Preparation of Standard *Clostridium perfringens* Antitoxin in the Sheep

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Summary

In Iran, strains of Clostridium perfringens *isolated from infected animals and specimens are typed by using antitoxins Type A, C and D. The procedure that lead to preparation, in the sheep, of standard Clostridium perfringens antitoxins Type A, C and D is described. The native breed of sheep used was highly tolerant to hyperimmunisation by different types of* Clostridium perfringens *toxins and produced hyperimmunne sera of very high titres*

Introduction

Antitoxins are necessary for typing strains of *Cl. perfringens* isolated from infected animals and specimens that are submitted to laboratories for determination. Horse and sheep are the animals that had been previously used for preparation of clostridial antitoxins by several workers(1,2,3,4). We intended to investigate the potentiality of Iranian native breeds of sheep in producing clostridial antitoxins. The results, that led to production and standardisation of Type A, C and D antitoxins of *Cl. perfringens*, are presented in this paper.

Materials and methods

Preparation of toxins: Toxigenic strains of *Cl. perfringens* Types A, C and D were used for preparation of toxins. Medium used for preparation of *Cl. perfringens* Type A, C and D toxins consisted of:

proteose peptone	3 %
$Na_2 HPO_4$	1 %

NaCl	0.25%
Glucose (for Type A and C)	1 %
Dextrine (for Type D)	1 %
Trace element and vitamin solution	0.7 %

The pH was adjusted to 7.5 for Type A and C; 8.2 for Type D. The prepared medium was autoclaved at 110°C for 30 minutes. Each type of

Cl. perfringens was cultured in a 500 ml flask containing 300 ml of the above medium for 5-6 h. After a period of incubation the cultures were centrifuged immediately and passed through sterile filter (EKS Seitz). A sample was removed from each type for determination of minimum lethal dose (MLD) in 18-20 Swiss white mice.

For determination of MLD of alpha, beta and epsilon toxins of

Cl. perfringens Type A, C and D several dilutions, from 1\10-1\200, 1\500-1\3000 and 1\1000-1\12000 were made of each. The results are shown in Table 1.

Hyperimmunisation schedule: Six healthy native sheep were selected for preparation of clostridial antitoxins. Each was given two injections of 2 ml enterotexemia polyvalent vaccine. Groups of sheep, 2 each, were selected for hyperimmunisation of Type A, C and D of *Cl. perfringens* toxins. Each group was injected, subcutaneously, 3 ml of *Cl. perfringens* Type A, C and D toxoid separately. Second and 3rd injections, of the same dose, were carried out at 3 weeks intervals(4). After seven weeks, each sheep was injected, intramuscularly at the thigh region, with 20 ml of *Cl. perfringens* Type A, C and D toxins . Six days later the second injections, intramuscularly at 2 sites, were carried with 40 ml of the toxins.

Two days later 3rd injections, intramuscularly at four sites, were carried out with 80 ml of the toxins(4). Seven days after completion of the cycle of immunisations, 300-500 ml blood was taken from each sheep. second, 3rd and 4th bleeding were also carried out at 3-day intervals (Table 2). At each bleeding, a total volume of 11,290 ml of blood was collected; 3,920 ml for Type A, 3,970 ml for Type C and 3,400 ml for Type D. Each type of *Cl. perfringens* serum was collected in a separate flask.

A sample was taken for determination of the level of antitoxin, 0.5% tricresol was added to the crude antitoxin, mixed well and stored at 4°C until use.

Concentration and Purification: The method described by Pope et al.(5) was used for purification and concentration of each type of collected crude antitoxin. Briefly, the crude antisera were dialysed against normal saline at pH 7.0 for 2 days at 4°C. To the final filtered product, 0.5% tricresol was added and samples were taken from each type of purified antiserum of *Cl. perfringens* for determination of the level of antitoxins. Aliquots of 10 ml were prepared, freeze-dried and sored at 4°C until use.

Standardization: For determination of the level of concentrated antitoxin of each type of *Cl. perfringens* dried *alpha*, *beta* and *epsilon* toxins were used.

The *alpha*, *beta* and *epsilon* toxins were standardised by International antiserum of *Cl. perfringens* Type A, C and D. The standard toxins were used for titration of the prepared concentrated *Cl. perfringens* Type A, C and D antiserum by serum-neutralisation test in 18-20 grams Swiss white mice (3).

Results

The minimum lethal dose of *Cl. perfringens* Type A, C and D toxins were 150, 3000, 12000 per ml, respectively. The titres of crude and purified antitoxin of each type of *Cl. perfringens* are summarised in Table 3. As the table shows crude titres of *alpha*, *beta* and *epsilon* antitoxins were 10, 3,500 and 65 international units per ml, respectively. After concentration and purification the levels increased to 40, 6000 and 600 for Type A, C and D, respectively. The prepared sera have been successfully used for identification of *Cl. perfringens* organisms isolated from animals and specimens received from different parts of Iran.

Discussion

There are few papers concerning preparation of standard *Cl. perfringens* in the sheep(2,4). This study proved that the sheep could be successfully used, instead of the horse, for preparation of Clostridial antitoxins. The native breed of sheep used in our work tolerated very well the injections from 20

Cl. perfringens Type A antitoxin was supplied by Statens Serum Institute, Copenhagen, Denmark. Type C and D antitoxins were obtained from Central Veterinary Laboratory, UK

ml up to 80 of high titre *Cl. perfringens* Type A, C and D toxins. The sheep responded well to *Cl. perfringens* antigens. After concentration and purification high level *Cl. perfringens* antitoxins, in comparison with the International Standard, were obtained. Other pathogenic clostridial antitoxins could be prepared by using the methods described in the communication.

Table 1. Determination of minimum lethal dose of *Cl. perfringens* Type A, C and D

Type	Minimum lethal dose per ml
A	150
C	3000
D	12000

Table 2. Bleeding of hyperimmunised sheep

Number of sheep collected	Cl. perfringens immunised	Cycle of types	Total volume bleeding	Serum
2	Type A	1st 2300 ml 2nd 1620 ml	3920 ml	1400 ml
2	Type C	1st 2150 ml 2nd 1820 ml	3970 ml	1400 ml
2	Type D	1st 1200 ml 2nd 2200 ml	3400 ml	1200 ml

Table 3. Titres of sera in International Units

Cl. perfringens	Number of sheep	Titres of sera in International units /ml	
		Hyperimmunisation	Concentration of pooled serum
Type A	2	10 Int. unit/ml	40 Int. unit per ml
Type C	2	3500 Int. unit/ml	6000 Int. unit per ml
Type D	2	65 Int. unit/ml	600 Int. unit per ml

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