



Preparation of Combined Inactivated Oil Adjuvanted *Pasteurella Spp.* and *Clostridium Spp.* Vaccine (Pneumoclost) in Sheep



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Abstract

THIS STUDY, aimed to prepare and evaluate a combined *Pasteurella Spp.* and *Clostridium Spp.* vaccine in two groups of one-year-old sheep. The first group was immunized with that vaccine and the second was kept as a negative control. All first group individuals were injected with two doses (1 month apart). This group showed the highest antibody titres after the second dose in the fourth month for *M. hemolytica* type A and *P. multocida* type A and the fifth for *P. multocida* (B6 and D) and *P. trehalosi*, when it was evaluated by IHA test. A passive mouse protection test was used for *P. multocida* (A, B6 and D) protection rate evaluation and revealed that 96% protection for A and B6 but 93% for D. SNT was used for *Clostridium Spp.* evaluation. The first month was the highest titers for *C. tetani*, *C. perfringens*, and by agglutination titer for *C. chauvoei*. The fourth month was the highest titer for *C. septicum*. The fifth month was the highest titer for *C. novyi* (B). ELISA test results revealed that the first month was the highest titers for *C. chauvoei*, *C. tetani*, and *C. perfringens*. The fourth month was the highest titer for *C. septicum*. The fifth month was the highest titer for *C. novyi* (B). The second group had no significant changes all over the experiment duration. Briefly, this vaccine has protective effects, achieving protection for sheep against illness and elevating the multi-handling and injection stress on the livestock and the workers efforts.

Keywords: Pasteurella, Mannheimia, Clostridia, Polyvalent, Vaccine.

Introduction

Pasteurella and Mannheimia are Gram negative, nonmotile, bipolar coccobacilli and facultative anaerobic bacteria. These bacteria can cause a contagious disease known as pneumonic pasteurellosis. They are commensal in the lung. The disease occurs under stress, like shipping, weaning, and nutrition changes. Also, respiratory disease caused by viruses that can be complicated by infection with *Pasteurella multocida* (*P. Multocida*), *Mannheimia haemolytica* (*M. haemolytica*), and *Pasteurella trehalosi* (*P. Trehalosi*) or other bacterial species [1]. Pneumonic pasteurellosis is considered one of the main infectious diseases from the

economic perspective with a widespread prevalence all over the world [2]. Also, Pasteurellosis can be caused by *Mannheimia haemolytica* (*M. Haemolytica*), which is considered one of the most common widespread bacterial infections of sheep [3]. Respiratory Mannheimiosis can be used as a synonym for Pneumonic Pasteurellosis with a wide spread in ruminants. That disease can induce adverse economic losses that reach up to 30% of the deaths globally [4].

Clostridium is Gram-positive, anaerobic bacteria and capable to form endospore [5]. Clostridium bacteria are found in the soil and in the guts of the animals. They are anaerobic (grow in the absence of oxygen). *Clostridium spp.* is non-invasive, but can

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cause the disease by toxins [6]. Gangrene and food-borne (stomach and intestine) diseases are caused by clostridia toxins leading to wasted costs. [7]. Such diseases include enterotoxaemia (*Clostridium perfringens*); lamb dysentery (*C. perfringens* type B); pulpy kidney (*C. perfringens* types D); blackleg (*C. chauvoei*); malignant edema (*C. septicum*); tetanus (*C. tetani*), and black disease (*C. oedematiens* type B) [8]. Diseases caused by *Clostridium* can be combated by different types of vaccines that can be mono or polyvalent [9]. Vaccines are required to give animals protection against these diseases. Vaccines are a suitable solution to decrease the occurrence and the disease intensity. The vaccination effects are based on many factors like types, routes and the injection site, in addition to the type of adjuvant [10]. Prevention and control of clostridial infection in bovine and ovine depend mainly on the administration of an effective amount of the vaccine which includes toxoids of *C. perfringens* types A, B, and D, *C. septicum*, *C. tetani*, *C. novyi* and high cellular density of formalized cultures of *C. chauvoei* with high immunogenic power because the immunity against *C. chauvoei* is generally considered to be antibacterial rather than antitoxic [11].

Pneumonic pasteurellosis control can be done with monovalent vaccines that consume operational costs, time and effort. So, the simultaneous immunization against aerobic and anaerobic infections gives a good solution with good immunity [12].

Montanide is an adjuvant that consists of a metabolizable oil with a highly emulsifier properties. Montanide is an incomplete adjuvant of Seppic, that give solid and long-live immunity. In comparison to common oil adjuvants, Montanide emulsions have more stability and easier in injection with high immunogenic ability with low adverse effects [13-16].

This study is aimed to produce and evaluate a combined polyvalent effective vaccine prepared from *Pasteurella* spp. and *Clostridia* spp. adjuvanted with Montanide™ ISA 206 VG oil for sheep.

Material and Methods

Experimental animals

Sheep

Twenty of one-year-old sheep that appeared in good health were obtained from Veterinary Serum and Vaccine Research Institute. They were used for the evaluation of the prepared vaccine. The sheep were not vaccinated against *Pasteurella* or *Clostridia* formerly.

Mice

Six hundred and ninety Swiss white mice were taken for this work from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These

mice were grouped as follows; Five hundred and forty mice group were used for the passive mouse protection test. The remaining 150 mice were used for the mouse toxicity test group.

Experimental design:

Twenty sheep were used for evaluation of the prepared combined polyvalent inactivated *Pasteurella* and *Clostridia* vaccine. The tested animals were grouped into two groups, 10 sheep for each. Group 1 (vaccinated group): The sheep were injected subcutaneously with two doses (4 weeks apart between each dose) of the vaccine (3 ml/dose). Group 2 (negative control group) was kept as a negative control. Blood samples were collected before the 1st dose, 2 weeks after the 1st dose, 1 month after 2nd dose, then monthly till the 7th month from both groups. The serum samples that were collected were used to evaluate the immunity after the vaccination in comparison to the other control one.

Vaccine preparation Strains:

P. multocida types A, D, B6, *M. haemolytica* type A and *P. trehalosi* type T were obtained kindly from the Aerobic Bacteria Research Department, VSVRI, ARC, Egypt.

C. perfringens types A, B, *C. perfringens* type D, *C. septicum* and *C. chauvoei* were obtained kindly from the Anaerobic Bacteria Research Department, VSVRI, ARC, Egypt.

The used adjuvant

Montanide™ ISA 206 VG (SEPPIC Co., France).

P. multocida and *M. haemolytica* antigen preparation according to [17]

All *Pasteurella* strains were cultured separately in nutrient broth with enrichment of yeast extract. For 24 hr at 37 °C, the cultured media were incubated, followed by formalin inactivation in final concentration of 0.5% for 24 hours. The inactivated cultures of *P. multocida* biotypes were adjusted to contain 1×10^7 CFU for each. But, *M. haemolytica* type A and *P. trehalosi* type T were adjusted to contain 1×10^8 CFU in the vaccine formula, according to [1].

Clostridia antigenic Culture preparation

It was prepared by using equal amounts of toxoids of *C. perfringens* type A, *C. perfringens* type B, *C. perfringens* type D, *C. septicum* and *C. chauvoei* and *C. oedematiens* type B according to [8,18] (the *Clostridia* are grown in suitable media (peptone 3%, sodium chloride 0.5%, yeast extract 0.5%, beef extract 0.5%, disodium hydrogen phosphate 0.4%, l- cysteine 0.05% and glucose 2%) under anaerobic condition and controlled condition of pH (7.5- 8) and temperature (35°- 37°C).

Clostridial culture was incubated for 4hrs (*C. perfringens* type A and B), 24hrs (*C. perfringens* type D, *C. chauvoei* and *C. septicum* and *C. oedematiens* type B). The preparation of *C. tetani* toxin were done according to [19] which was grown in brain heart infusion broth with yeast extract, cysteine, disodium hydrogen phosphate, potassium chloride and glucose under anaerobic condition with pH 6.9±0.2 at 35°C. *C. tetani* culture was incubated for 5 days.

Cultures were inactivated using 0.5% formalin at 37°C for 7 days except *C. tetani* for 1 month. The Killed bacteria were separated from the inactivated culture by centrifugation except *C. chauvoei* was taken as a whole culture.

Preparation of the combined vaccine

In this research, the inactivated polyvalent combined Pasteurellosis and Clostridial vaccine was adjuvanted with Montanide oil™ ISA 206 VG. The preparation process was done according to the instructions of Montanide manufacturer (SEPPIC). The aqueous antigenic cultures (*Pasteurella* and *Clostridia*) were mixed with the Montanide in ratio 50% in weight using a low shear rate and controlled temperature at 31° C in one step process to get a stable vaccine emulsion.

Quality control testing of the vaccine

According to the regulation of [20] safety and sterility tests were carried out.

Evaluation of the prepared vaccine efficacy

Evaluation of the generated antibodies against *Pasteurella* and *Mannheimia* in the sheep by the following tests:

Indirect hemagglutination (IHA) test for evaluating antibodies against *Pasteurella* and *Mannheimia* was done according to [21]. The test was done as follow:

- 1- Two-fold serial dilutions of the collected sample sera starting with 1/2 were prepared in stabilizer buffer pH 7.2 to give a final volume of 50 µl/well (microtiter plate) and 50 µl of sensitized RBCs were added to each well.
- 2- The plates were shaken, then left at room temperature for approximately 2 hours where the first reading was taken. The plates were then placed in refrigerator till the next morning where the second reading was taken.

Passive mouse protection test for evaluating *P. multocida*

It was used to evaluate the protection rate of the vaccinated serum against challenge with the 3 serotypes of *P. multocida* (A, D and B6) all over the collected blood as described by [22].

Evaluation of the generated antibodies against *Clostridia* in sheep as follows:

Serum neutralization test (SNT)

The antitoxic values expressed in (IU/ml) were determined in Swiss white mice by SNT according to [20] which serum sample was diluted in PBS (pH 7.0), then 0.5 ml of each toxin (0.1mg/ml) was added to 0.5 ml of diluted individual serum sample. The mixture was incubated at room temperature for 1 hr., then 0.2 ml of the toxin-serum mixture was injected intravenously into each of 2 mice, to determine the neutralizing titer the highest dilution of sera protecting more than 50% of inoculated mice. But for *C. tetani* antitoxin and *C. chauvoei*. *C. tetani* antitoxin was tested for measuring the antitoxin titer in the serum samples of the vaccinated group by a toxin neutralization test [23]. But, the immunity against *C. chauvoei* were measured by Plate agglutination test [24].

Indirect ELISA test

It was done according to [25] using different *Clostridial* antigens which were diluted with coating buffer then 100 µl of diluted cells was put in each well. The antigen coated plates were incubated at 4°C overnight. The plates were washed three times with washing buffer. Then plates were blocked by adding 100 µl of blocking buffer and incubated for one hour at room temperature. The plates were washed three times, then were incubated with 100 µl of diluted sera samples to each well for one hour at room temperature. The plates were washed three times. The plates were incubated with 100 µl to each well of horseradish peroxidase (HRPO) conjugated anti-sheep IgG diluted 1:7000 in washing buffer for 30 minutes at room temperature. The plates were washed as before three times. The plates were incubated with 100 µl to each well of substrate solution (phosphate citrate buffer) for 10 minutes at room temperature in a dark place. Colour development was terminated with adding 50 µl to each well of 12.5 % H₂SO₄. Optical density at 492 nm (OD 492) of each well was measured with an ELISA reader. The serum samples were approved to be positive when the absorbance values were equal or higher than the cut-off value (the cut value = the double of the mean of O. D. of negative sera). The immunity was determined as the last dilution of serum gave a positive result.

Results

Quality control testing results

Evaluation of safety

The test of safety was done on mice and guinea pigs. The findings showed that all the vaccinated animals survived all over the 7 days (period of observation).

Evaluation of sterility

No detection of growth has appeared after the tested vaccine inoculation in different types of media (cooked meat broth, Sabouraud agar, nutrient broth or agar and thioglycolate broth. These results were proved the purity of the vaccine (no bacterial, mycoplasma, or fungal growth).

Evaluation of the prepared vaccines against *Pasteurella* results

Indirect hemagglutination (IHA) test results

According to Table (1), in the vaccinated group, the mean titers of antibodies began to increase after the 1st dose of vaccination and the 2nd vaccination dose (booster dose). This increase was continued to reach the highest level for *P. multocida* types A and B6 in the 4th month (1843.2±457.9, 1638.4±560.9, respectively) and type D in the 5th month (1331.2±686.9). *M. haemolytica* type A reached the highest titer in the 4th month (1843.2±457.9), but *P. trehalosi* type T had the highest titer in the 5th month (921.6±228.9). The control group had 2 antibody titers with preference to the vaccinated group.

Passive mouse protection test results

According to Table (2), the overall means protection test results in the vaccinated sheep group serum in comparison to the control one, challenged with *P. multocida* (A, D and B6) virulent strains were as follow; 96% protection for the vaccinated group and the control group had 0% protection for A and B6. But, for type D, the protection was 93% for the vaccinated group and 0% for the control group.

Measuring of the immunity of the vaccinated sheep against *Clostridia* results

The present study revealed the absence of any side effects or symptoms of illness in all vaccinated animals. All the animals were free from any of the specific antibodies before vaccination. The results illustrated in Tables (3) and (4) showed the response of sheep against *C. perfringens* (A: alpha toxin, B: beta toxin and D: epsilon toxin) in addition to *C. septicum* (alpha toxin), *C. novyi* (B: alpha toxin, *C. tetani* and *C. chauvoei* stimulates detectable antibody response after 2 weeks of vaccination. After the 2nd dose of the combined vaccine (booster dose), rapid and powerful response occurred, raising the antibody level to the highest peak in the 1st month for *C. perfringens* (A: alpha toxin, B: beta toxin, D: epsilon toxin), *C. tetani* and *C. chauvoei*, at the 5th month for *C. novyi* (B: alpha toxin) and at the 4th month for *C. septicum* alpha toxin. Tables 3 and 4 data revealed that the prepared combined vaccine yielded the maximum rate of antibody titers that remained above the minimum protective level of all strains of polyvalent clostridial vaccine until the 7th month.

This study approved that the multi components of the vaccine had no side effects on the antigenic

component. Also, our results indicated that a combined vaccine adjuvanted with Montanide™ ISA 206 gave high antibody titer with a long duration.

Discussion

In this study, *Pasteurella* and *Clostridia* inactivated polyvalent combined vaccine was prepared and adjuvanted with Montanide™ ISA 206 VG. Sheep were grouped into 2 groups; vaccinated and control. The vaccinated group was vaccinated with two doses. The advantages of the successful combined vaccine are to relieve the stress on animals, decrease the labour of workers and saving time in addition to expense. Combined vaccines have multiple advantages such as protection against many diseases at the same time in one dose, reducing vaccination expenses and saving time. Moreover, the combined vaccines will protect the host from stress factors in the application of repeated mono vaccinations on several occasions, according to the vaccination program [26]. Also, [27] explained the protection effect of the polyvalent clostridia and *P. haemolytica* vaccine against the *P. haemolytica* (A6) infection. The several antigens content did not adverse the effect of each antigen.

During the present work, the vaccine was proved to be stable, pure from contamination and safe for sheep injection. These observations agree with the recommendation of [20]. In the present study, the IHA test measured the antibody titers against *Pasteurella* and *Mannheimia* acquired from the prepared vaccine. According to Table (1), there was a gradual increase in the antibody titers after the 1st and 2nd doses of vaccination in the vaccinated group till reaching the highest titers in the 4th month after the 2nd dose for *P. multocida* (A and B6) and *M. haemolytica* (A), then began to decrease from the 5th month. Also, for *P. multocida* (D) and *P. trehalosi* (T), the highest titer was in the 5th month, then began to decrease from the 6th month. In the same way, [1] compared the *Pasteurella* vaccine and combining *Pasteurella* with *Corynebacterium pseudotuberculosis ovis* bacterin using an IHA test, and found that the combined vaccine gave a higher immunity than the individual one. For the combined *Pasteurella* vaccine, the highest antibody titers for *P. multocida* (types A, D, B6) were 512 in the 5th month for type A, but in the 4th month for D and B6. Also, for *M. hemolytica* (A and T) the highest antibody titers (512) were at the 5th month. [28] evaluated their prepared combined vaccine against foot and mouth disease and *P. multocida*. They concluded that there was a difference in the significance between the combined vaccine and the *Pasteurella* vaccine was present. Also, they reported that the combined vaccine is safe and immunogenic with no side effects. [29] prepared a combined vaccine against *M. haemolytica*, *P. multocida*, and *H. somni*. They concluded that the booster dose of the vaccine had increased the antibody titer. [30] were evaluated the

polyvalent *Pasteurella* vaccine (Pneumo-bac® vaccine which is an inactivated polyvalent vaccine of *Pasteurella* (*P. Multocida*; A, B & D and *M. haemolytica*; A & T) with oil adjuvant by IHA and passive mouse protection test. They found that by using IHA test, the highest antibodies titers in the 5th month for *P. multocida* type A, 2nd month for type B, 2nd and 3rd month for type D and type A of *M. haemolytica* and 3rd month for type T. About evaluation with the passive mouse protection test, the vaccinated serum gave protection with 100%. [17] prepared a vaccine against *P. multocida* from the field isolates. They evaluated the titers of the antibodies by the passive hemagglutination inhibition test (134.86 ± 114.582 on the 4th week after vaccination). They concluded that the vaccinated animals may be protected against hemorrhagic septicemia. [31] compared inactivated *P. multocida* adjuvanted with Aluminum-based mineral salts (alum) adjuvant and Herbal adjuvant. They cited the antibody titer of the vaccinated animals with the *Pasteurella* vaccine with Alum gel adjuvant increased from the 4th week up to the 14th week. For the vaccine with the herbal adjuvant, the antibody titer increased from the 3rd month to the 4th month. Then, antibody titer decreased after that, but showed protection till the 6th month. In the same vein, [32] prepared *P. multocida* vaccines and used the IHA test to evaluate them. They reported that, Montanide as an adjuvant had a role in improving the quality of the vaccine. Also, the vaccinated animals that had a booster dose of the vaccine adjuvanted with oil had immunity. [12] prepared bivalent clostridial and *Pasteurella* (types A, D, and *M. haemolytica*) combined vaccine and Clostridial vaccine in sheep, for comparison between them. They had found no significant difference in titers of antibodies between both with priority to the combined vaccine for protection. The results also almost agreed with our result as they reached the peak of antibody titers between 3rd and 4th months for *Pasteurella* strains. [33] compared the immunity acquired from vaccination against hemorrhagic septicemia oil adjuvant and alum-precipitated vaccines. Serum samples from vaccinated and boosted animals were evaluated by Indirect Hemagglutination Test (IHA) and Passive Mouse Protection Test (PMPT). IHA titer and PMPT test results showed that alum precipitated vaccine gave protective titer of antibodies for up to 4 months and with PMPT gave 20% protection. The vaccinated animals with oil adjuvanted vaccine had protective antibody titers till 10 months and PMPT gave 60% protection. These results approved that oil adjuvanted vaccine gave long immunity and the booster dose was necessary for solid immunity with longer duration. [34] prepared a vaccine against *P. multocida* strain 6: B (from a local strain) adjuvanted with Montanide ISA-70. They evaluated the immunity acquired from this vaccine via a passive mouse protection test. The

results revealed that 100% protection on days 24, 90 and 150 post-vaccinations. [35] prepared a hemorrhagic septicemia vaccine with Alum. adjuvant. When they evaluated the antibody titers of the vaccinated cattle by passive mouse protection test, they found the protection rate for the first 2 months post-vaccination was 100%, for the 3rd and 4th months it was 83%, for the 5th month it was 50% and the 6th month was 40%. [36] prepared a combined FMD virus, rabies virus, *P. multocida* and *C. chauvoei* vaccine. They compared this combined vaccine against individual ones for each component. On the other hand, for *P. multocida*, the combined vaccine gave higher immunity than individuals on days 21 and 90 in the vaccinated calves when evaluated via an ELISA test. The Montanide has been used in vaccination trials in humans, animals and poultry [37] showing the immunogenic effect on vaccines in variety of animal species [38, 39]. Montanide is capable to stimulate the immunity (humoral and cellular) to challenge the infection [40, 41]. Also, in this study, the usage of serum neutralization (SNT) and ELISA tests were for evaluating the immunity against the Clostridia antigens of the prepared vaccine. The results of SNT showed that the immune response against different clostridial strains was high in the combined vaccine adjuvanted with Montanide ISA 206 and remained above the minimum protective level of all strains of poly valent clostridial vaccine until the 7th month which is 4 IU/ml for *C. perfringens* (A: α toxin), 5 IU/ml for *C. perfringens* (B: β toxin), 2.5 IU/ml for *C. perfringens* (D, ϵ toxin), *C. septicum* (α toxin) and *C. tetani*, 3.5 IU/ml for *C. novyi* (B: α toxin) according to United States Department of Agriculture in 2002 and 0.5 μ l/ml agglutination level or less for *C. chauvoei* according to [24]. This indicates that no competition was observed between Clostridia antigens and *Pasteurella* antigens in the combined vaccine. These results agreed with [12] which prepared bivalent clostridial and *Pasteurella* (types A, D, and *M. haemolytica*) combined vaccine and Clostridial vaccine in sheep for comparing between them and found no significant difference in titers of antibodies between both with priority to the combined vaccine for protection. Also [42] which had evaluated a combined vaccine in sheep against *P. multocida* (A, B and D) and *C. perfringens* (B and D) and found that there was no competition was observed between *C. perfringens* and *P. multocida* antigens as the non-significant difference ($P < 0.05$) could be noted between beta and epsilon antitoxin titers or anti-*P. multocida* antibodies in samples of the vaccinated sheep with a bivalent vaccine of *C. perfringens* (B and D), trivalent *P. multocida* (A, B, and D) vaccine and combined vaccine including all antigens. Also, [43] showed that SAT2 FMD Vaccines adjuvanted with Montanide ISA 206 are better than the vaccine adjuvanted with Quil-A Saponin for producing a

protection reaction of immunity response with more protection level and duration. ELISA test results almost confirmed the results of the Serum Neutralization test.

Conclusions

From the discussed results, it is concluded that, the combined polyvalent inactivated Pasteurella and Clostridial vaccine adjuvanted with Montanide™, ISA 206 VG revealed neither competition nor mutual interference between all strains, and the vaccine offered good protective immunity against the used strains and could be used safely for protection against pasteurellosis and clostridial diseases for sheep.

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Conflicts of interest

There are no conflicts to declare. The authors declared no competing interests.

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TABLE 1. Antibodies titers against *P. multocida* (A, D and B6), *M. haemolytica* type A and *P. trehalosi* in the vaccinated group and the control one using the indirect hemagglutination test (mean±SD), SD: standard deviation

Time intervals	The prepared combined vaccine					Control
	<i>P. multocida</i>			<i>M. haemolytica</i>	<i>P. trehalosi</i>	
	A	D	B6	A	T	
Pre vaccination	2±0	2±0	2±0	2±0	2±0	2±0
2 weeks after 1 st dose	102.4±35.1	83.2±42.9	102.4±35.1	102.4±35.1	70.4±35.1	2±0
Second dose (1 month after 1st dose)						
1 st month	665.6±343.5	166.4±85.9	332.8±171.7	512±0	140.8±70.1	2±0
2 nd month	819.2±280.4	332.8±171.7	409.6±140.2	819.2±280.4	230.4±57.2	2±0
3 rd month	1638.4±560.9	409.6±140.2	819.2±280.4	1638.4±560.9	409.6±140.2	2±0
4 th month	1843.2±457.9	665.6±343.5	1638.4±560.9	1843.2±457.9	768±362.0	2±0
5 th month	1638.4±560.9	1331.2±686.9	1433.6±560.9	1433.6±560.9	921.6±228.9	2±0
6 th month	1024±0	665.6±343.5	921.6±228.9	921.6±228.9	640±384.0	2±0
7 th month	819.2±280.4	409.6±140.2	614.4±388.2	614.4±388.2	422.4±372.1	2±0

TABLE 2. The Passive mouse protection test results in the vaccinated sheep group serum in comparison to the control one, challenged with virulent strains of *P. multocida* type A, D and B6.

Time intervals	<i>P. multocida</i> virulent strains											
	A				D				B6			
	Vaccinated group		Control		Vaccinated group		Control		Vaccinated group		Control	
	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live
Pre vaccination	10	0	10	0	10	0	10	0	10	0	10	0
2 weeks after 1 st dose	2	8	10	0	3	7	10	0	2	8	10	0
	2 weeks after 1 st dose											
1 st month	1	9	10	0	2	8	10	0	1	9	10	0
2 nd month	0	10	10	0	1	9	10	0	0	10	10	0
3 rd month	0	10	10	0	0	10	10	0	0	10	10	0
4 th month	0	10	10	0	0	10	10	0	0	10	10	0
5 th month	0	10	10	0	0	10	10	0	0	10	10	0
6 th month	0	10	10	0	0	10	10	0	0	10	10	0
7 th month	0	10	10	0	0	10	10	0	0	10	10	0
Overall means Protection %	96		0		93		0		96		0	

$$\text{Protection \%} = \frac{\text{No. of survived mice}}{\text{Total No. of mice}} \times 100$$

TABLE 3. Mean antitoxin titers of sheep serum against Clostridial spp. in combined vaccine.

Time intervals	Combined Clostridia spp. and <i>Pasturella</i> spp. oil adjuvant vaccine						
	<i>C. perfringens</i> type A (α toxin)	<i>C. perfringens</i> type B (β toxin)	<i>C. perfringens</i> type D (ϵ toxin)	<i>C. novyi</i> type B (α toxin)	<i>C. septicum</i> (α toxin)	<i>C. tetani</i>	<i>C. chauvoei</i> (agglutination titer (μ l/ml))
	Pre vaccination.	0	0	0	0	0	0
2 weeks after 1 st dose	22	22	12	8	12	11	0.01
	Second dose (1 month after 1 st dose)						
1 st month	35	28	16	11	15	15	0.01
2 nd month	30	26	16	11	15	12	0.02
3 rd month	26	24	14	13	18	12	0.05
4 th month	26	18	12	15	20	10	0.05
5 th month	22	16	11	19	16	8	0.1
6 th month	18	11	9	17	14	8	0.1
7 th month	15	9	7	11	11	6	0.2

TABLE 4. Antitoxin titers of sheep serum measured by ELISA against Clostridial spp. in combined vaccine (mean±SD) SD: standard deviation

Time intervals	Combined clostridia spp. and Pasturella spp. oil adjuvant vaccine						
	<i>C. perfringens</i> type A (α toxin)	<i>C. perfringens</i> type B (β toxin)	<i>C. perfringens</i> type D (ε toxin)	<i>C. novyi</i> type B (α toxin)	<i>C. septicum</i> (α toxin)	<i>C. tetani</i>	<i>C. chauvoei</i>
Pre-vaccination	0.235±0.01	0.142±0.01	0.046±0.0	0.173±0.05	0.068±0.0	0.077±0.001	0.218±0.004
2 weeks after 1 st dose	1.288±0.02	0.499±0.02	0.711±0.03	1.584±0.02	1.491±0.01	1.196±0.002	1.691±0.01
	Second dose (1 month after 1 st dose)						
1 st month	1.583±0.01	0.681±0.01	1.014±0.001	1.601±0.001	1.56±0.03	1.382±0.01	1.772±0.01
2 nd month	1.425±0.05	0.652±0.04	1.002±0.06	1.61±0.02	1.569±0.02	1.291±0.02	1.764±0.01
3 rd month	1.352±0.011	0.567±0.02	0.85±0.04	1.631±0.01	1.687±0.02	1.288±0.02	1.895±0.03
4 th month	1.315±0.022	0.393±0.04	0.772±0.01	1.656±0.03	1.775±0.01	1.179±0.01	1.889±0.02
5 th month	1.3±0.02	0.376±0.02	0.701±0.012	1.687±0.01	1.598±0.02	1.086±0.003	1.006±0.06
6 th month	1.019±0.004	0.309±0.01	0.697±0.024	1.669±0.05	1.557±0.04	1.081±0.003	0.998±0.04
7 th month	0.998±0.01	0.295±0.01	0.677±0.03	1.609±0.01	1.486±0.02	1.009±0.03	0.87±0.03

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اعداد لقاح زيتي مثبط جامع من مجموعة بكتريا الباستيريلا والكلوستريديا في الأغنام

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المستخلص

لقد هدفت هذه الدراسة الى انتاج وتقييم لقاح جامع ضد الباستيريلا والكلوستريديا في مجموعتين من الأغنام بعمر عام واحد. المجموعة الأولى تم تحصينها باللقاح بينما المجموعة الثانية لم تتلق اللقاح وتم الاحتفاظ بها كمجموعة سلبية ضابطة. كل أفراد المجموعة الأولى تلقت جرعتين من اللقاح يفصل بين الحقتين مدة شهر. ولقد أوضحت هذه المجموعة باستخدام اختبار تلزن الدم غير المباشر أعلى قياس للأجسام المناعية بعد الجرعة الثانية في الشهر الرابع للمانهيميا هيموليتيكا نوع A والباستيريلا ملتوسيدا نوع A والشهر الخامس للباستيريلا ملتوسيدا نوعي B6 و D والباستيريلا تريهالوزي. وباستخدام اختبار وقاية الفئران السلبي أوضح أنه نسبة الوقاية للباستيريلا ملتوسيدا كانت كالتالي 96% للنوعين A و B6 و 93% للنوع D. وعند استخدام اختبار تحييد المصل لتقييم الكلوستريديا كان الشهر الأول هو الأعلى لنوعين الكلوستريديا الكزازية والمطثية الحاطمة وأيضا للكلوستريديا شوفياي باختبار التلزن. وال شهر الرابع كان الأعلى لكلوستريديا التفسخ بينما الشهر الخامس كان الأعلى للكلوستريديا نوفياي النوع B. وعند استخدام اختبار الاليزا أوضح أن الشهر الأول كان الأعلى للكلوستريديا شوفياي و الكلوستريديا الكزازية والمطثية الحاطمة. والشهر الرابع كان الأعلى لكلوستريديا التفسخ. وأيضا الشهر الخامس كان الأعلى للكلوستريديا نوفياي النوع B. أما المجموعة الثانية لم يكن بها تغيرات تذكر طول مدة التجربة. وباختصار فان هذا اللقاح له تأثيرات وقائية محققا الوقاية للأغنام ضد المرض ومخففا عبء تكرار الامساك وتكرار الحقن للحيوان ومجهودات العمالة.

الكلمات الدالة: باستيريلا ، مانهيميا ، كلوستريديا ، لقاح جامع ، لقاح.