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Use of Montanide gel as an adjuvant for polyvalent clostridial vaccine

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Abstract

Clostridium bacteria are found in soil and in the guts of animals. They are anaerobic. They create a wide spectrum of poisons that cause a variety of diseases, some of them are invasive and others not invasive, but they produce a wide range of toxins responsible for varieties of diseases. Inactivated vaccines have long been used to prevent clostridial diseases in sheep and cattle, and this practice is regarded as crucial to the management of clostridial infection in many animals. This study attempts to increase the immunogenicity of the locally manufactured inactivated polyvalent clostridial vaccine in order to produce a high and prolonged duration of protection in vaccinated animals through concentration of clostridial toxoids by using ammonium sulphate and using an alternative adjuvant as Montanide Gel 01$^{TM.}$. Two vaccines were prepared, vaccine no.1 (polyvalent clostridial vaccine adjuvanted with alum (traditional vaccine)) and vaccine no.2 (polyvalent clostridial vaccine adjuvanted with Montanide Gel 01^{TM}). Both vaccines were evaluated in rabbit, sheep and cattle. After concentration of Clostridial toxoids and using Montanide Gel 01^{TM} as a new adjuvant, the components of the polyvalent clostridial vaccine were significantly higher compared with that elicited by the traditional polyvalent clostridial vaccine. Additionaly, the dose of vaccine was reduced from 5 ml to 3 ml for cattle and from 3ml to 2ml for sheep per dose.

Keywords: *Clostridial spp.*, concentration, Montanide Gel 01 TM, the vaccinal dose.

Introduction

Clostridium is classified as Gram-positive endospore-forming obligate anaerobes [1]. *Clostridium* bacteria are found in soil and in the guts of animals. They are anaerobic, Clostridia spp. some of them are invasive and other not invasive, but they produce a wide range of toxins responsible for varieties of diseases.[2]. Most clostridial toxins are pore-forming toxins responsible for a wide variety of gangrenes and gastrointestinal diseases in humans and animals, which leading to significant economic losses in farming industry [3]. Such diseases include enterotoxaemia (*Clostridium perfringens* type A); lamb dysentery (*Clostridium perfringens* types B); pulpy kidney (*Clostridium perfringens* types D); blackleg (*Clostridium chauvoei*); malignant oedema (*Clostridium septicum*); tetanus (*Clostridium tetani*), and black disease (*Clostridium novyi* type B) [4].The prevention and control of these different diseases caused by Clostridia spp. are done by using vaccine formulations containing one or more clostridial bacterins and toxoids [5]. Vaccines are required to give animals protection against these diseases and reduce the incidence and severity of these diseases. The effectiveness of immunization depends on several factors including the type of vaccine, the route of administration and the adjuvant used [6].

Prevention and control of clostridial infection for bovine and ovine depend mainly on the administration of an effective amount of the vaccine including toxoids of *C. perfringens* types A, B, D; *C. septicum*; *C. tetani*, *C. novyi* and adequate density of *C. chauvoei* bacterin because immunity to *C. chauvoei* is generally considered to be antibacterial rather than antitoxic [7].

Montanide Gel 01 ^{TM}, a new adjuvant based on the dispersion of polymeric gel in water, has the advantages of a high antigen load, high stability, and easy emulsification [8-10]. Due to polymer adsorption properties, this adjuvant improves the recruitment of the innate immune system, which provide a significant enhancement of the immune

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response in aqueous vaccines[11]. It was observed that using of Montanide Gel 01^{TM} as an adjuvant elicited a sufficient early immune response in vaccinated cattle with an excellent safety profile whatever the nature of the antigen[12]**,** and it was reported that the antibodies induced were higher than Aluminium-based vaccines and so it can be used associated with a wide range of antigenic media and recommended to be used as adjuvant for sensitive animal's vaccines [13, 14]**.** These results prompted the use of Montanide adjuvant for Clostridial vaccine formulation which could be helpful in controlling of clostridial diseases.

This work aims to improve and enhance the immunogenicity of the locally produced inactivated polyvalent clostridial vaccine to induce high and long-lasting immunity in vaccinated animals using Montanide Gel 01^{TM} as an adjuvant which in turn leads to reduce the vaccinal dose from 5ml to 3ml in cattle and from 3ml to 2ml in sheep.

Material and Methods

Bacterial strains

Clostridial strains were used in vaccine preparation which are *C. perfringens* types A, B and D; *C. novyi* type B; *C. septicum*, and *C. tetani*; and a bacterin from *C. chauvoei* were obtained from Anaerobic bacterial vaccine department, Veterinary Serum and Vaccine Research Institute (VSVRI), ARC, Egypt.

Animals

Mice

Three hundred (300) white Swiss mice weighing approximately 18-20 g were taken from the farm of VSVRI. These mice were utilized to determine the Minimum Lethal Dose (MLD) of Clostridial toxins, safety for prepared bacterin and to titrate antitoxin levels in the sera of vaccinated animals using the Serum Neutralization Test (SNT).

Rabbits

Twenty-five Bosket rabbits weighing 2–2.5 kg were kept in batteries in a well-ventilated, sterile place, where they were fed commercial pellets and provided with clean water. Be sure that they are free from clostridia. They were used for safety and potency of prepared vaccines.

Sheep

Twenty sheep were used for evaluation of prepared vaccines obtained from VSVRI had not received any Clostridial vaccine before and were selected on the bases of absence of detectable levels of clostridial antitoxin before the beginning of the experiment.

Cattle

Twenty cattle were used for evaluation of prepared vaccines obtained from VSVRI had not received any Clostridial vaccine before and were selected on the bases of absence of detectable levels of clostridial antitoxin before the beginning of the experiment.

Reagents

Ammonium sulfate

Used in concentration of Clostridial toxoids at saturation of 70% according to http://www.encorbio.com/protocols/AM-SO4.htm.

Formalin

Used as inactivating agent for inactivation of clostridial culture in ratio of 0.5- 1% [15]

Adjuvants

Potassium aluminum sulphate (Alum) used in a concentration of 1% according to [16]**.**

Montanide Gel 01^{TM} was obtained from **SEPPIC**, **Paris, France** and used in a concentration of 10%

Vaccine preparation

The bacterin was prepared using antigens, either toxoids or bacterin, obtained from cultures of *C. perfringens* types A was prepared according to [17], *C. perfringens* type B and D, *C. chauvoei* and *C. septicum* were prepared according to[4], *C. novyi* type B was prepared according to [18] while *C. tetani* was prepared according to [19].

All strains were cultured anaerobically in suitable clostridial medium and controlled condition of pH (7.5- 8) and temperature 37^{o}C for 4hrs (*C. perfringens* type A and B), 24hrs (*C. perfringens* type D, *C. chauvoei* and *C. septicum* and *C. novyi* type B) while C. tetani was cultured at pH 6.9±0.2 and incubated for 5 days at 35^{o}C.

The toxicity of each strain and determination of Minimal Lethal Dose (MLD) was done according to [20] using mouse toxicity test. Limit of flocculation was done according to [21], for determination the potency of tetanus toxin. Dose for *C. chauvoei* was determined by opacity or absorbency units, these units are based on the optical density (O.D.) of the culture, as measured at wavelength 625 nm according to [4]. Cultures were then inactivated with 37% formaldehyde at (0.5% v/v), and complete detoxification was verified by injecting intraperitoneally 0.5 ml of each preparation into mice.

The toxoids were clarified by using continuous flow centrifuge at 15,000 rpm and the concentration of Clostridial toxoids was done by using Ammonium sulphate at saturation of 70% according to http://www.encorbio.com/protocols/AM-SO4.htm.

Vaccine formulation

Polyvalent Clostridial vaccine was prepared by mixing different toxoids in ratio of one part for *C. tetani*, two parts for *C. perfringens* type A alpha toxin and four parts for each *C. perfringens* type B (beta toxin) and type D (epsilon toxin), *C. novyi* type

B (alpha toxin) and *C. septicum* then divided into two parts:

Vaccine No. 1 was adjuvanted with alum at 1% (w/v).

Vaccine No. 2 was adjuvanted with Montanide gel at 10% (v/v).

Quality control tests for the prepared vaccines

Sterility test

The prepared vaccines were used directly after being tested for free from any contaminants, i.e., aerobic or anaerobic bacteria and fungi according to [22]. This was done through inoculation in various types of media including Nutrient agar, Thioglycolate medium and Sabouraud dextrose agar. The media were incubated at 37°C for 10 days, while the medium used for fungal growth was incubated at 25°C for 14 days.

Safety test

The safety of the vaccines was evaluated by administering double the recommended dose of each vaccine subcutaneously to 5 rabbits lacking antibodies. The vaccines were monitored for possible adverse reactions, both locally at site of injection and systemically through monitoring any body temperature changes or generalized abnormal changes in the rabbits according to the directions of [23].

Evaluation of the prepared vaccines

- Rabbits divided into five groups each of 5 rabbits.

Group 1: Inoculated with 3ml of vaccine No.1

Group 2: Inoculated with 3ml of vaccine No. 2.

Group 3: Inoculated with 2ml of vaccine No. 2.

Group 4: Inoculated with 1ml of vaccine No. 2.

Revaccination after 3 weeks from first dose by same dose in each group.

Group 5: kept as control negative group.

- Sheep divided into four groups each of 5 sheep.

Group 1: Inoculated with 3ml of vaccine No.1

Group 2: Inoculated with 3ml of vaccine No.2

Group 3: Inoculated with 2ml of vaccine No.2

Revaccination after 3 weeks from first dose by same dose in each group.

Group 4: kept as control negative group.

- Cattle divided into four groups each of 5 cattle.

Group 1: Inoculated with 5 ml of vaccine No.1

Group 2: Inoculated with 5 ml of vaccine No.2

Group 3: Inoculated with 3 ml of vaccine No.2

Revaccination after 3 weeks from first dose by same dose in each group.

Group 4: kept as control negative group

All of these animals were kept under observation. Blood samples were collected on days 0, and 21 from second dose. The sera were stored at – 25°C until evaluation of antibody titres.

Evaluation of antitoxin titres

Serum antibody levels against different clostridial components included in the vaccines were assessed by using toxin neutralization test (TNT) in Swiss white mice. Titres were expressed in antitoxin units per ml (AU/ml). The minimum protective level of *C. perfringens* type A was 4 AU/ml [24], B was 10 AU/ml and D was 2 AU/ml. *C. septicum* was 1 AU/ml, *C. novyi* type B was 0.5 AU/ml and *C. tetani* was 2.5 AU/ml according to [25]**.** While antibodies against *C. chauvoei* were determined by plate agglutination test according [26]**.** Animals having agglutination titre of 2µl or less are considered protective.

Statistical analysis

Descriptive analysis of antitoxin titre for each component of polyvalent clostridial vaccine and data management presented as the mean ± Confidence Interval 95% of the mean and inference analysis between groups of animals and comparing the dose of vaccine done by ANOVA after performed normality test. All statistical analyses were performed by R version 2024.12.0+467. Statistical significance was set at $p < 0.05$.

Results

Quality control results of prepared vaccines

Results of sterility tests

No detection of growth has appeared after the tested vaccine inoculation in different types of media. These results proved the purity of the vaccine (no bacterial or fungal growth).

Result of safety

At the end of the safety study, every rabbit cohort was healthy and alive, with no negative reactions seen, and their food or water intake remained unchanged.

Result of vaccine evaluation

In rabbit

As shown in table (1) mean antitoxin titre against alpha toxin of *C. perfringens* type A in the group (1) received polyvalent clostridial vaccine alum adjuvant with 3 ml dose 4.4 IU/ml (CI 95%, 3.88 to 4.91), while rabbit in group (2) there was an increase in titre by 1.2 (CI 95%, 0.46 to 1.93), in addition in group (3) which received 2 ml dose of the vaccine the antibody titre was reduced by -0.8 (CI 95%, -1.5 to -0.06) from group (1). In group (4) there was great reduction in antibody titre -3 (CI 95%, -3.7 to -2.2).

Mean antitoxin titre against beta toxin of *C. perfringens* shown that in group (1) was 10.8 IU/ml (CI 95%, 1.36) while in groups (2) there was increased in titre 2.4 (CI 95%, 1.33). In group (3) there was no difference in titre with group (1).

Mean antibody titre against epsilon toxin of *C. perfringens* shown that in group (1) was 5.4 IU/ml (CI 95%, 0.68) while in groups (2) there was increased in titre 1.2 (CI 95%, 0.66). In group (3) there was slight reduction about (-0.2) in titre compared to group (1).

Mean antitoxin titre against alpha toxin of *C. novyi* type B shown that in group (1) was 4.2 IU/ml (CI 95%, 1.03) while group (2) increased in titre 1.2 (CI 95%, 0.79) and group (3) increased about 0.4 (CI 95%, 0.79) in titre compared to group (1).

Mean antitoxin titre against alpha toxin of *C. septicum* shown that in group (1) was 3.0 IU/ml (CI 95%, 0.87) while group (2) increased in titre 1.2 (CI 95%, 0.84) and in group (3) there was increased about 0.6 (CI 95%, 0.84) in titre compared to group (1).

Mean antitoxin titre against tetanus toxin shown that in group (1) was 4.0 IU/ml (CI 95%, 1.24) while group (2) increased in titre 1.2 (CI 95%, 0.95) . In group (3) there was reduced in titre about 0.4 (CI 95%, 0.95) in titre compared to group (1).

In sheep

Through the results shown in table 2, mean antitoxin titre against alpha toxin of *C. perfringens* type A in the group (1) received polyvalent clostridial vaccine alum adjuvant with 3 ml dose gave 5.2±1.03 IU/ml, while in group (2) which received polyvalent clostridial vaccine Montanide gel adjuvant with 3 ml dose give 7.6±0.68, in addition in group (3) which received 2 ml dose of the vaccine give 5.6±0.68. In group (4) there was great reduction in antibody titre (0.3±0.34).

Mean antitoxin titre against beta toxin of *C. perfringens* shown that in group (1) was 11±1.96 IU/ml, while in group (2) gave 15.6. In group (3) the antibody titre was 11.6±1.42. In group (4) there was great reduction in antibody titre which was 0.5±0.62.

Mean antitoxin titre against epsilon toxin of *C. perfringens* shown that in group (1) was 3.4±1.41 IU/ml. while in group (2) gave 5.6±1.42. In group (3) the antibody titre was 4.6±1.11. In group (4) there was great reduction in antibody titre (0.2±0.34)

Mean antitoxin titre against alpha toxin of *C. novyi* type B shown that in group (1) was 5±1.24 IU/ml. while in group (2) gave 6.6±1.11, In group (3) the antibody titre was 5.2±0.55. In group (4) there was great reduction in antibody titre (0.1±0.11)

Mean antitoxin titre against alpha toxin of *C. septicum shown* that in group (1) was 3.6±1.4 IU/ml while in group (2) gave 5.6±1.88, in group (3) the antibody titre was 4.0±0.88. In group (4) there was great reduction in antibody titre (0.2±0.34)

Mean antitoxin titre against tetanus toxin shown that in group (1) was 3.8 ±1.62 IU/ml while in groups (2) gave 5.4±1.42, In group (3) the antibody titre was 3.4±1.41. In group (4) there was great reduction in antibody titre (0.3±0.55)

In cattle

As shown in table (3) mean antitoxin titre against alpha toxin of *C. perfringens* type A in the group (1) received polyvalent clostridial vaccine alum adjuvant with 3 ml dose give 3.8±1.04 IU/ml, while in group (2) which received polyvalent clostridial vaccine Montanide gel adjuvant with 3 ml dose give 6.0±0.88, in addition in group (3) which received 2 ml dose of the vaccine give 3.8±0.55. In group (4) there was great reduction in antibody titre (0.3±0.34).

Mean antitoxin titre against beta toxin of *C. perfringens* shown that in group (1) was 9.4±1.11 IU/ml, while in groups (2) gave 13.4±1.11. In group (3) the antibody titre was 11±1.24. In group (4) there was great reduction in antibody titre which was 0.5±0.62.

Mean antitoxin titre against epsilon toxin of *C. perfringens* shown that in group (1) was 2.6±0.68 IU/ml. while in groups (2) gave 4.8±1.03. In group (3) the antibody titre was 3.2±1.03. In group (4) there was great reduction in antibody titre (0.2±0.34)

Mean antitoxin titre against alpha toxin of *C. novyi* type B shown that in group (1) was 4.2±1.03 IU/ml. while in groups (2) gave 5.8±1.o3, In group (3) the antibody titre was 4.6±0.68. In group (4) there was great reduction in antibody titre (0.6±0.11)

Mean antitoxin titre against alpha toxin of *C. septicum* shown that in group (1) was 2.6±0.68 IU/ml while in groups (2) gave 4.8±1.03, In group (3) the antibody titre was 3.6±0.68. In group (4) there was great reduction in antibody titre (0.2±0.34)

Mean antitoxin titre against tetanus toxin shown that in group (1) was 3.0 ±0.87 IU/ml while in groups (2) gave 4.8±1.03, In group (3) the antibody titre was 3.6±0.68. In group (4) there was great reduction in antibody titre (0.2±0.34)

Discussion

Prevention of clostridial diseases with inactivated vaccines has been documented for many years in sheep and cattle [27] and is considered a critical point in the control of clostridial infection in different animals. Thus, using a novel adjuvant and different vaccinal volume dosages, vaccination trials have been conducted in cattle, sheep, and rabbits to investigate and quantify a particular antibody response to clostridial antigen.

For many years, it has been known that inactivated vaccine can prevent clostridial infections in sheep, goats and cattle [28].

Vaccine adjuvants play a critical role in enhancing the immune response to vaccines and improving their efficacy. Montanide is a family of

adjuvants used in the development of vaccines and other immunological applications. These adjuvants are designed to enhance the immune response to an antigen and make vaccines more effective [29]. Montanide Gel 01 ™ as an adjuvant aims to increase the effectiveness of aqueous- type vaccines by acting as a water-based adjuvant of immunity. It is a water-soluble dispersion of a synthetic polymer with a low Montanide adjuvant content that falls under the high molecular weight polyacrylic acid category. Thus, the purpose of this work is to create an inactivated polyvalent clostridial vaccine using 10% Montanide Gel 01 ™ as an adjuvant and compare the immunological response in rabbit, sheep and cattle to that of the alum precipitated inactivated vaccine. Also, consider reducing the vaccinal dose. In this study, two vaccine formulae were prepared to determine which provides the greatest efficacy. All the toxoid used was concentrated with ammonium sulphate 70 %. The first one is the inactivated polyvalent clostridial vaccine of adjuvanted with aluminum potassium sulphate (Alum) (local vaccine) (vaccine no. 1) and 2nd one is inactivated polyvalent clostridial vaccine adjuvanted with Montanide Gel 01 ™ (vaccine no.2). All vaccines were evaluated in rabbits, sheep and cattle with different doses by using TNT.

The sterility testing results of the prepared vaccines showed that they were free from any bacterial, or fungal contamination on inoculated media, and this agreed with what is recommended by [22]. It was also found that the use of 2x field dose in rabbit was safe. These findings met the recommendations and requirements of safety of [23]

As indicated in tables (1) and (2), the neutralized antibody titre in sera of rabbits and sheep reached the USDA- recommended minimum permissible limit for group 1 (vaccinated with 3 ml alum adjuvant vaccine (local vaccine)) and group 3 (vaccinated with 2 ml Montanide Gel 01™ adjuvant vaccine) for almost all of the vaccine's components. While in group 2 which received a dosage of 3 ml of the Montanide Gel 01™ adjuvant vaccination, exhibited a noticeably greater antibody titre than the other two groups.

The results presented in table (3) demonstrated that although cattle's response to the polyvalent clostridial vaccine adjuvanted with either alum or Montanide Gel 01™ adjuvant was lower than that of rabbits and sheep for all antigen vaccine components, they achieved antibody level above the minimum protective level and this results agreed with [30] who found that the immune response of cattle for polyvalent clostridial vaccine was less than that of sheep.

From the previous results, it was noticed that using polyvalent clostridial vaccine adjuvanted with Montanide Gel 01 ™ gave better immunity than the local vaccine at a lower vaccinal dose when it was evaluated in rabbits, sheep and cattle. These results agreed with [12] who observed that using of Montanide Gel 01 ™ as adjuvant gave sufficient early immune response in vaccinated cattle and with [31] who found that Montanide Gel 01 ™ adjuvant Entero-3 vaccine gave an intense immune response and showed prolongation of antibody secretion in comparison to the aluminum hydroxide gel vaccine. Also, our results agreed with [32, 13, 14 and 33] who reported that antibodies production induced by Montanide Gel 01™ based vaccine was higher than aluminum based vaccines. Also, these results agreed with [34] who mentioned that alum precipitated vaccine gave poor result than aluminum hydroxide gel and this may indicate that localized antigen in case of alum adjuvant vaccine dose not continuously stimulate the immune response. It has been hypothesized that aluminum adjuvanted antigens is rapidly encapsulated into a granuloma, thus excluding it from the antibody producing mechanism [35]. The use of concentrated clostridial toxoids besides using Montanide Gel 01 ™ as adjuvant led to a reduction of in the dose of vaccine from 5 ml and 3 ml for cattle and sheep respectively to became 3ml and 2 ml per dose which reduces stress on the animal.

Conclusion

Regarding to the previous results, it could be concluded that, Montanide Gel 01™ was highly immunogenic when used as an adjuvant in preparation of polyvalent clostridial vaccine through inducing a higher level of antibody titre in comparison with poly valent clostridial vaccine adjuvanted with alum. Also, using Montanide Gel 01 ™ with concentrated toxoid had a great impact on reducing the vaccinal dose.

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Declaration of Conflict of Interest

The authors declare that there is no potential conflict of interest.

Ethical of approval

This study was approved by the Agricultural Research Center- Institutional Animal Care and Use Committee (ARC- IACUC). It was reviewed and supervised by the Ethical Committee of Veterinary Serum and Vaccine Research Institute (VSVRI) (ARC- VSVRI- 2- 25).

TABLE 1. Antitoxin titre expressed by IU/ml in sera of rabbits after 14 days from second dose of polyvalent clostridial vaccine.

Groups	Antitoxin titres in sera of vaccinated rabbits expressed as (IU/ml)						Agglutination titre of *C. chauvoei* (µl/ml)
	Alpha toxin C. perfringens A	*Beta toxin C. perfringens B*	*Epsilon toxin C. perfringens D*	*Alpha toxin of C. novyi type B*	*Alpha toxin of C. septicum*	*Tetanus toxin of C. tetani*	
Group 1	4.4 ±0.68	10.8 ±1.36	5.4 ±0.68	4.2 ±1.03	3.0 ±0.87	4 ±1.24	0.05
Group 2	5.6 ±0.68	13.2 ±1.61	6.6 ±0.68	5.4 ± 0.68	4.2 ±1.03	5.2 ±1.03	0.05
Group 3	3.6 ±0.68	10.8 ±1.03	5.2 ±0.55	4.6 ± 0.68	3.6 ±0.68	3.6 ±0.68	0.05
Group 4	1.4 ±0.68	5.6 ±1.11	1.4 ±0.68	1.2 ± 0.55	1.4 ±0.68	1.2 ±0.55	0

TABLE 2. Antitoxin titre expressed by IU/ml in sera of sheep after 14 days from second dose of polyvalent clostridial vaccine.

Groups	Antitoxin titres in sera of vaccinated rabbits expressed as (IU/ml)						Agglutination titre of *C. chauvoei* (µl/ml)
	Alpha toxin C. perfringens A	*Beta toxin C. perfringens B*	*Epsilon toxin C. perfringens D*	*Alpha toxin of C. novyi type B*	*Alpha toxin of C. septicum*	*Tetanus toxin of C. tetani*	
Group 1	5.2±1.03	11±1.96	3.4±1.41	5±1.24	3.6±1.4	3.8±1.62	0.05
Group 2	7.6±0.68	15.6±1.42	5.6±1.42	6.6±1.11	5.6±1.88	5.4±1.42	0.05
Group 3	5.6±0.68	11.6±1.42	4.6±1.11	5.2±0.55	4.0±0.88	3.4±1.41	0.05
Group 4	0.3±0.34	0.5±0.62	0.2±0.34	0.1±0.11	0.2±0.34	0.3±0.55	0

TABLE 3. Antitoxin titre expressed by IU/ml in sera of cattle after 14 days from second dose of polyvalent clostridial vaccine.

Groups	Antitoxin titres in sera of vaccinated rabbits expressed as (IU/ml)						Agglutination titre of *C. chauvoei* (µl/ml)
	Alpha toxin C. perfringens A	*Beta toxin C. perfringens B*	*Epsilon toxin C. perfringens D*	*Alpha toxin of C. novyi type B*	*Alpha toxin of C. septicum*	*Tetanus toxin of C. tetani*	
Group 1	3.8 ± 1.04	9.4 ± 1.11	2.6 ± 0.68	4.2 ± 1.03	2.6 ± 0.68	3.0 ± 0.87	0.05
Group 2	6.0 ± 0.88	13.4 ± 1.11	4.8 ± 1.03	5.8 ± 1.03	4.8 ± 1.03	4.8 ± 1.03	0.02
Group 3	3.8 ± 0.55	11 ± 1.24	3.2 ± 1.03	4.6 ± 0.68	3.6 ± 0.68	2.8 ± 1.03	0.04
Group 4	0.3 ± 0.34	0.5 ± 0.62	0.2 ± 0.34	0.6 ± 0.11	0.2 ± 0.34	0.3 ± 0.55	0

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استخدام جل المونتانيد كمحفز مناعي للقاح الكلوستريديا الجامع

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الملخص

توجد الكلوستريديا في التربة وفي أمعاء الحيوانات. الكلوستريديا تُنتج مجموعة واسعة من السموم المسؤولة عن أنواع مختلفة من الأمراض. لطالما استُخدمت التطعيمات المثبطة للوقاية من أمراض الكلوستريديا في الأغنام والماشية، وتُعتبر هذه الممارسة أساسيةً في إدارة عدوى الكلوستريديا لدى العديد من الحيوانات. تسعى هذه الدراسة إلى زيادة مناعة لقاح الكلوستريديا متعدد العترات المثبط المُصنّع محليًا، بهدف توفير حماية طويلة الأمد للحيوانات المُلقحة من خلال تركيز سموم الكلوستريديا باستخدام كبريتات الأمونيوم ومادة مساعدة بديلة مثل جل المونتانيد. تم تحضير لقاحين: اللقاح رقم 1 (لقاح الكلوستريديا متعدد العترات مضاف إليه الشبة (اللقاح المحلي)) واللقاح رقم 2 (لقاح الكلوستريديا متعدد العترات مضاف إليه جل المونتانيد)، وتم تقييمهما على الأرانب والأغنام والأبقار. بعد تركيز سموم الكلوستريديا واستخدام جل المونتانيد كمادة مساعدة جديدة، خُفِّضت جرعة اللقاح من 5 مل إلى 3 مل للأبقار، ومن 3 مل إلى 2 مل للأغنام لكل جرعة. كما كانت مستويات الأجسام المضادة ضد أحد مكونات لقاح الكلوستريديا الجامع أعلى بشكل ملحوظ مقارنة بلقاح الكلوستريدي الجامع المحلي.

الكلمات الدالة: عترات الكلوستريديا، التركيز، جل المونتانيد، جرعة اللقاح.