



## Comparative evaluation of antibody response in sheep vaccinated by bivalent clostridial and pasteurilla combined vaccine

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### ABSTRACT

The protective effect of both bivalent Clostridial vaccine and Pasteurella combined vaccine was investigated in sheep by using ELISA test. The antibody titers against *C. Chauvoei* and *C. septicum* in sera of sheep either vaccinated with bivalent Clostridial or Pasteurella combined vaccines reach to its peak at day 90 post vaccination. There was no significant difference in *C. chauvoei* and *C. septicum* antibody titers between bivalent Clostridial and Pasteurella combined vaccines during entire period of study. In addition, the antibody titers against *P. multocida* type A and D and *M. haemolytica* type A in sera of sheep vaccinated with both vaccines were high at period between day 30 -180 post vaccinations. The result revealed that there is no significant difference in antibody titers in both vaccines.

**Keywords:** *C. chauvoei*, *C. septicum*, *P. multocida*, *M. haemolytica*, ELISA

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### 1. INTRODUCTION

Clostridial and Pasteurella diseases are two major diseases affecting sheep having an adverse effect on their management. The control of these diseases is conducted by vaccination with specific monovalent vaccines, consuming considerable time, labor and operations cost. Many authors recommended the use of a combined vaccine against some infectious diseases that revealed good immunity as well as single vaccine (Olivia Cooper 2011). Simultaneous immunization of farm animals against anaerobic and aerobic infection gave good immunity (Burdov 1962; Darie et al. 1979). In addition, simultaneous administration of haemorrhagic septicaemia and FMD vaccines resulted in no adverse effect (Joseph and Hedger 1984).

In Egypt some authors studied the effect of combined vaccine in cattle which induced a high level of antibodies in sera of cattle vaccinated either with monovalent or bivalent vaccine of blackleg and FMD as studied by Abdel-Hamid et al. (1991). Another work studied the ability to prepared combined vaccine from *C. perfringens* B and C in combination with *P. multocida* type A, B and D to assist the sheep farmer to control the most major bacterial diseases of sheep (Hussein et al. 2000).

The present work was planned to study the immune response of sheep vaccinated with Clostridial and Pasteurella combined vaccine and compared them with that produced with correspond bivalent.

### 2. MATERIALS AND METHODS

## 2.1. Preparation of Vaccines:

### 2.1.1. Bivalent Clostridial vaccine (*C. chauvoei* and *C. septicum*).

#### a) Preparation of *C. chauvoei* whole cell culture vaccine:

Guinea pigs were injected with 0.5 ml *C. chauvoei* cultured strain suspended in 0.5 ml CaCl solution. The heart blood of guinea pig collected after death and cultures on cooked meat media at 37°C for 18 h. The cultured media inoculated into peptone thioglycolate media supplied with 2% glucose solution and incubated at 37°C for 18 h. The culture media of *C. chauvoei* inactivated using 0.5% formalin and incubated at 37°C for 7 days with agitation for complete inactivation of cultured media as described by Gadalla et al. (1974). Thiomerosal 0.01% was added as preservative. The obtained *C. chauvoei* culture had opacity 6.0 units/ml.

#### b) Preparation of *C. Septicum* toxoid vaccine:

Toxid vaccine of *C. septicum* was prepared with the same method of *C. Chauvoei* as described by Gadalla et al. (1974). Detoxification of *C. septicum* vaccine was done by addition of 0.5% formalin and the culture incubated at 37°C for 5 days. The whole culture was subjected to ultra-filtration by using 0.22 µl Millipore filter to obtain *C. septicum* toxoid only without the bacterial cells. The required dose for *C. septicum* toxoid vaccine should be contained 10L+/dose according to Merk (1998).

#### c) Preparation of bivalent Clostridial vaccine

Bivalent vaccine was prepared by addition of 4 volume *C. chauvoei* to one volume *C. septicum* as described by Merk (1998). Aluminum hydroxide gel 25% was added as adjuvant and the prepared vaccine kept at 4°C.

### 2.1.2. *P. multocida* type A and D and *M. haemolytica* type A vaccine.

Fresh strains from *P. multocida* type A and D and *M. haemolytica* type A were inoculated into Bain and Jones media and incubated at 37°C for 24 hrs. Then, formalin 37% was added to inactivate the culture as described by Confer et al. (1993). The concentration was adjusted to  $5 \times 10^{10}$  CFU/ml using MacFarland standard tube method. The culture was mixed thoroughly with aluminum hydroxide gel 25% according to Blackall and Reid (1987) using magnetic stirrer at 300 rpm.

### 2.1.3. Preparation of combined vaccine.

Equal volume from bivalent Clostridial vaccine and Pasteurella vaccine were mixed together according to Ghanem (1987).

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

## 2.2. Preparation of antigens

### 2.2.1. Preparation of *C. chauvoei* antigen for use in ELISA

*C. chauvoei* strain was cultured on agglutinating broth medium at 37°C for 18-24 hrs. The culture was centrifuged at 4000 rpm at 4°C for 10 min. The cells were collected and washed twice with phosphate buffer saline (PBS) as described by (Mattar et al. 2002). The cells were sonicated using cell sonicator for 4 min. The supernatant was collected by centrifugation at 4000 rpm for 10 min. The protein concentration was adjusted to 20µg/ml in carbonate bicarbonate buffer pH 9.6.

### 2.2.2. Preparation of *C. septicum* antigen for use in ELISA

Alpha toxin was purified from culture supernatant of *C. septicum* strain as described by Hong et al. (2002); the toxin was precipitated from supernatant of 18 hrs brain heart infusion broth by 60% saturated

ammonium sulphate, dissolved in 10 mM sodium sulphate pH7. The protein concentration was adjusted to 10µg/ml in carbonate bicarbonate buffer.

#### 2.2.3. Preparation and titration of *P. multocida* type A and *D M. haemolytica* antigen type A used for ELISA

The different strain were grown on brain heart infusion agar for 18 hrs and suspended in carbonate bicarbonate buffer pH 9 according to Voller et al. (1976). The suspension was sonicated for 15 min at 35% power using a cell disrupter with microtip probe.

#### 2.3. Vaccination Schedule

In a clean Twenty five sheep aged of 4-6 months were divided into four groups; First group composed of 7 sheep which vaccinated with bivalent Clostridial vaccine S/C in two doses (the 1st dose is 3 ml, the 2nd dose is 2 ml) with 21 days interval. The second composed of 7 sheep which vaccinated S/C with Pasteurella vaccine in two doses (1st dose is 1ml, and 2nd dose is 2ml) with 21 days interval. The third group composed of 7 sheep vaccinated S/C with combined vaccine in two doses (1st dose is 4 ml and 2nd dose is 4ml) with 21 days interval. In addition to, control group which composed of 4 sheep kept as non-vaccinated control.

#### 2.4. Blood Sampling

Blood samples were collected from all animals before vaccination and immediately after the injection of 2<sup>nd</sup> dose of different vaccines at day 14, 30, 60, 90, 120, 150, 180, 210 and 240.

#### 2.5. Evaluation of different prepared vaccines

Enzyme linked immunosorbent assay (ELISA). It was carried out according to (Voller et al. 1976; Walls 1977; Wood 1991; Matter et al. 2002) for measuring antibodies against *C. chauvoei*, *C. septicum*, *P. multocida* and *M. haemolytica* type A. The

results were calculated according to formula described by (Grabowska et al. 2002).

#### 2.6. 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

All the inoculated guinea pigs and mice with different prepared vaccine were survived all over the observation period (7 days).

##### b) Sterility test

All plates of nutrient agar, cooked meat broth, thioglycolate broth, nutrient broth and sabouraud agar media which were inoculated with different prepared vaccines were found free from any bacterial, mycoplasma, or fungal contamination.

#### 3.2. Determination of immune response of different prepared vaccines in sheep by ELISA test

##### 3.2.1. The immune response against *C. chauvoei* and *C. septicum* in vaccinated sheep

The results of ELISA test for measuring the antibodies titer of *C. chauvoei* and *C. septicum* in sheep vaccinated with different vaccines showed that both vaccines elicited antibody titer (3.75 and 3.05 for *C. chauvoei* and 3 and 2.9 for *C. septicum*) at day 14 post vaccination as shown in table 1. The titers subsequently become higher to reach its peak (49.7 and 44.5 for *C. chauvoei* and 5 and 4.4 for *C. septicum*) at day 90 post vaccination. Finally, the titer gradually decline to reach to low titer at day 180 post vaccination.

Moreover, the comparison of antibody titer in both group of vaccines in case of *C. chauvoei* and *C. septicum* by t-test revealed

Comparative evaluation of antibody response in sheep

that there is no significant difference during the entire period of the study as shown in table 1.

3.2.2. The immune response against *P. multocida* type A and D and *M. haemolytica* type D in vaccinated sheep

The antibody titers against *P. multocida* type A and D and *M. haemolytica* type A in vaccinated sheep with both type of vaccines

were measured by ELISA. The result revealed that the antibody titers were high at period from day 30 to day 180 post vaccination with both vaccines and in *P. multocida* and *M. haemolytica* as shown in table 2. The analysis of obtained results by t-test revealed that there was no significant difference between antibody titers in both types' vaccines.

Table 1: ELISA titers of *C. chauvoei* and *C. septicum* in sera of sheep vaccinated with bivalent Clostridial and combined vaccines.

Day post 2 <sup>nd</sup> dose of vaccination	Mean of ELISA titer			
	<i>C. Chauvoei</i>		<i>C. septicum</i> alpha antitoxin	
	bivalent Clostridial vaccine	combined vaccine	bivalent Clostridial vaccine	combined vaccine
Zero day	0.038±0.216	0.0875±0.105	0.045±0.011	0.041±0.055
14 days	3.75±1.15	3.05±0.75	3±0.4	2.9±0
30 days	9.60±0.4*	8.50±0.255*	3.6±1.2*	3.2±0.2*
60 days	18.7±1.0	18.1±1.9*	3.8±1.45*	3.3±0.7*
90 days	49.7±2.1*	44.5±0.5	5.0±0.4*	4.4±0.3*
120 days	20.65±1.95*	19.755±1.245*	3.1±0.3*	2.85±0.15*
150 days	11.5±0.8*	11.05±0.25*	2.7±0.1*	2.55±0.25*
180 days	0.85±0.15*	1.1±0.1*	2.6±0.33*	2.35±0.5*
210 days	0.343±0.02	0.350±0.15	0.4±0.1	0.38±0.2
240 days	0.125±0.1	0.109±0.5	0.22±0.2	0.19±0.11

\*Non-significant difference between vaccinated groups ( $p>0.05$ ).

Table 2: ELISA titer for *P. multocida* type A, D and *M. hemolytica* type A in sera of sheep vaccinated with Pasteurella and combined vaccines.

Day post 2 <sup>nd</sup> dose of vaccination	Mean of ELISA titer					
	<i>P. multocida</i> type A		<i>P. multocida</i> type D		<i>M. haemolytica</i> type A	
	Pasteurella vaccine	combined vaccine	Pasteurella vaccine	combined vaccine	Pasteurella vaccine	combined vaccine
Zero day	16.7±1.2	11.2±2.0	17.9±2.5	15.5±3.0	13.5±1.5	12.55±2.0
14 days	230±70	160±0.00	210±24	170±15.5	89.5±16.5	93±23
30 days	502±50*	490±30*	470±40*	465±30*	119±3.5	117±20
60 days	2020±60*	1860±40	1930±60	1799±20.5	153±17	176±6.5
90 days	2595±45*	2679±50*	2250±45*	2010±50.5*	219±3.5*	213±3.0*
120 days	2040±36*	2190±35*	3710±35*	3690±30*	243±0*	219±3.5
150 days	1850±15*	1915±25*	2260±40.5*	2110±45.5*	223±7*	210±0
180 days	1090±20*	1180±15*	1180±55*	1202±37*	159±3.5	139±3.5
210 days	910±60*	890±20.5*	930±39.5*	899±20.5	97±2*	89±1*
240 days	190±30*	190±10.5*	195±30*	189±25*	44±1	37±2.5

\*Non-significant difference between vaccinated groups ( $p>0.05$ ).

#### 4. DISCUSSION

The development of effective multi-component Clostridial vaccines and their widespread use 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.

The protection afforded by Clostridial and Pasteurella vaccines does not last long and immunity from these two vaccines is not long lasting 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 breeders, Veterinarians from an economic point of view. Thus, the ideal method of control is vaccination. Inactivated bacterial whole-cell vaccines have been the most widely studied (Chandran et al. 2010). 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).

ELISA is suitable for measuring the immune response to different antigenic functions instead of serum neutralization test because it has two advantages. First, it will allow a significant reduction in the number of mice. Second, ELISA allows an open ended fully quantitative estimation of potency for vaccines (Makhareta and Hammam 2001). The calculation of ELISA titer by weighted parallel line model is preferable for evaluation of antibody level for ELISA data (Grabowska et al. 2002). Thus, the ELISA test was used as model test to evaluate the antibody titer against different bacteria in vaccinated sheep.

Regarding to the results of ELISA for measuring of antibody titer against *C. chauvoei* and *C. septicum* as shown in table (1), all sheep began to have antibody titer at day 14 post vaccination, the titer against both bacteria was reaching to its peak at day 90 post vaccination for both bivalent Clostridial vaccine and combined vaccine.

The results of the present study are comparable to that of Osman et al. (1997) who found that the presence of antibodies in cattle sera by plate agglutination test, indirect haemagglutination test, complement fixation test and ELISA gave the best results with no great difference between them. Furthermore, El-Helw et al. (2012) concluded that the potency for *C. chauvoei* and *C. septicum* vaccine can be done by ELISA and replaced the traditional methods for evaluations due to its precision, sensitivity, rapid and save animals used. The immunity conferred by combined vaccine of blackleg and haemorrhagic septicaemia was similar to that obtained by each vaccine alone (Ghanem 1987).

Regarding to ELISA titers to *P. multocida* type A and D and *M. haemolytica* type A in the sera of sheep vaccinated with Pasteurella and combined vaccine in table (2), all sheep had antibody titer at day 14 post vaccination. The antibody titer was reaching to its peak at day 120 post vaccination for both Pasteurella and combined vaccine.

These results agreed with those 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 antibody titers against *P. multocida*, *M. haemolytica* and *Histophilus somni* were significantly increased 4 weeks post vaccination.

**Conclusion:** The experimental combined vaccine was reliable for protection against

blackleg, gas gangrene and pneumonic Pasteurellosis caused by *C. chauvoei*, *C. septicum*, *P. multocida* types A, D and *M. haemolytica* type A. Additionally, ELISA test can be used as an alternative method for evaluation of different prepared vaccines.

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### تحضير وتقييم لقاح الكلوستريديا والباستريلا المركب في الأغنام.

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### الملخص العربي

إن إنتاج اللقاحات المركبة هي اتجاه العلماء لما لها من فائدة اقتصادية كبيرة ولذلك تم في هذا البحث إنتاج لقاح مركب من الكلوستريديا والباستريلا ودراسة رد الفعل المناعي في الأغنام باستخدام اختبار الاليزا لقياس الاجسام المضادة ضد ميكروب الكلوستريديم سبتكم وشوفياي وأوضحت النتائج ان اعلي مستوي للاجسام المناعية يصل عند اليوم 90 بعد التحصين علاوه علي أنه لا يوجد فروق جوهرية في الاجسام المناعية الناتجة عن تحصين الاغنام باللقاح الثنائي للكلوستريديم منفردا أو اللقاح المركب للكلوستريديم والباستريلا معا، وتم أيضا قياس الاجسام المضادة ضد ميكروب الباستيريل مالتوسيدا نوع (أ؛ د) المانيهيميا هيموليتكا نوع (أ) باستخدام اختبار الاليزا وأوضحت النتائج انه يوجد ارتفاع ملحوظ في الاجسام المضاده من اليوم 30 حتي اليوم 180 من حقن التحصين. بالاضافه الي أنه لا يوجد فروق جوهرية في معدل الاجسام المناعية الناتجة عن تحصين الاغنام بلقاح الباستريلا منفردا أو اللقاح المركب للباستريلا والكلوستريديا معا.

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