The effectiveness of bivalent clostridial and pasteurella combined vaccines in the laboratory animals

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A B S T R A C T

Two types of vaccines were prepared. The first one is a bivalent clostridial vaccine (*C. chauvoei* and *C. septicum*). The second vaccine is a combined vaccine from *C. chauvoei*, *C. septicum*, *P. multocida* type A and D, and *M. haemolytica*. The effectiveness of both bivalent clostridial vaccine and pasteurella combined vaccine was investigated in guinea pigs, rabbits and mice. To the bacterial immunity of C. chauvoei component, the immune response of guinea pig was conducted. The results revealed that both types of vaccines (bivalent clostridial vaccine and combined one) gave 100% protection for guinea pigs, when challenged with up to 128 MLD spore suspension. The immune response of the prepared vaccines was investigated in rabbits based on the mean antitoxin titer for *C. septicum*. The results revealed that the both vaccines showed almost similar antibody titer. The immune response of mice was evaluated based on challenge test for estimation of bacterial immunogenicity of *P. multocida*. The results revealed that there was no difference in log protection in both vaccines.

Keywords: *Clostridium chauvoei*, *Pasteurella multocida*, challenge test

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1. INTRODUCTION

Development of effective multi-component clostridial vaccines and their widespread use by farmers resulted in marked reduction in losses from clostridial diseases in sheep of all ages (Hussein et al., 2000). Pasteurellosis remain one of the most significant causes of losses in sheep population (Gilmour 1978).

The control of pneumonic pasteurellosis that conducted by vaccination with specific monovalent vaccines is consuming considerable time, labor and operational cost. Many authors recommended the use of a combined vaccine against some infectious diseases in cattle that revealed good immunity as well as single vaccine (Cooper 2011). The simultaneous immunization of farm animals against aerobic and anaerobic infections gave good immunity (Burdov 1962; Darie et al., 1979). Moreover, simultaneous immunization of lambs against anaerobes, anthrax and FMD induced strong immunity against these diseases (100% protection) (Joseph and Hedger 1984). As well as, the immunity conferred by combined vaccine of blackleg and hemorrhagic septicemia was similar to that obtained by each vaccine alone (Ghanem 1987).

The present work the effectiveness of both clostridial and pasteurella combined vaccines was investigated in guinea pig rabbit, and mice.

2. MATERIALS AND METHODS

2.1. Laboratory animals:

2.1.1. Guinea pigs:

A total number of 90 guinea pigs weighted 350-450 gm were used for evaluation of potency of *C. chauvoei* in the prepared vaccines, passage of *C. chauvoei* strain to augment their virulence, evaluation of spore suspension of *C. chauvoei* and also in safety test of prepared vaccines.

2.1.2. Rabbits:

Twelve boskat rabbits, their weight ranging from 2.5-3 kg were used for evaluation of C. septicum toxoid-containing vaccines.

2.1.3. Mice:

Swiss white mice of about 18-22 gm body weights were used for evaluation of potency of *P. multocida* types (A and D) vaccine and also used in the safety test of prepared vaccines.

2.2. Preparation of the Vaccines

Three aluminum hydroxide gel vaccines were prepared

a) Bivalent Clostridial vaccine (*C. chauvoei* and *C. septicum*). It was prepared according to Gadalla et al., (1974)

b) Pasteurella vaccine: *P. multocida* type (A and D) and *M. haemolytica* type A. It was prepared according to Confer (1993).

c) Combined vaccine of bivalent clostridial vaccine and pasteurella vaccine (*P. multocida* type (A and D) and *M. haemolytica* type (A)). It was prepared according to Ghanem (1987).

All vaccines were subjected to sterility and safety testing before using them in immunization according to British Veterinary Pharmacopoeia (2010).

2.3. Preparation of Antigens

a) Preparation and titration of *C. chauvoei* spore suspension. It was prepared according to (Cooper et al., 1960)

b) Preparation and titration of *P. multocida* (type A and D). It was prepared according to (Ose and Muenster (1968).

2.4. Evaluation of the prepared vaccines

2.4.1. Challenge test in guinea pigs using C. chauvoei spore suspension

It was carried out to evaluate the vaccines containing *C. chauvoei* based on challenge assay in guinea pigs according to British Veterinary Pharmacopoeia (2010). Forty five guinea pigs were divided into 3 groups, each of 20 in group (1and 2) and 5 in group (3). Group (1) was vaccinated with Bivalent *C. chauvoei* and *C. septicum* vaccine in 2 doses (2ml/dose) with 14 days interval. Animals were subdivided into 5 subgroups each of 4 guinea pigs. The five subgroups were challenged with 16, 32, 64, 128 and 256 MLD of *C. chauvoei* spore suspension respectively, after 21 days from 2nd dose. Group (2) was vaccinated with combined Pasteurella and Bivalent Clostridial vaccine, in 2 doses (2ml/dose) with 14 days intervals. Animals were subdivided into (5) subgroups each of 4 guinea pigs. The five subgroups were challenged with 16, 32, 64, 128 and 256 MLD of *C. chauvoei* spore suspension respectively, after 21 days from 2nd dose. Group (3) (control group) was inoculated with 0.1 ml of 1 MLD intramuscularly with *C. chauvoei* spore suspension.

2.4.2. Challenge test in rabbits using C. septicum toxin

Twelve boskat rabbits were divided into three groups, each of five animals in groups (1and 2) and 2 in group (3). Group (1) was vaccinated with bivalent *C. chauvoei* and *C. septicum* vaccine in 2 doses (2ml/dose) with 21 day intervals. Blood samples were taken after 14 days from the 2nd dose. Group (2) was vaccinated with combined Pasteurella and bivalent Clostridial vaccine in 2 doses (2 ml/dose) with 21 day intervals. Blood samples were taken after 14 days from the

2nd dose. Group (3) was kept unvaccinated as control group. SNT was applied on serum samples for determination the antitoxin titer against *C. septicum* toxin.

2.4.3. Mouse protection test

This test was performed to evaluate the potency of Pasteurella monovalent and polyvalent vaccines. The test performed according to Ose and Muenster (1968) as follows: Groups of ten mice each were inoculated S/C with 0.2 ml of the prepared vaccines per mouse with 2 doses, 21 days apart and challenged 10 days after last dose. Challenge exposure consisted of S/C inoculation of 0.1 ml/mouse of dilution of *P. multocida* strain grown on tryptose broth. Dilutions of challenge inoculations ranged from 10-1 to 10-10. Non-vaccinated control group was challenged. Mice were observed each day for 7 days after challenge exposure, and mortality was recorded. The LD_{50} was calculated according to Reed and Muench (1938) for each group of mice based on the accounted death on the 7^{th} day. The LD_{50} of the vaccinated mice was compared with the LD_{50} of the non-vaccine mice. At least 2.0 log protections were needed for a vaccine to be approved.

2.5. Statistical Analysis

The result of ELISA in all experiments was analysis with t-test according to (Freund 2001).

3. RESULTS

3.1.Evaluation of the prepared vaccines

a) Safety test

The safety test was carried out in guinea pig and mice. The results revealed that all the inoculated animals with different vaccine formulas were survived all over the observation period (7 days).

b) Sterility test

No growth was detected after inoculation of the prepared vaccine formulas in nutrient agar, cooked meat broth, thioglycolate broth, nutrient broth and sabouraud agar media. These results indicated that the vaccines were free from any bacterial, mycoplasma, or fungal contamination.

3.2. The protective effect of the prepared vaccines in guinea pigs

The results revealed that both vaccines (bivalent clostridial vaccine and combined vaccine) gave 100% protection for guinea pigs, which challenged with up to 128 MLD spore suspension. All control animals (Group 3) were died within 18-24 hours after inoculation with 1 MLD spore suspension and *C. chauvoei* was re-isolated from their heart blood (Table 1).

3.3. The protective effect of the prepared vaccines in rabbits

The results revealed that the mean antitoxin titer for *C. septicum* in group (1) and group (2) was 3.68 IU/ml and 3.34 IU/ml, respectively, while serum samples of control group had a zero titer. Analysis of the data by t-test revealed that there was no significant difference between the both groups vaccinated by bivalent Clostridial and combined vaccines (Table 2).

3.4. The protective effect of the prepared vaccines in mice

The log protection of mice challenged with *P. multocida* type (A and D) were within protection level starting from day- 30 till day-180 post vaccination. Analysis of results by t-test revealed that there was no difference in log protection between pasteurella and combined vaccines (Table 3).

Table (1): Surviving rate of g. pigs vaccinated with bivalent clostridial and combined vaccines and challenged with *C. chauvoei* spore suspension

Group	Vaccine	Minimum lethal dose (MLD)														
		16			32			64			128			256		
		S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
G1	Bivalent	4	-	100	4	-	100	4	-	100	4	-	100	2	2	50
G2	Combined	4	-	100	4	-	100	4	-	100	4	-	100	1	3	33

S = Survived, D = Dead, G3 (Control) were dead.

Table (2): Mean alpha antitoxin units of *C. septicum* in the sera of rabbits vaccinated with bivalent clostridial and combined vaccines.

Type of vaccine	No. of rabbits	*C. septicum* antitoxin titer (I.U.)
Bivalent vaccine	5	3.68 ± 0.44*
Combined vaccine	5	3.34 ± 0.46*
Control (Non-vaccinated)	2	0

*Non-significant difference between vaccinated groups (p>0.01).

Table (3): Immunizing potency of mice vaccinated with bivalent clostridial and combined vaccines and challenged with *P. multocida* type (A and D).

Days post vaccination	Pasteurella vaccine		Combined vaccine	
	Log protection			
	Type A	Type D	Type A	Type D
Zero day	0.5	0.6	0.4	0.4
14 days	1.3	1.5	1.2	1.4
30 days	3.2	3.5	3.5	3.9
60 days	4.2	4.0	4.3	3.9
90 days	4.1	3.7	4.0	3.8
120 days	3.8	3.2	3.8	3.5
150 days	3.5	3.1	3.7	3.4
180 days	3.2	3.1	3.2	3.1
210 days	2.1	2.0	2.0	2.1
240 days	1.9	1.7	1.7	1.8

4. DISCUSSION

Using effective multi-component Clostridial vaccines by farmers resulted in a marked reduction in losses from Clostridial diseases in sheep of all ages (Hussein et al., 2000). With successful control of these diseases, Pasteurellosis remains one of the most significant causes of losses in sheep population (Gilmour 1978).

The protection afforded by Clostridial and Pasteurella vaccines does not long lasting immunity, and these two vaccines are employed separately every year, a matter which consumes considerable time, effort and cost. Therefore, a suitable combined vaccine which can confer a dependable degree of immunity against both diseases is more convenient for sheep breeder, veterinarians from economic point of view (Chandran et

al., 2010). Therefore, the development of a combined vaccine against those diseases is needed. In this study, three vaccines were prepared including Pasteurella vaccine (*P. multocida* types A and D and *M. haemolytica* type A, Clostridial vaccine (*C. chauvoei* and *C. septicum*) and combined vaccine (Clostridial and Pasteurella vaccines). The prepared vaccines were subjected to sterility and safety. The prepared vaccines were sterile when inoculated into thioglucolate media, nutrient agar, and Sabouraud agar media and vaccines were safe when inoculated into mice and guinea pigs. Guinea pigs and rabbits were used as laboratory animals for evaluation of *C. chauvoei* and *C. septicum*. The results showed that the protection percentage in guinea pigs vaccinated with Clostridial vaccine (*C. chauvoei* and *C. septicum*) and combined vaccine (*P. multocida* types A and D, *M. haemolytica* type A, *C. chauvoei* and *C. septicum*) was 100% after challenge with 128 MLD of C. chauvoei spore suspension. However, British Veterinary pharmacopoeia (2010) approved C. chauvoei containing vaccines that give protection to guinea pigs against challenge with only one MLD of spore suspension. The results for the mean antitoxin titer of C. septicum was 3.68 and 3.34 U/ml in rabbit vaccinated with Clostridial vaccine and combined vaccine, respectively. The requirement was tabulated in British Veterinary pharmacopoeia (2010) was 2.5 U/ml for the approved vaccines containing *C. septicum* toxoid. These findings indicated that there was neither antagonistic nor synergistic effect between different antigens of combined vaccine.

Regarding to results of immunizing potency among mice vaccinated with pasteurella and combined vaccines and challenged with *P. multocida* type (A), it was clear that the log of protection mice was 3.2 and 3.5 at day 30 post vaccination for both pasteurella and combined vaccines, respectively. The log protection was reaching to its peak at day 90 post vaccination which was 4.1 and 4.0, respectively for both pasteurella and combined vaccines. There was significant higher log protection during period from day 30 to day 180 post vaccination for pasteurella and combined vaccines. After that there was significant decline in log protection from day 210 post vaccination so to maintenance the log protection at higher level to protect animals against disease; revaccination will be done at day 180. The log protection of mice challenged with *P. multocida* type (D) was 3.5 and 3.9 at day- 30 post vaccination for both pasteurella and combined vaccines. The log protection was reaching to its peak at day-90 post vaccination which 3.7 and 3.8 respectively for both pasteurella and combined vaccines. These results agreed with these of Wells et al., (1984) who found that the requirement of multivalent vaccine including the common serotypes of *P. multocida* in the field was providing protection against infection with *P. multocida* serotypes included in the vaccine. Otomaru et al., (2012) found that the antibodies titers against *P. multocida*, *M. haemolytica* and *H. somni* were significantly increased 4 weeks post vaccination. Overall, the results in this report indicated that there were no differences in the immunological response of the bivalent and the combined vaccines in the laboratory animals. Therefore, further field studies on these vaccines in sheep are required. The results will be helpful in controlling in both clostridial diseases and pasteurellosis.

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فاعليلية اللقاح المركب للكلوستريديم والباستريلا فى الحيوانات المخبرية.

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الملخص العربي

فى هذه الدراسة تم تقييم المناعة الناتجة عن تحصين خنازير غينيا باللقاح الثنائى للكلوستريديم واللقاح المركب للكلوستريديا والباستريلا مستخدما اختبار تحدى المناعة عن طريق العدوى الاصطناعية بميكروب كلوستريديم شوفياى. أوضحت النتائج أن كل من اللقاح الثنائى من الكلوستريديم شوفياى والكلوستريديم سبتكم وكذلك اللقاح المركب من الكلوستريديم والباستريلا أعطت حماية كافية فى الارانب الهندى عند استخدام اللقاح الثنائى للكلوستريديم منفردا أو اللقاح المركب من الكلوستريديم والباستريلا معا. أوضحت النتائج أنه لا يوجد فروق جوهرية فى نسبة الحماية الوقائية للفئران المحصنة بلقاح الباستريلا منفردا أو اللقاح المركب لكل من الكلوستريديم والباستريلا معا.

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