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Improvement of Polyvalent Clostridial Vaccine

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SUMMARY

Rabbits, sheep and cattle were vaccinated with a prepared polyvalent clostridial vaccine adjuvenated with either alum or gel after addition of alpha toxoid of *C. perferingens* type A to polyvalent vaccine. The immune response of rabbits and sheep to the different components of the two vaccines was higher than these in cattle. The antitoxic values of rabbits, sheep and cattle for alpha and beta toxoid of *C. perfringens* type A and B, alpha toxoid of *C. septicum* and alpha toxoid of *C. novyi* type B in rabbits, sheep and cattle were higher in gel vaccine than in alum one, but the response to epsilon toxoid of *C. perferingens* type D was the same in both vaccines. Also the immune response of tetanus toxoid of *C. tetani* antigens and antigens of *C. chauvoei* bactrein and toxoid were higher in alum vaccine than in gel one. Finally both vaccines (alum and gel adjuvenated) gave satisfactory antibody titres which were higher than the minimum protective levels for all antigen components of both improved polyvalent clostridial vaccine.

INTRODUCTION

Clostridial diseases have been a concern of sheep and cattle producers for many years, because these diseases are often rapidly fatal and usually affect cattle from six months to two years of age (Troxel *et al.*, 1997). Enterotoxaemia caused by *C. perfringens* types A, B and D is characterized by high fatality rate, sudden death and lesions of necrotic and hemorrhagic enteritis (Popoff, 1990; Manteca and Duab, 1994; Songer, 1996 and Manteca *et al.*, 2000, 2001). Black-quarter disease is characterized by severe myositis and toxaemia which is caused by *C. chauvoei* and *C. septicum* (Williams, 1977; Blood *et al.*, 1983 and Harwood, 1984). Black disease of sheep due to *C. novyi* type B causing deaths due to toxaemia is caused by alpha toxin produced by the organism in the

liver. In Egypt, Tetanus is more common disease of all species of domestic animals caused by the toxin of *C. tetani* (El Nahas, 1960). Controlling of these diseases by active immunization is of a considerable importance (Freriches and Gray, 1975; Webster and Frank, 1985 and Rahman *et al.*, 1998). Vaccination against clostridial diseases has been practiced for many years in sheep (Blackwell *et al.*, 1983) and cattle (Stokka *et al.*, 1994). The conventional locally produced polyvalent clostridial vaccine is used only for sheep in Egypt (El-meneisy *et al.*, 2004). Polyvalent clostridial vaccine containing six toxoids mixtures protected sheep against *C. chauvoei*, *C. septicum*, *C. novyi* type B, *C. perfringens* type B, and D and *C. tetani*. The effectiveness of immunization depends on several factors as type of vaccine, route or site of vaccination and adjuvant used (Chirase *et al.*, 2001). Adjuvant have been used to enhance the immune response of veterinary vaccine for many years. The advantage of using adjuvant in the vaccine mixture is that smaller quantities of the antigen are usually required to stimulate good response (Andrew *et al.*, 1996 and Gaddalla *et al.*, 1971) used potassium aluminum sulphate (alum) as an adjuvant for a polyvalent clostridial vaccine. Aluminum salts have been used for many years in both human and veterinary vaccines. The only problem with their use has been the formation of persistent granulomes when products containing high level of aluminum were administered subcutaneously (El-Meneisy *et al.*, 2005). Aluminum hydroxide gel adjuvant vaccine induced good immune response for most antigenic vaccine in addition to its immuno-stimulant effect (Abdallah *et al.*, 2005).

So the aims of this work are to fulfill the following points:-

1- Adding of alpha toxoid of *C. perfringens* type A to the currently produced polyvalent clostridial vaccine.
2- Addition of two adjuvants to the prepared polyvalent clostiridial vaccine

which are alum and aluminium hydroxide gel.

3- Determining the immune response of these two vaccines in rabbits, sheep and cattle.

MATERIALS AND METHODS

Vaccine preparation

Polyvalent clostridial vaccine containing five toxoid components of *C. septicum*; *C. perfringens* types A & B and D and *C. novyi* type B and whole culture of *C. chauvoei* were prepared as described by (Gaddalla *et al.*, 1974) while tetanus toxoid was prepared according to instruction of Rijks institute (1980). Equal amounts of these components were mixed together except tetanus toxoid which was added as 25

LF/dose. This mixture of antigens was divided into two parts and formulated with two different types of adjuvant.

1- Potassium aluminum sulphate (Alum) at 1% according to (Gaddalla *et al.*,1974)
2- Aluminum hydroxid gel (gel) was added at 20% concentration according to (El-Sehemy *et al.*, 2004)

The two types of vaccine were subjected to sterility and safety test before using them in immunization according to (The European Pharmacopeia, 2001).

Vaccination schedules.

Two groups of rabbits (10 rabbits for each), sheep and cattle (five animals for each) were injected as follow:

1- The first group of rabbits, sheep and cattle were injected with polyvalent vaccine adjuvenated with alum.
2- The second group of rabbits, sheep and cattle were injected with polyvalent vaccine adjuvenated with gel.

Rabbits and sheep received two doses of 3 ml of the vaccine, three weeks apart, while cattle were injected with two doses of 5 ml, three weeks apart.

Antitoxin Assay.

Blood samples were collected from each animal of each group separately before immunization and then two weeks after the second dose. Sera of each group of animals were pooled and tested for detection of antibodies against all antigens of both vaccines component by using serum neutralization test (SNT) in Swiss white mice. The antitoxin values for these components were expressed in international units (IU) as described by British Veterinary Pharmacopeia (1985). Clostridium tetani antitoxin was titrated according to the Rijks institute protocol (1989), while antibody against C. chauvoei was determined by plate agglutination test according to Claus and Macheak (1972).

RESULTS AND DISCUSSION

Clostridial diseases are characterized by sudden onset, short disease fade and high fatality rate which make the probability of treatment success at minimal level (Abdallah, *et al* 2005). Such diseases cause losses among sheep, goat and cattle flocks. Polyvalent clostiridal vaccine is widely recommended as prophylaxix against such diseases caused by clostiridial species (Alaa, 1996). The prepared polyvalent clostiridial vaccine containing six antigens achieved the most suitable requirements to produce high yield of antibodies (Gaddalla, *et al.*, 1974).

Alpha toxoid of *C. perferingens* type A was added to the routinely produced polyvalent clostiridial vaccine to increase the spectrum of the vaccine in prophylaxis of the susceptible hosts against enterotoxaemia. Part of this vaccine was adjuvenated with gel, while the other part was adjuvenated with

alum. The two vaccines were evaluated in rabbits, sheep and cattle. The immunogenic response of gel and alum vaccines in rabbits as shown in Table (1) revealed that the antibody titre of alpha and beta antitoxin of *C. perferingens* types A and B, alpha antitoxin of *C. novyi* type B and alpha antitoxin of *C. septicum* were higher in gel vaccine than in alum one. These results agree with (Mario, 1969) who mentioned that alum precipitated Vaccine gave poor result than aluminium hydroxide gel vaccine. The results also show that antibody titre of epsilon antitoxin of *C. perferingens* type D was nearly the same in both gel and alum vaccines. The antibody titer of antitoxin of *C. tetani* and agglutination titre of *C. chauvoei* were higher in alum vaccine than gel one. These results agree with (Grigoriu, *et al.*, 1965) who stated that the superiority of alum precipitated vaccine to that concentrated with aluminium hydroxide gel.

From the obtained results it was noticed that the two different adjuvenated vaccines induced good antibody response to each corresponding group of rabbits for all vaccine components.

Table (2) illustrated the results of immune response of sheep vaccinated with polyvalent vaccine adjuvenated with either gel or alum. The same results of rabbits were obtained in sheep respectively in addition the antibody levels for all antigen vaccine components were higher in sheep than those of rabbits. However both antibody titres in rabbits and sheep for all antigen vaccine component were higher than the level required for protection.

The results obtained in Table (3) showed that the response of cattle against polyvalent clostiridial vaccine adjuvenated either with gel or alum is less than those obtained in rabbit and sheep for all antigen vaccine component but it achieved antibody level more than the minimum protective level { The minimum protective level for *C. perferingens* types B and D and *C. septicum* was 0.25 IU/mL (Cooper, 1976 and Oxer, *et al* 1971) ; for *C. perferingens* type A is 0.1 IU/mL (Weipers, *et al* 1964); for *C. novyi* and *C. tetani* it is 0.5 IU/mL and 0.1 IU/mL, respectively (Macheak, 1976) and for C. chauvoei it is 2.0 uL (Farrag, 1975). This results agree with (El-Meneisy, *et al.*, 2004) who found that the immune response of cattle for polyvalent vaccine was less than that of sheep.

It could be concluded from the present study that the addition of *C. perferingens* type A toxoid to the routinely produced polyvalent clostiridial vaccine to protect the susceptible animals aginst enterotoxaemia is more useful and beneficial. In the same manner both adjuvants (gel and alum) can be used with the polyvalent clostiridial vaccine as both gave satisfactory results in different animals.

Table (1):- Antibodies titers in sera of Rabbits vaccinated with different adjuvenated Polyvalent clostiridial vaccine

Antibody titer of polyvalent clostiridial vaccine in Rabbits IU/mL							
Type of Adjuvant	Alpha antitoxin of C.P.A	Beta Antitoxin of C.P.B	Epsilon antitoxin of C.P.D	Alpha antitoxin of C.N.B	Antitoxin of *C. tetani*	Alpha antitoxin of C.S.	Aglutt. titer of C.Ch (Ul)
Gel Vaccine	2	12	3	3.5	4	3	0.1
Alum Vaccine	1.5	10	3.5	3	5	2	0.05

C.P.A. : *C. perfringens* type A
C.P.B. : *C. perfringens* type B
C.P.D. : *C. perfringens* typed
C.N.B. : *C. novyi* type B
C. S. : *C. septicum*
C.T. : *C. tetani*
C. Ch. : *C. chauvoei*
Agg. : agglutination

Table (2):- Antibodies titers in sera of Sheep vaccinated with different adjuvenated Polyvalent clostiridial vaccine

Antibody titer of polyvalent clostiridial vaccine in Sheep Iu/mL							
Type of polyvalent vaccine	Alpha antitoxin of C.P.A	Beta Antitoxin of C.P.B	Epsilon antitoxin of C.P.D	Alpha antitoxin of C.N.B	Antitoxin of *C. tetani*	Alpha antitoxin of C.S.	Aglutt. titer of C.Ch (Ul)
Gel Vaccine	2.5	17	7	5	7	4	0.04
Alum Vaccine	2	15	7	4	9	3	0.02

Table (3):- Antibodies titers in sera of Cattle vaccinated with different adjuvenated Polyvalent clostiridial vaccine

Antibody titer of polyvalent clostiridial vaccine in Cattle IU/mL							
Type of polyvalent vaccine	Alpha antitoxin of C.P.A	Beta Antitoxin of C.P.B	Epsilon antitoxin of C.P.D	Alpha antitoxin of C.N.B	Antitoxin of *C. tetani*	Alpha antitoxin of C.S.	Aglutt. titer of C.Ch (Ul)
Gel Vaccine	1.5	9	3	3	3	2	0.5
Alum Vaccine	1	7	3	2	4	1	0.1

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تطوير لقاح الكلوستريديا الجامع

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تم تحصين مجموعات من الأرانب و الأغنام و الماشية بلقاح الكلوستريديا الجامع المضاف اليه كل من المحسنين المناعيين الجل و الشبة و ذلك بعد إضافة توكسيد الألفا المحضر من الكلوستريديم برفرينجنز نوع أ الى اللقاح الجامع. وقد وجد أن الإستجابة المناعية لكل من الأرانب و الأغنام تجاه جميع المكونات الأنتيجينية للقاحى الجل و الشبة كانت أعلى من نظيرتها فى الماشية. كما وجد أن الإستجابة المناعية لكلا من الأرانب و الأغنام و الماشية تجاه كل من توكسيد الألفا و البيتا للكلوستريديم برفرينجنز نوعى أ و ب و الألفا للكلوستريديم سيبتكم و الألفا للكلستريديم نوفياى نوع ب كانت أعلى فى اللقاح المحسن بالجل عن نظيره المرسب بالشبة بينما كان هناك تقارب فى النتائج الخاصة بأنتيجين الكلوستريديم برفرينجنز نوع د. كما وجد ايضا أن الإستجابة المناعية تجاه توكسيد التيتانوس و أنتيجين الكلوستريديم شوفياى كانت أعلى فى اللقاح المرسب بالشبة عن اللقاح المحسن بالجل. فى النهاية وجد أن جميع المكونات الأنتيجينية للقاحى الجل و الشبة أعطت أجسام مناعية عند مستوى جيد و كانت أعلى بكثير من أدنى مستوى مطلوب للحماية.