء عملية التنقية. كما وجد أن اختبار الإليزا يمكن إستخدامه كبديل لإختبار السم المتعادل في تقييم وقياس الأجسام المناعية في الأمصال المضادة للكلوستريديا.