IMMUNE RESPONSE OF MODIFIED POLYVALENT CLOSTRIDIAL VACCINE

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ABSTRACT

Three different vaccinal formulations were evaluated by administration to experimental rabbits comprising alhydrogel adsorbed toxoids vaccine (V.A) , Alhydrogel (Aluminuium hydroxide gel) adsorbed vaccine containing epsilon protoxoid and treated with L-lysine (V.B) and the alum precipitated vaccine (V.C). It was found that Alhydrogel adsorbed vaccine containing epsilon as protxoid treated with L-lysine resulted in a comparatively higher antitoxic titers for beta and epsilon toxoids of ***C. perfringens*** types B and D (V.B) than the other two vaccines.

INTRODUCTION

Clostridial diseases are characterized by sudden onset, short disease fade, and high fatality rate which make the probability of treatment success become at minimal level. Enterotoxaemia caused by ***C.perfringens*** is considered to be the worst killer disease in sheep, cattle and goat and can result in serious economic losses (**Hatheway, 1990; Kumar *et al.*, 1992; Uzal *et al.*, 1994; Manteca *et al.*, 1999; and Manteca *et al.*, 2001**). The disease occurs due to the production of major lethal toxins as Beta (β) and Epsilon (Є) which are produced by ***C. perfringens*** type B and D respectively. Epsilon toxin is responsible for a rapidly fatal enterotoxaemia called pulpy kidney or overeating disease in sheep **Timoney *et al.*, (1988)**. The toxin is normally produced as relatively inactive protoxin (non toxic) which is activated by proteolytic enzymes such as trypsin and chemotrypism to be converted to the active lethal form (**Habeeb *et al.*, 1973; Worthington and Maria, 1977; and Mc Donel. 1986**). Beta toxin (β) is the causative agent of dysentery (**Dailing, 1926; Tunnicliff, 1933; and Frank, 1956**). The beta toxin is a highly trypsin-sensitive protein (**Sakurai and Duncan, 1978; Jolivet-Reynaud, 1986; Sakurai and Fujii, 1987; Bougo *et al.*, 1995 and Minami *et al.*, 1997**). The majority of clostridial vaccines being prepared and used as multicomponent formulations which commercially are

multivalent vaccines typically consisting of inactivated cells, toxin or both bacterin and toxoid (**Brown *et al.*, 1976; Hjerpe, 1990; and walker, 1992**). Recently the toxoid vaccines have been developed for sheep and goats (**Uzal and Kelly, 1998; and Uzal *et al.*, 1999**) and for cattle (**Stokka *et al.*, 1994; and Troxel et al, 1997**) and also for buffaloes (**Rahman *et al.*, 2001**).

The currently local produced polyvalent clostridial vaccine is consisted of toxoids of ***C. perfringens*** types B and D (beta and epsilon toxins), ***C. oedematiens*** type B (alpha toxin) ***C. septicum*** (alpha toxin) and toxoid of ***C. tetani*** toxin while the ***C. chauvoei*** component is added as whole culture. This study was planned to improve the efficacy of the currently produced polyvalent clostridial vaccine through changing different parameters as using of the epsilon protoxoid instead of toxoid and using of alhydrogel as an alternative adjuvant instead of alum and comparing the immune response of these modified vaccine formulations with the immune response induced by the currently routinely used alum precipitated vaccine in rabbits.

MATERIAL AND METHODS

Preparations of clostridial cultures and toxins:

Cultures and toxins of ***C .perfringens*** types B and D, ***C. septicum, C. oedematiens*** type B and ***C. chaucoei*** were prepared according to **Gaddalla *et al.*, (1974)** while ***C. tetani*** toxin was prepared according to **Rijks Instruction, (1980)**. ***C. perfringens*** type D culture was divided into two parts, trypsin was added to the first part in 0.05% concentration according to **Fathia Shafie *et al.*, (2003)**, while the other part left without activation with tryspin. 0.5 % formalin was added to each prepared culture and toxin for inactivation. After toxoiding process was completed, each prepared toxoid was further divided into two parts, L.lysine was added to the first part of each prepared toxoid and to the prototoxiod formulation in 1mM final concentration according to **Percival *et al.*, (1990)** to stop the reaction of formalin. Other part of each toxoid was left without L.lysine treatment. Inactivated cultures and toxoids were separated and concentrated by using ultrafilteration system (Millipore Corporation USA). Merthiolate was added in 1: 1.0000 final concentration as preservative. Alhydrogel (aluminium hydroxide gel) was added as adjuvant to the concentrated toxoids of the first part and to the protoxoid formulation in 20% concentration according to **EL-Sehemy *et al.*, (2004)**, while potassium aluminium sulfate (alum) was added in 1% concentration to the another part of the prepared toxoids of each type.

Preparation of polyvalent clostridial vaccines:

Three vaccinal formulations were prepared with diffent treatments as follow:

Vaccine (A); It is a polyvalent clostridial vaccine prepared from clostridial toxoids mixtures with whole culture of ***C. chauvoei*** treated with L-lysine and adjuvanated with alhydrogel. While the Vaccine (B) containing clostridial toxoids of all components except in case of ***C. perfringens*** type D it contained the epsilon protoxoid instead of epsilon toxoid with whole culture of ***C.chauvoei*** and L-lysine and adjuvinated with alhydrogel . The last one, vaccine (C) was the routinely prepared polyvalent clostridial vaccine alum precipitated vaccine. Sterility and safety tests were carried out according to the regulation of **British veterans Codex, (1970)**.

Vaccination Schedules:

Three groups, each of 10 Boscat rabbits (2.5-3kg) were vaccinated with each vaccine (A,B and C) Each animal in each group was injected S/C with two doses of 5 ml and 3 ml at three weeks apart from each vaccine. All groups were bled before vaccination and then two weeks post-boostering and polled sera of each group was kept in refrigerator until used.

Antitoxin assays:

The antitoxic values expressed in (IU/ml) were determind in Swiss white mice by serum neutralization test (SNT) as described by *Brithish veterinary pharmacopiea, (1985)* for all clostridial antitoxins except ***C. tetani*** antitoxin which was tiatrated according to *Rijks Instruction, (1989)* while antibodies against ***C.chauvoei*** were determined by plate agglutination test according to **Claus and Macheak, (1972)**.

RESULTS AND DISCUSSION

In the present study, three multicomponent clostridial vaccines, each containing six antigens were prepared with different formulations. The immunogenic responses of these different formulated vaccines in experimental rabbits were evaluated and compared as shown in the table.

The results revealed that the vaccine (B) which is the polyvalent clostridial vaccine which comprising epsilon as prototoxid and treated with L-lysine as formalin inhibitor and incorporated an alhydrogel as an adjuvant induced the highest antitoxic titres for beta and epsilon toxoids of ***C. perfringens*** types B and D than alhydrogel adsorbed vaccine (V.A) as well as alum precipitated vaccine (V.C). These results agreed with **Percival *et al.*, (1990)** as they used formalinized epsilon protoxoid for immunization of mice and rabbits and indicated that it afforded a good degree of protection.

Also agree with **Worthington *et al.*, (1973)** who indicated that the purified epsilon protoxoid had the properties of typical protein. The significantly highest titre of beta toxoid induced by (V.B) than the other two vaccines (V.A) and (V.C), come in agreement with the finding of **(Jolivet Reynayd, 1986; Buogo *et al.*, 1995; and Minami *et al.*, 1997)** who proved that the beta toxin is highly- trypsin sensitive protein and the trypsin decrease its antigenicity as a vaccine component. Therefore in the formulation of vaccine (B), the use of trypsin as epsilon activator was avoided so the harmful effect of trypsin on beta toxoid was prevented.

To avoid the deteriorating effect of formalin on inactivated antigens L-lysine was added to stop formalin reaction , which had a good effect on the immunity of animals as shown in the table. This result have been confirmed with the finding of **Percival *et al.*, (1990)** who indicated that the addition of L-lysine had a remarkable degree on integrity of the antigenic components of the vaccine and thus maximize their immunogenicity.

It was clear that alhydrogel adjuvant vaccine induced a good immune response for most antigenic vaccine components. These results agree with the finding of **(Hepple, 1967; and Terry *et al.*, 1967)** as they used alhydrogel as an adjuvant for epsilon toxoid and proved that the epsilon toxiod antigen is released from alhydrogel over relatively short period in addition to its immuno-stimulant effect. Also agree with **(Freriches and Gray, 1975; Kerry and Craig, 1979; Blackwell *et al.*, 1983; Farrage *et al.*, 1984; Webester and Frank, 1985; and Green *et al.*, 1987)** who compared the immune response of sheep, goats, rabbits and G. pigs to a number of various batches of multicomponent clostridial vaccines with different formulations, they found that alhydrogel adsorbed vaccine induced a good immunogenic response for all vaccine components. Also agree with **(Verma, 1986; and EL-Sehemy *et al.*, 2004)** as they used alhydrogel as an adjuvant for preparation of enterotoxaemia vaccine for rabbits caused by ***C.perfringens*** type A, and they found that the vaccine was safer, potent and confer good degree of protection and with less local reaction when compared with that of alum.

From above mentioned results, it could be concluded that preparation of polyvalent clostridial vaccine including epsilon as protoxoid, treated with L-lysine as formalin inhibitor and incorporating an alhydrogel as an adjuvant increase its efficacy and antigenicity for all its components.

In conclusion, the best formulation for polyvalent clostridial vaccine is the use of epsilon in the protoxoid form and treatment of the formalized cultures with L-Lysine as formaline inhibitor followed by incorporation into alhydrogel as an adjuvant, could increase the antigenicity and the immunogenicity of its internal component.

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Table (1): Antitoxic values of pooled rabbit sera vaccinated with different formulations of polyvalent clostridial vaccines.

Vaccine used	Antiloxin titre (IU/ml)					Agglutination titre of *C.chauvoei* ML/ml
	C. perfringens		*C.oedemations*	*C. Septicum*	*C.tetani*	
	Beta	Epsilon	Alpha	alpha		
Prevaccination samples	Non-detectable	Non-detectable	Non-detectable	Non-detectable	Non-detectable	Non-detectable
A	40	5	5	2.5	20	0.05
B	50	8	5	2.5	20	0.05
C	40	3	5	2.5	20	0.05

A=Alhydrogel adsorbed toxoids vaccine treated with L-lysine.
B= Alhydrogel adsorbed vaccine containing epsilon protoxoid, treated with L-lysine .
C= Alum precipitated vaccine (routine used).
* Agglutination titre is defined as the number of microliters (MC) reqires to provide defense agglutination (75%) of 0.03 ml of standardized antigen.

الملخص العربى

الاستجابة المناعية للقاح الكلوستريديا الجامع المطور

تم حقن ثلاثة لقاحات من اللقاح الجامع المحضرة بمعالجات مختلفة فى الأرانب وتشمل لقاح جامع يحتوى على توكسيدات الكلوستريديا ومرسب بالألومنيوم هيدروكسيدجيل لقاح (أ) و اللقاح الجامع والذى يحتوى على سم الابسيلون الخام (بروتوكسيد) بالاضافة الى توكسيدات الكلوستريديا المختلفة مضاف اليه مادة ال ل-ليسين ومرسب بمادة الألومنيوم هيدروكسيد جيل (كمحسن) واللقاح الثالث الجامع الروتينى والمرسب بالشبة.

وجد أن اللقاح الجامع الذى يحتوى على سم الايسيلون الخام (بروتوكسيد). والمعالج بمادة ال-ليسين والمرسب بالإلومنيوم هيدروكسيد جيل كمحسن يعطى رد فعل مناعى لكل توكسيدات البيتا والإبسيلون للكلوستريديم بيرفرنجينز نوع ب و د عن اللقاحين الآخرين.