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Comparative Immunological Studies for Evaluation Enterotoxemia Vaccine in Rabbit

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ABSTRACT

Enterotoxemia caused by *Clostridium perfringens* is an important disease in rabbits. *Clostridium perfringens* type A induces severe diarrhea, bloat, and high mortalities, especially among weaned rabbits. This study investigated the potency of inactivated vaccine formulations that protect rabbits against *Clostridium perfringens* type A by preparing two monovalent vaccines with different adjuvants (Montanide gel 01^{TM} and aluminum hydroxide gel). Three groups of rabbits consisted of a control group that received 2mL phosphate buffer saline subcutaneously and two groups injected with 2mL of the prepared monovalent vaccines subcutaneously in 2 doses at three weeks intervals with two different adjuvants (Montanide gel and aluminum hydroxide gel). Antibody titers of the vaccinated rabbits were determined up to 11 months using ELISA and serum neutralization tests. The aluminum hydroxide gel vaccine was protected till 6 months with a protection rate of 80%, and the Montanide gel 01 TM vaccine was protected till 10 months with a protection rate of 90%. It is concluded that the Montanide gel adjuvant vaccine has a longer-lasting immunity than the aluminum hydroxide gel vaccine.

Key words: Aluminum hydroxide gel, *Clostridium perfringens* type A, Montanide gel, Serum neutralization test, ELISA

INTRODUCTION

Clostridium spp. is a normal inhabitant in the animal's intestinal tract, harmless until it activates and releases its toxin under stress conditions or changes in diet (Asadi et al. 2023). *Clostridium perfringens* was related to specific enteric diseases in animals and humans named foodborne diseases which are associated with specific symptoms like diarrhea, enterotoxaemia, and enteritis (Grenda et al. 2023). In animals, enterotoxemia is frequently the most common disease caused by *Clostridium perfringens* (Uzal et al. 2018). *Clostridium perfringens* is a pathogen responsible for causing enterotoxaemia, enteritis, and gangrene in both humans and animals (Mehdizadeh Gohari et al. 2021). *Clostridium perfringens* secrets enterotoxins that are responsible for inducing severe lesions and damage in rabbit's intestinal tract tissues associated with accumulation of fluid (Garcia et al. 2014). Diab et al. (2003) isolated *Clostridium perfringens* type A toxin from caecal contents of dead rabbits suffering from diarrhea with enterotoxaemia at different farms. Al-Jindan et al. (2023) isolated a strain of *Clostridium perfringens* from a rabbit fecal sample suffering from severe gastrointestinal disturbance. *Clostridium perfringens* type A was an important pathogen that responsible of causing acute enterotoxaemia and bloat in rabbits accompanied by high mortalities that may reach to 50% especially at age 5-7 weeks and significant economic losses, also this strain was classified as multi-drug resistant as antibiotic treatment ineffective. As a result of this finding, vaccination was the proper choice for protection. Vaccine production progress is associated with choosing a proper adjuvant that elaborates the long duration of high immunity. The effective factor in vaccine manufacture as an adjuvant which was responsible for stimulating both humoral and cellular immunity (Dalsgaard et al. 1990). Aluminum compounds widely used as adjuvants specifically in manufacturing of human vaccines for 70 years and more based on their ability to enhance antibody production (Ulanova et al. 2001). Aluminum adjuvant vaccines are widespread and are related to excellent safety, cheap availability and a long-life shelf (Laera et al. 2023). Aluminum adjuvanted vaccines were used in both human and veterinary vaccines for a long time (Pérez et al. 2012; Di Pasquale et al. 2015). Also, aluminum hydroxide adjuvant was used in many licensed vaccines such as

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COVID-19 (Laera et al. 2023). Aluminum hydroxide adjuvants may cause adverse reactions if the vaccines are stored under specific conditions like unfavorable temperatures (He et al. 2015). Montanide gel is a polymer-adjuvant that is already used in vaccine production due to its safety and high immune response (Deville et al. 2011; Téllez-Martínez et al. 2019). Montanide gel 0.1^{TM} is a recent water-based adjuvant that improved vaccine efficacy. It elicits high long-duration immunity without any adverse effects (Parker et al. 2009). The Montanide Gel 01 ^{TM} was safer than oil adjuvants and fewer adverse effects were observed in vaccinated animals (Matsiela et al. 2022).

The aim of the current study was to determine and compare the potency of the prepared inactivated *Clostridium perfringens* type A monovalent vaccines by adding Montanide gel and aluminum hydroxide gel as adjuvants.

MATERIALS AND METHODS

Ethical approval

All procedures are performed according to Egyptian animal ethical standards of the committee of national research. Ethical issues are reviewed according to the ethics guidelines and protocol of (NLQO) and by the Ethical Committee of veterinary serum and vaccine research.

Bacterial strain

Clostridium perfringens type A isolate was identified by the committee of the institute VSVRI from dead rabbits that suffered from bloat and severe diarrhea. The strain identification was dependent on culture characters, Nagler test, dermonecrotic reaction, and toxin neutralization test (Eyre 2009).

Vaccine preparation

The lyophilized isolated strain was rehydrated in cooked meat medium and incubated under the anaerobic condition at 37°C then examined on sheep blood agar aerobically and anaerobically at 37°C for 24 hours. Then transferred to the starter primary peptone medium and incubated for 4 hours then transferred to the secondary production medium containing 1% sucrose for 4-5 hours at pH 8 at 37°C to achieve optimum growth for toxin production and filtered by using a Millipore filter for cell separation. According to Fu et al. (2004) the toxin minimal lethal dose (MLD) was determined in mice by measuring the minimum amount of the alpha toxin which was responsible for killing two mice from three within two days. The prepared toxin was inactivated by the addition of 0.5% formalin for 7 days till inactivation completely occurred. The Merthiolate (thiomersal 0.01%) was added as a preservative agent on the second day of formalin addition. Then the toxoid was filtered by using a Millipore filter for cell separation, then the adjuvant was added. The prepared toxoid was divided into two parts one of them added aluminum hydroxide gel adjuvant (20% of the solution) and the other was added with Montanide gel adjuvant (20% of the solution), both vaccines were homogenized by magnetic stirring then the vaccine was examined for safety and sterility.

Quality control of the prepared vaccines

Sterility test

The test was carried out by the British Pharmacopeia Commission (2007). One milliliter (ml) of the prepared toxoid was transferred into two tubes called fluid thioglycollate and nutrient agar slope; incubated aerobically at 37°C and 1mL of the prepared toxoid was incorporated into cooked meat broth and anaerobically incubated at 37°C. Two Sabouraud agar tubes were inoculated, one incubated at 37°C and another incubated at room temperature. All tubes were observed for ten days for bacterial or fungal growth, the vaccine is considerably passable if no growth appears in any of the inoculated media.

Safety test

There were two safety tests, the first one was carried out by injecting five male Swiss white mice (22g) with 0.5mL of toxoid intraperitoneally. The second safety test was carried out by injection of five male Guinea pigs (250-300g) intramuscular with 3mL of the prepared toxoid. The inoculated animals were observed for 14 days.

The potency of the vaccines

Thirty representative Bosket rabbits (2-2.5kg) free from *Clostridium perfringens* type A were used. The experimental rabbits were reared in a hygienic environment with restricted precautions, suitable ventilation, and sterilized water (Bennegadi et al. 2003). The rabbits were assigned to three groups (10/group); group (1) was vaccinated with aluminum hydroxide gel adjuvanted toxoid, group (2) was vaccinated with Montanide gel adjuvanted toxoid, and group (3) served as control group negative unvaccinated group. The groups of vaccinated rabbits were injected with 2 doses of 2ml (S/C) with intervals of three weeks of the prepared vaccines and the control group was injected with phosphate buffer saline. Blood samples (2mL) were collected from the ear vein after vaccination monthly and the sera were separated, pooled, and kept in sterile screw-capped bottles at -20ºC until determination of humoral immune response by serum neutralization test and ELISA.

Determination of humoral immunity

Serum neutralization test

In this test serially dilution of serum samples occurs with the addition of an equal volume of alpha toxin incubated for 1 hour at 37°C then injected into two male Swiss white mice I/V with 0.2 mL from each dilution and observed mice for a day. The highest serum dilution that causes mice death was observed as the antibody titer measured by the International Unit (IU) (Barile et al. 1970).

Enzyme-linked immunosorbent assay

The assay was measured in accordance with Lee et al. (2012) and calculation of values in standard curve linear regression that represented values of antibody titer (Grabowska et al. 2002) by using of weighted parallel line model $Y=\alpha+B$ x is a straight line in which Y was Logarithm of dilution absorbance, α and B represented observed data and X was unknown value.

Challenge test

This test was done according to El-Maghraby et al. (2019) after the second week post boostering. The vaccinated groups and the unvaccinated control group were challenged by S/C injection with 0.1mL of the virulent alpha toxin of *Clostridium perfringens* type A strain and observed for 15 days post-challenge and mortality rate was recorded till the end of the experiment. Then the protection percentages of the prepared vaccines were calculated.

Statistical analysis

One-way ANOVA used to analyze the data by SPSS version 25. Differences were considered to be significant for values of $P<0.05$.

RESULTS

Minimum lethal dose

The local isolated strain was confirmed to be *Clostridium perfringens* type A and the Minimum Lethal Dose (MLD) of the toxin production media gives 100 MLD/mL.

Sterility and safety tests of the prepared toxoid

Sterility tests

All sterility tubes are free from any bacterial and fungal growth aerobically and anaerobically.

Safety tests

All mice and guinea pigs survived without showing any symptoms of the disease.

Determination of antibody titer

No antibodies against alpha toxoid were detected in the sera of rabbits before immunization. Standard alpha antitoxin (National Institute for Biological Standards and Control, United Kingdom) was used for the determination of SNT and ELISA. Table 1 and Fig. 1 indicated that the mean *Clostridium perfringens* type A alpha antitoxin titers as measured by ELISA in rabbits received vaccine with aluminum hydroxide gel adjuvant was 2.66 and titer in rabbits received vaccine with Montanide gel adjuvant was 3.63. It is clear that the mean antibody titers of both vaccinated groups measured by ELISA increased in the first month post-vaccination and then declined gradually till they reached the non-protective level, which is less than 0.5IU. In the case of the aluminum hydroxide gel adjuvanted vaccine, the antibody titer level decreased after seven months (0.54), while in the Montanide gel adjuvant vaccine, the titer decreased after 10 months (0.50). The results in Table 2 and Fig. 2 showed that mean antibody titer measured in vaccinated rabbits by SNT confirms the results of ELISA. It is clear that the mean antibody titers of both vaccinated groups increased after vaccination and then decreased gradually till reached to the non-protective level (less than 0.5IU) after seven months in the aluminum hydroxide gel adjuvant vaccine group, compared to ten months among Montanide gel adjuvant vaccine group. Table 3 illustrates that the protection rate of the prepared vaccines was 90% and 80% among the Montanide gel adjuvant vaccine group (G2) and aluminum hydroxide gel adjuvant vaccine group (G1) respectively. In all vaccinated rabbits a strong antibody response against alpha toxoid was detected six months after immunization.

Table 1: Determination of humoral immunity of the prepared vaccines against *Clostridium perfringens* type A in rabbits by ELISA

Duration(months)	Groups		
	G1	G2	G3
1	2.66±0.64	3.63±0.88	0
2	1.80±0.73	2.62±0.78	0
3	1.33±0.48	1.95±0.57	0
4	1.30±0.47	1.69±0.32	0
5	0.76±0.18	1.31±0.22	0
6	0.59±0.09	1.06±0.30	0
7	0.52±0.07	0.84±0.16	0
8	0.43±0.04	0.70±0.11	0
9	0	0.54±0.09	0
10	0	0.50±0.089	0

ELISA: Enzyme-linked immunosorbent assay, G1: Vaccinated with *C. perfringens* type A toxoid adjuvanted with aluminum hydroxide gel vaccine, G2: Vaccinated with *C. perfringens* type A toxoid adjuvanted with Montanide gel vaccine and G3: control unvaccinated group.

Table 2: Determination of humoral immunity of the prepared vaccines against *Clostridium perfringens* type A in rabbits by serum neutralization test (SNT)

Duration (months)	Groups		
	G1	G2	G3
1	2.5±0.80	3.3±0.90	0
2	1.70±0.78	2.35±0.83	0
3	1.32±0.64	2.05±072	0
4	1.30±0.60	1.5±0.44	0
5	1.10±0.30	1.3±0.45	0
6	0.76±0.24	1.2±0.33	0
7	0.45±0.26	1.0±0.30	0
8	0.40±0.20	0.95±0.15	0
9	0	0.70±0.24	0
10	0	0.5±0.15	0

SNT: Serum neutralization test, G1: Vaccinated with *C. perfringens* toxoid adjuvanted with aluminum hydroxide gel vaccine, G2: Vaccinated with *C. perfringens* toxoid adjuvanted with Montanide gel vaccine. and G3: control unvaccinated group.

Table 3: Results of the challenge test among the vaccinated rabbits using *Clostridium perfringens* type A.

Groups	No. of challenged rabbits	Dead rabbits/ total no.	Protection rate (%)
G1	10	2/10	80
G2	10	1/10	90
G3	10	8/10	20

G1: Vaccinated with *C. perfringens* toxoid adjuvanted with aluminum hydroxide gel vaccine, G2: Vaccinated with *C. perfringens* toxoid adjuvanted with Montanide gel vaccine, and G3: Unvaccinated control group.

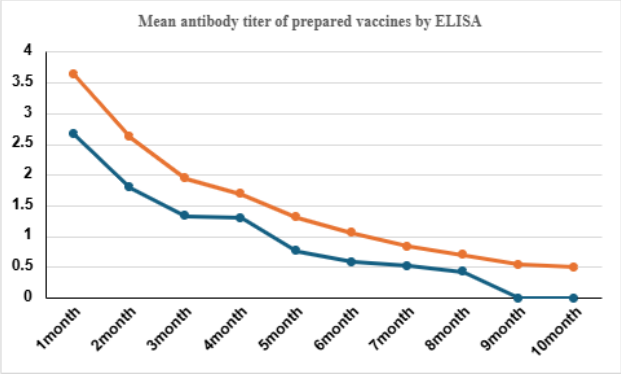

Fig. 1: Mean antibody titer of aluminum hydroxide gel compared with Montanide gel *Clostridium perfringens* type A vaccines measured by ELISA. Aluminum hydroxide gel (blue)and Montanide gel (orange).

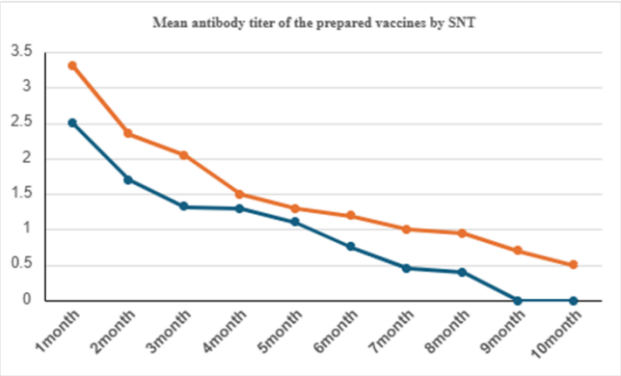

Fig. 2: Mean antibody titer of aluminum hydroxide gel compared with Montanide gel *Clostridium perfringens* type A vaccines measured by serum neutralization test (SNT). Aluminum hydroxide gel (blue) and Montanide gel (orange).

DISCUSSION

The rabbit industry has been developed to face the human requirement for protein with great attention to the important reason for economic losses like enterotoxaemia (Finzi and Amici 1991). Enteritis was a common disease in rabbits with clinical signs such as diarrhea, enterotoxemia, and death. *Clostridium perfringens* is an anaerobic bacterium that is characterized by forming spores and causing an acute disease named enterotoxaemia **(**Rosskopf-Streicher et al. 2004). It is classified into seven types (A, B, C, D, and E) depending on the production of four major toxins (alpha, beta, epsilon, and iota) (El-Jakee et al., 2013). *Clostridium perfringens* is a virulent bacterium, and its pathogenicity refers to releasing a potent toxin that has a great role in inducing enteric diseases in domestic animals with a high significant mortality rate in rabbits (Romero et al. 2011).

Vaccination is one of the most striking health achievements of animal history. The availability of an effective *Clostridium perfringens* vaccine could improve profitability for the rabbit's producers. The vaccine efficacy is determined by the availability of both antigen and adjuvants for induction immune response effectively (Facciolà et al. 2022). The adjuvant is a substance that plays an important role in enhancing immune responses against a vaccine (He et al. 2015). Many adjuvants are available now in vaccine production as aluminum hydroxide gel, and Montanide gel (Nooraei et al. 2023; Verma et al. 2023). The aluminum hydroxide adjuvants used in humans for many years as a result of its safety (Laera et al. 2023). Aluminum hydroxide stimulates the production of cytokines which is responsible for T cell activation (Ulanova et al. 2001). *Brucella abortus* S19 aluminum hydroxide gel adjuvanted vaccine exhibited high protective potency greater than standard S19 vaccine (Jain et al. 2015). Aluminum hydroxide adjuvanted vaccines are beneficial for vaccine manufacturing (He et al. 2015). Montanide is a new ready-to-use adjuvant which induces long-lasting responses in animals (Khorasani et al. 2016). Inactivated Rift Valley fever vaccine adjuvanted with Montanide gel 01™ Gel induced protective antibody titers in sheep after seven days of primary inoculation, which lasted for 13 months (Abd El Rahman et al. 2020). Also, Hamdi et al. (2020) and Wolff et al. (2021) published data on the evaluation of an inactivated lumpy skin disease vaccine in cattle using Montanide adjuvant from Seppic (A company supplies specialty chemical products for health and wellbeing) as a vaccine delivery system that showed Montanide gel gives high immune response with long duration.

This study is designed to evaluate the potency of both prepared vaccines with Montanide gel and aluminum hydroxide gel vaccines for the control of enterotoxaemia and bloat diseases in weaned rabbits. The quality control of the prepared vaccine was conducted according to the British Pharmacopeia Commission (2007). No evidence of local side effects among rabbits vaccinated with the prepared toxoid was observed. The antibody titer against alpha toxin was measured by ELISA and SNT. It is clear that rabbits vaccinated with Montanide gel 01 ™ showed an increase in antibody titers than that vaccinated with aluminum hydroxide gel. The mean antibody titers of both vaccinated groups increased after vaccination and then decreased gradually till they reached to the non-protective level (less than 0.5IU) (Enany et al. 2014) after seven months in rabbits vaccinated with aluminum hydroxide gel vaccine group (G1), while reached to ten months in rabbits vaccinated with Montanide gel vaccine group (G2). The results showed that the Montanide gel 01 ™ gave a high prolonged immunity with a safe profile in comparison with aluminum hydroxide gel and this finding agreed with the results of the challenge test and the results of Abul Magd et al. (2014) who concluded that the Montanide gel vaccine gave longer and higher immunity in sheep than aluminum hydroxide gel in Rift valley fever vaccine. Abd El Rahman et al. (2020) concluded that Montanide gel 01™ induces a high antibody titration which indicates that it gives long duration of immunity and recommends that it will be useful in vaccine production to enhance high immunity. Guzmán et al. (2021) concluded that Montanide gel 01 ™ was a powerful adjuvant and safer for manufacturing dog vaccines in comparison with oily Montanide ™ ISA 50.

Conclusion

This study concluded that the immune response of Montanide gel 01 ™ was safe, prolonged immunity, and a higher protection rate with no post-vaccination reaction than the immune response of the aluminum hydroxide gel vaccine. It could be suggested that the convenience of using Montanide gel 01™ in the vaccination of sensitive animals like rabbits as it was safe and gave significant immunity with high protection.

Competing interests: The authors declared that this study has no conflict of interest.

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Authors' contributions: El Jakee J. designed experiments and wrote and reviewed the manuscript. Riham Wahied applied the manuscript experiments, performed data analysis and wrote. Reviewed and accepted for the final version by Al.

REFERENCES

Abd El Rahman SE, Abul Magd DM, Atwa MH and Soliman SM, 2020. Evaluation of the cellular and humoral immune

response of sheep vaccinated with inactivated Rift Valley fever vaccine adjuvanted with Montanide Gel. Journal of Applied Veterinary Sciences 5(1): 22-34. https://doi.org/10.21608/javs.2020.21145.1002

Abul Magd DM, Ebeid M, Moustafa AM, Shalakam EMS and Abdel Wahab MG, 2014. Efficacy of Montanide gel inactivated Rvf vaccine in comparison with aluminum hydroxide gel inactivated one. Benha Veterinary Medical Journal 27(1): 239-247.

Al-Jindan R, AlEraky DM, Farhat M, Almandil NB, AbdulAzeez S and Borgio JF, 2023. Genomic insights into virulence factors and multi-drug resistance in *Clostridium perfringens* IRMC 2505A. Toxins 15(6): 359. https://doi.org/10.3390/toxins1506039

Asadi A, Khiav LA, Emadi A and Dadar M, 2023. Evaluation of humoral immune responses against *C. perfringens* epsilon toxin in Iranian sheep and goats after vaccination. Veterinary Animal Science 21: 100305. https://doi.org/10.1016/j.vas.2023.100305

Barile MF, Hardegree MC and Pittman M, 1970. Immunization against neonatal tetanus in New Guinea. Bulletin of the World Health Organization 43(3): 453-459.

Bennegadi N, Fonty G, Millet L, Gidenne T and Licois D, 2003. Effects of age and dietary fiber level on caecal microbial communities of conventional and specific pathogen-free rabbits. Microbial Ecology in Health and Disease 15(1): 23-32. https://doi.org/10.1080/08910600310015574

British Pharmacopeia Commission, 2007. British veterinary pharmacopoeia. The Pharmaceutical Press, London, pp: 112-115.

Dalsgaard K, Hilgers L and Trouve G, 1990. Classical and new approaches to adjuvant use in domestic animals. Advances in Veterinary Science and Comparative Medicine 35:121-160. https://doi.org/10.1016/B978-0-12-039235-3.50011-3

Deville S, Carneaux E, Bertrand F, Cauchard S, Cauchard J and Dupuis L, 2011. Adjuvant formulation for companion animals vaccines. Procedia in Vaccinology 4: 104-112. https://doi.org/10.1016/j.provac.2011.07.015

Diab RA, El-Sehemy MM, Nadia ME, Shafie F and Hussein AZ, 2003. Enterotoxaemia in rabbits and trials for preparing vaccines from the isolated strains. Journal of the Egyptian Veterinary Medical Association 63(2): 59-64.

Di Pasquale A, Preiss S, Tavares Da Silva F and Garçon N, 2015. Vaccine adjuvants: From 1920 to 2015 and beyond. Vaccines 3(2): 320-343. https://doi.org/10.3390/vaccines302032

El-Jakee J, Ata NS, El Shabrawy MA, Abu El Naga ASM, Hedia RH, Shawky NM and Shawky HM, 2013. Characterization of *Clostridium perfringens* isolated from poultry. Global Veterinaria 11(1): 88-94. https://doi.org/gv/gv11(1)13/16.pdf

El-Maghraby AS, Abd El-moneimWS, Abd El-Moneam MM, Khalaf NM, Abo-Dalal SE and Omar LM, 2019. Preparation and evaluation of locally prepared inactivated combined vaccine of rabbit Haemorrhagic disease virus, *Pasteurella multocida* and *Clostridium perfringens* type A. Bioscience Research 16(4): 3973-3986.

Enany ME, Abdalla YA and Wahid RM, 2014. Detection of maternal immunity of enterotoxaemia vaccine of *Clostridium perfringens* type A in serum of pregnant dams and offspring of rabbits. Suez Canal Veterinary Medical Journal XIX(1): 123-136.

Eyre JWH, 2009. The Elements of Bacteriological Technique. A Laboratory Guide for Medical, Dental, and Technical Students. 2nd Ed. Rewritten and Enlarged.

Facciolà A, Visalli G, Laganà A and Di Pietro A, 2022. An overview of vaccine adjuvants: Current evidence and future perspectives. Vaccines 10(5): 81. https://doi.org/10.3390/vaccines10050819

Finzi and Amici A, 1991. Traditional and alternative rabbit breeding systems for developing countries. Rivista di Agriculture Subtropicale e Tropicale, (1): 103-125.

Fu SW, Xue J, Zhang YL and Zhou DY, 2004. Simplified purification method for *Clostridium difficile* toxin A. World Journal of Gastroenterology 10 (18): 2756-2758. https://doi.org/10.3748%2Fwjg.v10.i18.2756

Garcia JP, Li J, Shrestha A, Freedman JC, Beingesser J, McClane BA and Uzal FA, 2014. *Clostridium perfringens* type A enterotoxin damages the rabbit colon Infection and Immunology 82(6): 2211-2218. https://doi.org/10.1128/iai.01659-14

Grabowska K, Wang X, Jacobsson A and Dillner J, 2002. Evaluation of cost-precision rations of different strategies for ELISA measurement of serum antibody levels. Journal of Immunological Methods 271(1-2):1-15. https://doi.org/10.1016/S0022-1759(02)00334-4

Grenda T, Jarosz A, Sapała M, Grenda A, Patyra E and Kwiatek K, 2023. *Clostridium perfringens*-opportunistic foodborne pathogen, its diversity and epidemiological significance. Pathogens 12(6): 768. https://doi.org/10.3390/pathogens12060768

Guzmán PEE, Martin AP, Soto YB, Luis F, Bravo L, Herrera WF, Heredia CP, Pérez LG, González N and Alina Rodríguez-Mallon MPE, 2021. Comparison of Montanide™ GEL 01 and oily MontanideISA50 in presenting a peptide to the immune system of dogs. Journal of Veterinary Medicine and Animal Health 13(1):28-33. https://doi.org/10.5897/JVMAH2020.0867

Hamdi J, Boumart Z, Daouam S, El Arkam A, Bamouh Z, Jazouli M, Tadlaoui KO, Fihri OF, Gavrilov B and El Harrak M, 2020. Development and evaluation of an inactivated lumpy skin disease vaccine for cattle. Veterinary Microbiology 245: 108689. https://doi.org/10.1016/j.vetmic.2020.108689

He P, Zou Y and Hu Z, 2015. Advances in aluminum hydroxide-based adjuvant research and its mechanism. Human Vaccines and Immunotherapeutics 11(2): 477-88. https://doi.org/10.1080/21645515.2014.1004026

Jain L, Rawat M, Prajapati A, Tiwari AK, Kumar B, Chaturvedi VK, Saxena HM, Ramakrishnan S, Kumar J and Kerketta P, 2015. Protective immune-response of aluminum hydroxide gel adjuvanted phagelysate of *Brucella abortus* S19 in mice against direct virulent challenge with B. abortus 544. Biologicals 43(5):369-376. https://doi.org/10.1016/j.biologicals.2015.06.006

Khorasani A, Madadgar O, Soleimanjahi H, Keyvanfar H and Mahravani H, 2016. Evaluation of the efficacy of a new oil-based adjuvant ISA 61 VG FMD vaccine as a potential vaccine for cattle.Iranian Journal of Veterinary Research 17(1): 8-12.

Laera D, HogenEsch H and Hagan DTO, 2023. Aluminum adjuvants-Back to the future. Pharmaceutics 15 (7): 1884. https://doi.org/10.3390/pharmaceutics15071884

Lee KW, Lillehoj HS, Park MS, Jang SI, Ritter GD, Hong YH, Jeong W, Jeoung HY, An DJ and Lillehoj EP, 2012. *Clostridium perfringens* alpha-toxin and NetB toxin antibodies and their possible role inprotection against necrotic enteritis and gangrenous dermatitis in broiler chickens. Avian Diseases 56(1): 230-233. https://doi.org/10.1637/9847-070711-ResNote.1

Matsiela MS, Naicker L, Dibakwane VS, Ntombela N, Khoza T and Mokoena N, 2022. Improved safety profile of inactivated Neethling strain of the lumpy skin disease vaccine. Vaccine 2: 100209. https://doi.org/10.1016/j.jvacx.2022.100209

Mehdizadeh Gohari I, A Navarro M, Li J, Shrestha A, Uzal F and McClane BA, 2021. Pathogenicity and virulence of *Clostridium perfringens*. Virulence 12(1): 723-753. https://doi.org/10.1080/21505594.2021.1886777

Nooraei S, Lotfabadi AS, Akbarzadeh Moallem Kolaei M and Rezaei N, 2023. Immunogenicity of different types of

adjuvants and nano-adjuvants in veterinary vaccines: A Comprehensive review. Vaccines 11(2): 45. https://doi.org/10.3390/vaccines11020453

Parker R, Devilleb S, Dupuisb L, Bertrand B and Aucouturier JB, 2009. Adjuvant formulation for veterinary vaccines: Montanide ™ gel safety profile. Procedia in Vaccinology 1(1): 140-147. https://doi.org/10.1016/j.provac.2009.07.026

Pérez O, Batista-Duharte A, González E, Zayas C, Balboa J, Cuello M, Cabrera O, Lastre M and Schijns VE, 2012. Human prophylactic vaccine adjuvants and their determinant role in new vaccine formulations. Brazilian Journal of Medical and Biological Research 45(8): 681-692. https://doi.org/10.1590/S0100-879X2012007500067

Romero C, Nicodemus N, Jarava mL, Menoyo D and de Blas C, 2011. Characterization of *Clostridium perfringens* presence and concentration of its α-toxin in the caecal contents of fattening rabbits suffering from digestive diseases. World Rabbit Science19: 177-189. http://www.doi.org/10.4995/wrs.2011.941

Rosskopf-Streicher U, Volkers P, Noeske K and Werner E, 2004. Quality assurance of *C. perfringens* epsilon toxoid vaccines-ELISA versus mouse neutralization test. ALTEX-Alternatives to Animal Experimentation 21(Suppl 2): 65-69.

Téllez-Martínez D, Leandro Portuondo D, Loesch ML, Batista-Duharte A and Zeppone Carlos I, 2019. Recombinant Enolase-Montanide™ PetGel a vaccine promotes a protective Th1 immune response against a highly virulent Sporothrix schenckii by Toluene Exposure. Pharmaceutics 11 (3): 144. https://doi.org/10.3390/pharmaceutics11030144

Ulanova M, Tarkowski A, Hahn-Zoric M and Hanson LA, 2001. The common vaccine adjuvant aluminum hydroxide up-regulates accessory properties of human monocytes via aninterleukin-4-dependent mechanism. Infection and Immunology 69(2): 1151-1159. https://doi.org/10.1128/iai.69.2.1151-1159.2001

Uzal FA, Navarro MA, Li J, Freedman JC, Shrestha A and McClane BA, 2018. Comparative pathogenesis of enteric clostridial infections in humans and animals. Anaerobe 53: 11-2. https://doi.org/10.1016/j.anaerobe.2018.06.002

Verma SK, Mahajan P, Singh NK, Gupta A, Aggarwal R, Rappuoli R and Johri AK, 2023. New-age vaccine adjuvants, their development, and future perspective. Frontiers in Immunology 14: 1043109. https://doi.org/10.3389/fimmu.2023.1043109

Wolff J, Moritz T, Schlottau K, Hoffmann D, Beer M and Hoffmann B, 2021. Development of a safe and highly efficient inactivated vaccine candidate against lumpy skin disease virus. Vaccines 9(1): 4 https://doi.org/10.3390/vaccines9010004