Iraqi Journal of Veterinary Sciences, Vol. 39, Supplement II, 2025 (135-142)
Proceedings of the 6th Scientific Conference, College of Veterinary Medicine, University of Karbala

Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Development of oral-DNA-based vaccine against *Clostridium perfringens* using Lactobacillus expressing genetically modified beta toxin

D. Al-Sallami[1], T.I. Alsaedi[1], A. Alsultan[2], R. Alenazy[3], N.M. Almimoni[4], A.M. Almotayri[4], A.H. Alghamdi[4] and P. Tahmasebi[5]

[1]Department of Physiology, Pharmacology and Biochemistry, [2]Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Al- Dewaniyah, Iraq, [3]Department of Medical Laboratory, College of Applied Medical Sciences-Shaqra, Shaqra University, Shaqra, [4]Faculty of Science, Biology Department Al-Baha University, Al-Baha, Saudi Arabia, [5]Department of Biology, Faculty of Science, Ilam University, Ilam, Iran

Article information

Article history:
Received 09 May, 2025
Accepted 09 September, 2025
Published 11 November, 2025

Keywords:
DNA based vaccine
Clostridium perfringens
Beta toxin
Oral immunization
Mucosal immune response

Correspondence:
A. Alsultan
amjed.talib@qu.edu.iq

Abstract

Enteritis in bovine is resulting from *Clostridium perfringens* and may lead to death in calves. Beta toxin has been recognized as the main toxin involves in the pathogenesis. The study aims to induce mucosal immunity and protect calves against necrotic enteritis caused by *Clostridium perfringens*. Vaccine against *C. perfringens* was designed by deletion of signal peptides from beta toxin of *C. perfringens*. C terminal, (CPB-C 143-311) was selected as vaccine candidate with 170 amino acids. Modified toxin was tested about its antigenicity, toxicity, physiochemical and immunological properties. Also, docking of the modified protein with the immune receptor was determined using Insilco approach. The results of the immune informatics analysis show that the modified toxin is antigenic, non-toxic, non-allergic and stable. The modified protein was synthesized, cloned in pUC expression plasmid and transformed in to *E. coli* pet 21 strain. The recombinant plasmid was then transformed in our lab in to clinically isolated *Lactobacillus* strain. In vivo experiment was performed, 3 groups of mice (V1, V2 and T) were orally immunized with bacterial suspension of both *E. coli*, *Lactobacillus*, and PBS consequently. The immunized mice showed normal behavior and activity. Also, there is no any anatomical lesions after 14 days of the experiment which indicates safety of the modified toxin. Furthermore, Oral administration of both bacterial suspensions was able to induce the immune response in the mice. This study suggests that the modified beta protein may play a protective role against C. perfringens infection in mice.

DOI: 10.33899/ijvs.2025.159979.4296,

Introduction

One of the common reasons of sickness in cattle especially calves is diarrhea. Diarrhea in animals can cause huge economic losses around the world yearly (1-4). There are various causes of diarrhea in farm animals as pathogenic bacteria, viruses and intestinal parasites. *Escherichia coli* and *Salmonella* spp. are considered as the most known bacterial causes of diarrhea (5). In addition, *C. perfringens* type B, C and E is considered as a dangerous cause of haemorrhagic necrotizing enteritis in calves, sheep, chicken and piglets (6,7). *C. perfringens* is positive to Gram staining, sporogenic and anaerobic pathogen may cause many diseases in animals and human including diarrhea (8,9). *C. perfringens* subdivided into five types; type A, type B, type C, type D, and type E depending on type of toxins that produced; alpha (CPA), beta (CPB), *C. perfringens* enterotoxin (CPE), epsilon (ETX), Iota (ITX),

and necrotic enteritis B-like toxin (NetB) which can lead to gas gangrene, necrotic enteritis and enterotoxaemia (4,10). Importantly, β toxin (CPB) is a crucial virulence factor in *C. perfringens* pathogenesis in addition to the other toxins causes intestinal lesions (11,12). CPB toxin produced by types B, C and E of *C. perfringens* and it is encoded by *cpb* gene, it is beta-pore-forming toxins (BPFT) (13,14). Beta toxin is vulnerable to trypsin, posing a threat of CPB-mediated disease in newborn animals due to their limited production of gastric proteases, compounded by the presence of trypsin inhibitors in colostrum (15,16). Recent studies indicate that vaccinating calves with a blend of native toxins from *C. perfringens* generates antibodies that confer protection against *C. perfringens* challenges in bovine intestinal models with enteritis. Furthermore, immunization solely against alpha toxin offers significant protection against experimental gas gangrene, although both alpha toxin and perfringolysin O play roles in the disease's pathogenesis (17-19). Consequently, developing vaccines against *C. perfringens* is crucial in animal husbandry, as it can decrease treatment costs and improve animal welfare (20,21).

This study aims to evaluate the capacity of genetically modified *Clostridium perfringens* beta toxin (CPB) to induce an immune response in an animal model and immunize against *C. perfringens* safely.

Materials and methods

Ethical approval

Based on the data for the submitted research project detailed below and approved by the Scientific Research Ethics Committee (established by administrative decision number 4914 on 14/11/2024), which is based on relevant regulations and systems in the United States of America concerning the care and use of animals and humans in scientific research, the Dean of the College of Veterinary Medicine at Al-Qadisiyah University, have decided to approve and accept the research plan.

Bacterial strains preparation

The bacteria that used as a live vector was isolated from new born calf stool. *Lactobacillus* was selectively isolated from the stool samples using MRS selective agar. Then Gram stain was performed to recognize the *Lactobacillus.* Also, laboratory *E. coli* pet 21 strain was used to transform the constructed plasmid pUC19 and also used for oral immunization of the experimental animals. Five feces samples were collected from new born calves in AL-Qadisiyah governorate using sterile borosilicate glass bottles. The samples were placed in a nutrient medium (nutrient broth). Then it is placed in the incubator for a full day after that, the bacteria were grown in a special culture medium (selective media). MRS selective medium was used to isolate *Lactobacillus*. Also, LB agar was used for *E. coli* growth. The media were prepared according to the manufacturer's instruction and pH was adjusted to 7.5 and then autoclaved at 121ºC, at 15 psi steam for 20 min.

Experimental design

Beta toxin protein (CPB) was selected among different toxins secreted by *C. perfringens* to design vaccine candidate against this pathogen. The protein was modified by deleting signal peptides following (18). CPB-C (143-311) was synthesized and cloned into pUC expression plasmid by biological company (SYNBIO, USA). Mice were used as an experimental animal, three groups of mice (V1, V2 and control,) were obtained as 5 mice in each. Oral administration of bacterial suspension, 5×10^{10} cfu/mL was given at day 0, 4 and 7 to the animals. V1 was immunized with 200 µL of *E. coli* carrying the constructed plasmid, V2 with *Lactobacillus* carrying the constructed plasmid, and control group with PBS. Blood samples were collected from the animals at days 4,7 and 14. Complete blood count (CBC) test and total IgG measuring by ELIZA were performed.

Sequence retrieval of protein

We used NCBI to get the beta toxin sequence of *C. perfringens* strain FDAARGOS-903. Beta toxin is a main virulence factor responsible for intestinal lesions of hemorrhagic necrotizing enteritis in animals affected by *C. perfringens* (22,23). Consequently, we utilized the expression of the C-terminal domain (CPB-C 143-311) for the development of an oral DNA-based vaccine.

Antigenicity prediction

The antigenicity of the modified protein was evaluated using the IEDB analysis. The target organism was designated as bacteria, with a prediction threshold set at 0.4.

Profile of beta toxin properties

The modified protein's features were analysed using the ProtParam web server. The vaccine's antigenicity was confirmed with the VaxiJen v2.0 web server. The modified protein's secondary structure elements, including beta-turns, random coils, and beta-helices, were predicted using the PSIPRED v3.3 web server.

Forecasting the three-dimensional structure (Tertiary structure)

The three-dimensional structure of the modified protein was created using the I-TASSER web server. A high-quality model was chosen based on a confidence score that varied from -5 to 2.

Model vaccine validation and refinement

The optimization of the altered protein was performed utilizing the Galaxy web server. The Ramachandran plot was analysed through the RAMPAGE web server to validate the refined structure.

Docking of the modified protein with the immune receptor TLR-3

The ClusPro v2.0 web server was employed to explore the interaction affinity between the modified toxin and the TLR-3 receptor, which was sourced from the PDB database. Comprehensive evaluation of the binding affinity between the modelled vaccine and the TLR-3 receptor was performed utilizing the HDOCK web server.

Plasmid construction

Beta-toxin gene of *C. perfringens* (GenBank: CP065680.1) was synthesized by a biological company (SYNBIO, USA). After deleting the toxic amino acids, the resulted fragment of beta-toxin was CPB-C (143-311). Then, the fragment was cloned in to pUC expression vector and transformed into *E. coli* pet 21 strain by the same company. Conjugation between the *E. coli* that carrying the recombinant plasmid and the isolated *Lactobacillus* strain was performed in our lab following Samperio *et al*. (19). ApaLI enzyme was used to cconfirm presence of the constructed gene.

Oral immunization

Five-week-old female mice, each weighing between 45 and 50 grams, were randomly assigned to three groups (n = 5 per group): two treated groups and a PBS control group. The recombinant *Lactobacillus* and *E. coli* were cultured and centrifuged, washed with PBS, and re-suspended in PBS at a concentration of 5×10^{10} cfu/mL. The treated groups mice received 200 µL of the recombinant bacteria and the PBS for the control group. The immune and sampling procedures for the mice were conducted following (20) with some modification. Briefly, all mice were immunized orally at day 0, 4 and 7.

Detection of total IgG by ELISA

On day 4, 7 and 14 after immunization, serum samples were collected from 15 mice. Serum total IgG was detected by ELISA. In brief, to determine total Immunoglobulin G (IgG) levels in mice, a standard protocol involves several steps. First, collect blood samples from the mice, usually via tail vein. Allow the blood samples to clot at room temperature for approximately 30 minutes, then centrifuge at 3000 rpm for 10 minutes to separate the serum. Next, use an enzyme-linked immunosorbent assay (ELISA), which is a common method for quantifying total IgG. Prepare the ELISA plate by coating them with an IgG capture antibody specific to mouse IgG and incubate overnight at 4°C. After washing the plates, add the serum samples diluted in assay buffer, followed by incubation for 1 h to allow the antigen-antibody binding. A secondary antibody conjugated to an enzyme is then added, followed by a substrate solution that produces a colorimetric change proportional to the amount of IgG present. Followed by two times washing using wash buffer supplied with the kit. Measure the optical density of the wells with a microplate reader at the 450 nm wavelength, $p \leq 0.05$. Finally, calculate the total IgG concentration by comparing the results to a standard curve derived from known concentrations of mouse IgG.

Results

Sequence of beta toxin

The selected beta toxin (accession no. CP065680.1) of *C. perfringens* strain FDAARGOS_903 has 336 amino acids was modified manually. The modifying was by deleting the signal amino acids of the protein, C-terminal domain (CPB-C(143-311)) with 170 amino acids was selected (Figure 1). The antigenicity was assessed using the VaxiJen v2.0 webserver. The results indicated that the toxin, following deletion, is likely to be an antigen, exhibiting an antigenic score of 0.7483, which exceeds the established threshold.

```
      1  mkkkfislvi vssllngcll sptlvyandi gktttitrnk tsdgytiitq
ndkqiisyqs
     61 vdsssknedg ftasidarfi ddkyssemtt linltgfmss kkedvikkyn
lhdvtnstai
    121 nfpvrysisi lnesinenvk ivdsipknti sqktvsntmg ykiggsieie
enkpkasies
    181 eyaesstiey vqpdfstiqt dhstskaswd tkftettrgn ynlksnnpvy
gnemfmygry
    241 tnvpatenii pdyqmsklit gglnpnmsvv ltapngtees iikvkmerer
ncyylnwnga
    301 nwvgqvysrl afdtpnvdsh iftfkinwlt hkvtai
```

Figure 1: Amino acids sequence of CPB toxin (336 A.A.). The green highlighted part is the selected part.

Analysis of physiochemical and immunological properties of the modified toxin

The prediction results indicate that the protein is non-allergenic, antigenic, water-soluble, and non-toxic (Table 1). The physicochemical characteristics of the modified toxin were analysed using the ProtParam tool. The protein has a molecular weight of 19,178.39 Da, an estimated in vitro half-life of 20 hours in mammalian cells, and an isoelectric point (pI) of 9.34.

Forecasting the secondary structure

The secondary structure of the modified protein was predicted using PSI-PRED v3.3 and the NPS@ server. The analysis indicated that the structure comprises 37% helix, 48% coil, and 13% strand. During infection, antibodies recognize the unfolded regions of the protein within the host. As illustrated in Figure 2, these unfolded areas are represented by the random coil, depicted as grey amino acids in the figure 2.

Construction, verification, and enhancement of the three-dimensional model

The structural predictions relied on locating similar protein structures within the PDB database. Five leading models were produced, each accompanied by confidence

scores of -3.19, -2.09, -3.65, -4.15, and -3.91. The C-score for these models ranges from -5 to 2, with structures exhibiting a C-score below -1.5 typically classified as reliable. Of the five models, Model 1 was selected as the recommended 3D representation of the vaccine construct, boasting a C-score of -3.19, an estimated TM-score of 0.36 ± 0.12, and an estimated RMSD of 14.2 ± 3.8 Å (Figure 3). This model underwent additional refinement through the Galaxy web server, with the Ramachandran plot analysis illustrated in Figure 3B. In summary, the structural analysis of the modified protein reveals a significant level of flexibility.

Table 1: Immunogenic and physicochemical characteristics of the modified vaccine

Characteristic	Measurement	Remark
Total Amino Acid Count	170	Suitable
Molecular Weight	19178.39	Appropriate
Theoretical Isoelectric Point	5.26	Basic
Chemical Formula	$C_{842}H_{1307}N_{223}O_{275}S_7$	-------
Estimated Half-Life (*Escherichia coli*, in vivo)	10h	-----
Estimated Half-Life (Mammalian Reticulocytes, in vitro)	20h	------
Estimated Half-Life (Yeast Cells, in vivo)	30 min	-----
Average Hydropathicity Score (GRAVY)	-0.648	Water-Soluble
Vaccine Instability Index	40.66	Stable
Antigenic Potential	0.7438	Immunogenic
Allergenic Potential	Non-allergic	Hypoallergenic
Toxicity Assessment	Nontoxic	Non-Toxic

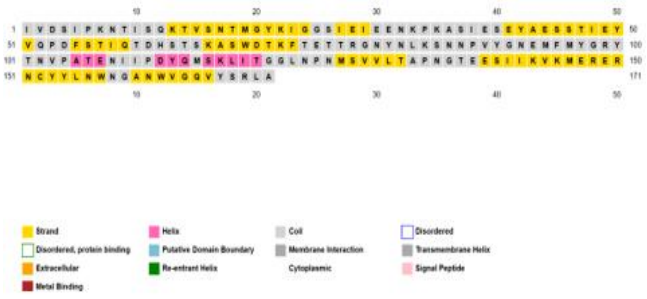

Figure 2: Prediction of the secondary structure elements of the engineered modified beta toxin (CPB) utilizing PSIPRED 4.0. The colour coding is as follows: grey represents coils, pink indicates helices, and yellow denotes strands.

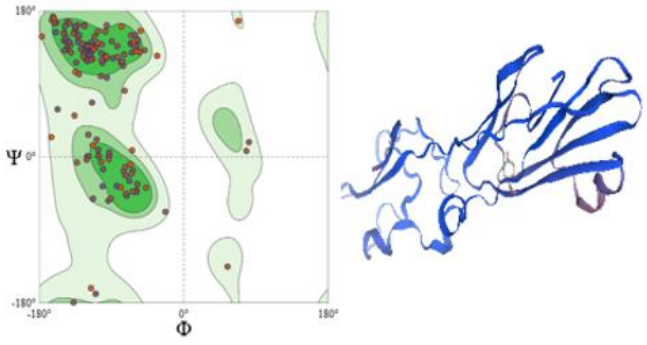

Figure 3: Refinement and validation of the 3D model. A: Development of the vaccine construct model using I-TASSER. B: Ramachandran plot analysis of the model.

Molecular docking of the constructed model with the TLR-3 receptor

The server analysis identified ten promising docking models between the designed vaccine and the TLR-3 receptor. The selected model included 91 components and demonstrated the lowest binding energy of -694. Additional assessment was performed using the HDOCK web server to examine the interaction between TLR-3 and the modelled vaccine in greater detail, yielding a minimum binding energy of -299 (Figure 4). The schematic diagram of vector constructed in this study was shown in figure 5 and their structures were confirmed by restriction enzyme digestion analysis.

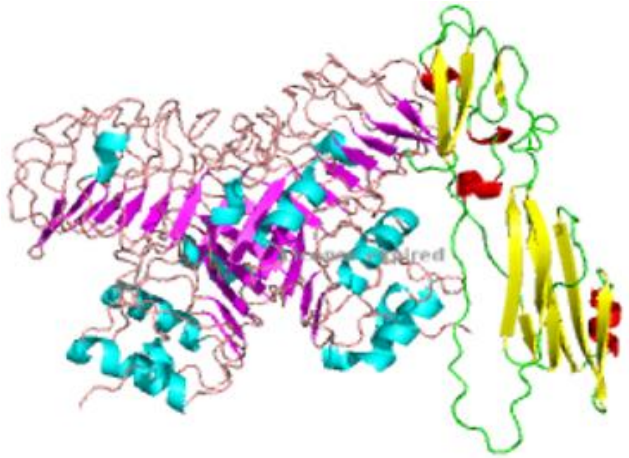

Figure 4: Molecular docking of the modelled CPB toxin with TLR2 receptor Plasmid construction.

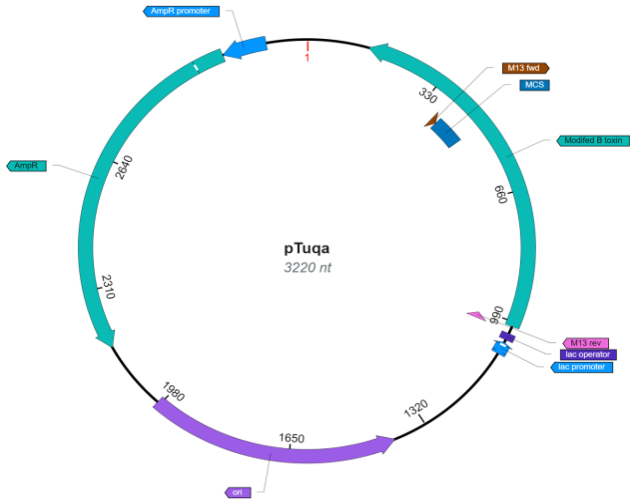

Figure 5: pUC vector constructed with CPB-C 143-311.

***Lactobacillus* isolation**

Lactobacillus strain that used in the study as a life bacterial vector was isolated from clinical feces samples were collected from healthy calves. The bacteria were diagnosed by MRS selective media and Gram staining (Figure 6).

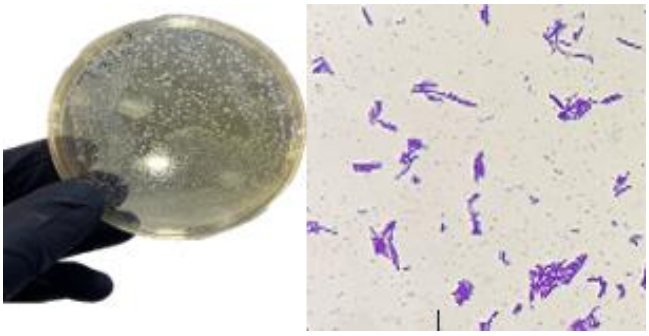

Figure 6: *Lactobacillus* on MRS selective media, and under microscope after Gram staining.

Using of live bacterial vector

The constructed plasmid pTuqa was prepared and transformed in to *E. coli* pet 21 strain by biological company (SYNBIO, USA). The recombinant *E. coli* was grown on LB plate with ampicillin 50 µl/ml and incubated overnight. Broth culture was performed with the same antibiotic and plasmid was extracted then digested to confirm presence of the inserted protein. Conjugation between the *E. coli* pet 21 and *Lactobacillus* was performed and the resulted *Lactobacillus* was tested by plasmid extraction to confirm presence of the plasmid. In addition to its growth on the plates with ampicillin (Figures 7 and 8).

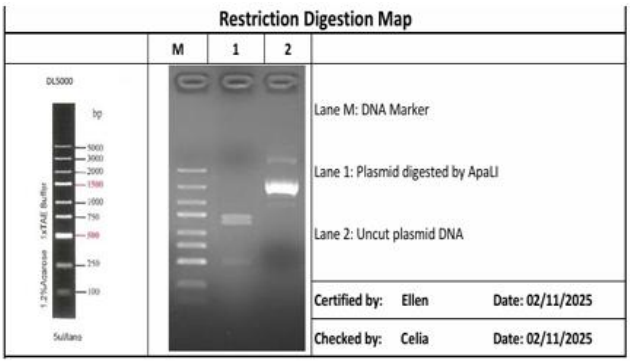

Figure 7: *E. coli* pet 21 carrying the constructed plasmid p Tuqa (pUC with CPB) on gel electroporation shows closed and linear plasmid.

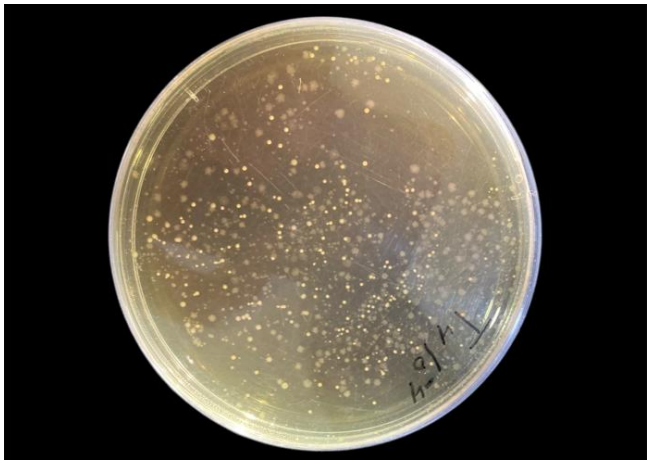

Figure 8: *Lactobacillus* carrying the constructed plasmid p Tuqa (pUC with CPB) on LB agar plate with ampicillin after conjugation.

Evaluation of vaccine safety and efficiency

There are no any adverse clinical signs observed in any of the groups after oral administration of the vaccine. There is no post-mortem changes were observed in the intestine or other internal organs after 7 days of immunization. Utilizing Complete Blood Count (CBC) as a biomarker for evaluating cellular immune response (On the 14th day of oral immunization). Lymphocytes percentage was significantly different between the groups. It was significantly increase in the lymphocyte's percentage of treated groups (V1 and V2) compared to the control. While other blood elements' values were similar between all animals' groups (Figures 9-11).

Detection of IgG in serum samples after immunization

The bar chart illustrates the progression of IgG values over time (Day 0 to Day 14) for three groups: control, v1, and v2. Both v1 and v2 show a steady and substantial increase in IgG values across all time points, while the control group exhibits only a slight rise. On Day 7 and Day

14, the values for v1 and v2 are significantly higher than those of the control group, as indicated by the red asterisks, suggesting a statistically meaningful difference likely due to treatment effects. By Day 14, v1 reaches the highest value (1200 pg/ml), followed closely by v2 (1100 pg/ml), while the control group remains much lower at (560 pg/ml). This pattern indicates that the interventions represented by v1 and v2 are not only effective but increasingly impactful over time.

Furthermore, the early divergence seen from Day 4 onward suggests that both treatments begin to exert measurable effects shortly after initiation, with the effects becoming more pronounced as time progresses. Overall, the results support the efficacy of v1 and v2 treatments in enhancing the measured outcome compared to the control (Figure 12).

Figure 9: Experimental mice look normal and active after immunization (day 7).

Figure 10: Anatomical section of experimental mice, normal colour and appearance after 7 days of immunization.

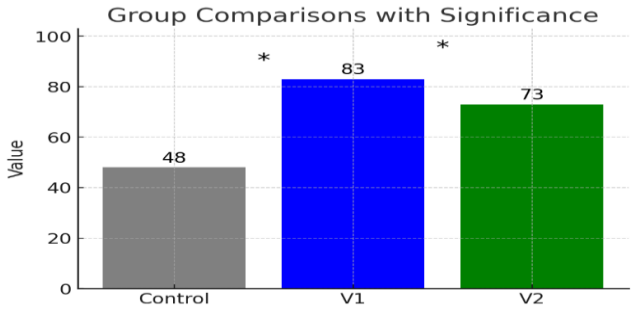

Figure 11: Lymphocyte percentage (%) of control, V1: mice immunized with *E. coli* carrying the constructed plasmid and V2: groups mice immunized with *Lactobacillus* carrying the constructed plasmid.

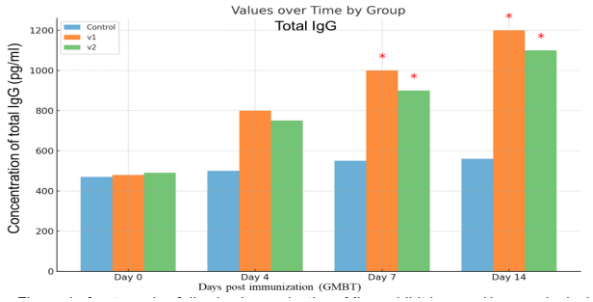

Figure 12: Fourteen days following immunization. Mice exhibit humeral immunological response 1: 200 serum dilution of total IgG from various groups of mice.

Discussion

Clostridium perfringens is a zoonotic pathogen commonly found in various environments and as part of the normal gut flora in humans and animals. It can lead to severe conditions such as muscle necrosis, necrotizing enteritis, and foodborne illness in humans. Consequently, its impact can result in significant economic losses in livestock sectors (24-28). The primary pathogenic mechanism of this bacterium involves the release of toxins, which have recently been categorized into seven distinct types A through G (29-31). Unfortunately, there are no effective treatments for severe *C. perfringens* infections, and while antibiotics have been used both preventively and therapeutically, their overuse has raised significant global public health concerns (32,33). Therefore, discovering new strategies that could replace antibiotics is becoming increasingly crucial.

Vaccination is a proven strategy for disease prevention. Several toxoid and subunit vaccines aimed at *C. perfringens* are available and have shown varying degrees of efficacy. Structural vaccinology, a field of study focusing on the epitopes that induce immunity, allows for the engineering of chimeric vaccines that incorporate only protective epitopes from different toxins while omitting irrelevant domains. This

streamlines the production process, enabling the creation of a single vaccine capable of providing immunity against multiple toxins rather than requiring multiple vaccine formulations. Research efforts have concentrated on elucidating the mechanism of toxicity associated with the CPB toxin, yielding valuable insights for vaccine development (34,35).

In the present study, the CPB toxin was selected for immunization against *C. perfringens*. In addition to the immune response that is induced by the modified toxin, using *Lactobacillus* as a vector may improve the microflora when the bacterial suspension has given orally. In a previous study, an oral vaccine was able to enhance the immunity in human and mice. *S. cerevisiae* and yeast-cell microcapsule (YCM) increased the Firmicutes and Bacteroides in the gut when they used in oral vaccination in rats (36,37). We found a high level of IgG and a significant increase in the lymphocyte percentage of the treated groups compared with the control, which indicates the role of the constructed vaccine to induce the mucosal immunity of the experimental rats. Furthermore, oral administration of the vaccine did not negatively impact the mice's behaviour or lead to any clinical signs or anatomical abnormalities. Overall, these findings suggest that the designed vaccine is non-lethal and capable of inducing an immune response in the host. Nonetheless, further tests and parameters must be evaluated to ensure its safety and efficacy.

Conclusion

In the current study, oral vaccine based on DNA toxin using *lactobacillus* expressing modified beta toxin was developed. At first step, sequence of beta toxin was obtained, then modified was performed of this sequence. The modified toxin was evaluated in term antigenicity, sensitivity and Allergenicity using immunoinformatic tool. The results show that it was immunogenic and it can be binding to the selected immune cell receptor. Furthermore, the modified toxin was non-toxic and non-allergic. At the second step, the modified toxin sequence was synthesised, cloned in plasmid and transformed into *E coli* then transferred to *Lactobacillus* via conjugation. Finally, the candidate toxin was evaluated using animal model (mice). Taken together, the candidate vaccine induced immune response in the animal model with no evidence of toxicity or Allergenicity on the animal model.

Acknowledgments

The authors would like to thank the Deanship of college of veterinary medicine al-Qadisiyah university for supporting this work.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Alawadi ZI, Alsultan A, Alsallami D, Alnomasy SF, Alqasmi M, Almufarriji FM, Alotaibi BS, Mazhari BB, Alenazy R. Designing a multiepitope mRNA-based vaccine against enterotoxigenic Escherichia coli infection in calves: Immunoinformatics and molecular modeling approach. Open Vet J. 2024;14(6):1417. DOI: 10.5455/OVJ.2024.v14.i6.9
2. Dargatz D. Beef '97, Part I: Reference of 1997 Beef Cow-Calf Management Practices. USDA; 1997. DOI: 10.22004/ag.econ.32742
3. Alsultan A, Walton G, Andrews SC, Clarke SR. *Staphylococcus aureus* FadB is a dehydrogenase that mediates cholate resistance and survival under human colonic conditions. Microbiol. 2023;169(3):001314. DOI: 10.1099/mic.0.001314
4. Cho YI, Yoon KJ. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. J Vet Sci. 2014;15(1):1. DOI: 10.4142/jvs.2014.15.1.1
5. Coşkun MR, Şahin M. Prevalence of neonatal calf diarrhea caused by *Escherichia coli* and investigation of virulence factors, serotypes, and antibiotic susceptibility. Pol J Vet Sci. 2023;26(3):335-341. DOI: 10.24425/pjvs.2023.145058
6. Alsultan A, Alsallami D. Efflux-mediated bile resistance in Gram-positive pathogens. J Pure Appl Microbiol. 2022;16(1):10-8. DOI: 10.22207/JPAM.16.1.07
7. Li J, McClane BA. Toxin plasmids of Clostridium perfringens. Microbiol Mol Biol Rev. 2013;77(2):208-233. DOI: 10.1128/MMBR.00062-12
8. Revitt-Mills SA, Rood JI, Adams V. Clostridium perfringens extracellular toxins and enzymes: 20 and counting. Microbiol Aust. 2015;36(3):114-117. DOI: 10.1071/MA15039
9. Vidal JE, Ohtani K, Shimizu T, McClane BA. Evidence that the Agr-like quorum sensing system regulates the toxin production, cytotoxicity and pathogenicity of *Clostridium perfringens* type C isolate CN3685. Mol Microbiol. 2012;83(1):179-194. DOI: 10.1111/j.1365-2958.2011.07925.x
10. Asadollahi P, Kalani BS. Novel toxin-based mRNA vaccine against Clostridium perfringens using in silico approaches. Toxicon. 2024;238:107584. DOI: 10.1016/j.toxicon.2023.107584
11. Lan-Xin OU, Zhang L, Wang Y, Li H, Zhu S. Mechanisms of intestinal epithelial cell damage by *Clostridium perfringens*. Anaerobe. 2024;102856. doi:10.1016/j.anaerobe.2024.102856
12. Sayeed S, Uzal FA, Fisher DJ, Saputo J, Vidal JE, Chen Y, Gupta P, Rood JI, McClane BA. Beta toxin is essential for the intestinal virulence of Clostridium perfringens type C disease isolate CN3685 in a rabbit ileal loop model. Mol Microbiol. 2008;67(1):15-30. DOI: 10.1111/j.1365-2958.2007.06016.x
13. Gurjar AA, Hiney RP, Casey TA. Real-time multiplex PCR assay for rapid detection and toxintyping of *Clostridium perfringens* toxin producing strains in feces of dairy cattle. Mol Cell Probes. 2008;22(2):90-95. DOI: 10.1016/j.mcp.2007.10.002
14. Gurtner C, Popescu F, Wyder M, Sutter E, Zeeh F, Frey J, Von Schubert C, Posthaus H. Rapid cytopathic effects of *Clostridium perfringens* beta-toxin on porcine endothelial cells. Infect Immun. 2010;78(7):2966-2973. DOI: 10.1128/IAI.00142-10
15. Ogawa S, Tsukahara T, Imaoka T, Inoue R, Nakayama K, Ushida K. The effect of colostrum ingestion during the first 24 hours of life on early postnatal development of piglet immune systems. Anim Sci J. 2016;87(12):1511-1515. DOI: 10.1111/asj.12585
16. Sakurai J, Nagahama M. *Clostridium perfringens* beta-toxin: characterization and action. Toxin Rev. 2006;25(1):89-108. DOI: 10.1080/15569540600590777
17. Uzal FA, Vidal JE, McClane BA, Gurjar AA. Clostridium perfringens toxins involved in mammalian veterinary diseases. Open Toxinol J. 2010;2:24. DOI: 1016/j.anaerobe.2015.01.006
18. Stevens DL, Tweten RK, Awad MM, Rood JI, Bryant AE. Clostridial gas gangrene: Evidence that α and θ toxins differentially modulate the immune response and induce acute tissue necrosis. J Infect Dis. 1997;176(1):189-195. DOI: 10.1086/514505

19. Schoepe H, Schotte U, Menge C, Genth H, Frey J. Naturally occurring *Clostridium perfringens* nontoxic alpha-toxin variant as a potential vaccine candidate against alpha-toxin-associated diseases. Infect Immun. 2001;69(11):7194-7196. DOI: 10.1128/IAI.69.11.7194-7196.2001
20. Dempster R. The manufacture of veterinary clostridial vaccines. Microbiol Aust. 2015;36(3):120-121. DOI: 10.1071/MA15040
21. Shreya D, Poonam R, Rajesh K, Sanjay K, Neelam T, Ravi P. Generation and characterization of recombinant bivalent fusion protein r-Cpib for immunotherapy against *Clostridium perfringens* beta and iota toxemia. Mol Immunol. 2016;70:140-148. DOI: 10.1016/j.molimm.2015.11.019
22. Samperio S, Guzmán-Herrador DL, May-Cuz R, Martínez-García JJ, Martín MC, Álvarez MA. Conjugative DNA transfer from *E. coli* to transformation-resistant Lactobacilli. Front Microbiol. 2021;12:606629. DOI: 10.3389/fmicb.2021.606629
23. Du L, Jia S, Zhang W, Cai C, Liu Y, Wang C, Zhu Y, Ma X, Yang X, Wei Z, Xu K. Oral Yeast-Cell Microcapsule-Mediated DNA Vaccines Against *Clostridium perfringens* Induce Effective Intestinal Immunity and Modulate Gut Microbiota. Vaccines. 2024;12(12):1360. DOI: 10.3390/vaccines1212136021
24. Zhao L, Guo Z, Wang Z, Wang R, Li Y, Wang L, Xu Y, Tang L, Qiao X. Recombinant Lactobacillus casei expressing *Clostridium perfringens* toxoids α, β2, ε and β1 gives protection against *Clostridium perfringens* in rabbits. Vaccine. 2017;35(32):4010-21. DOI: 10.1016/j.vaccine.2017.05.076
25. Stevens DL, Tweten RK, Awad MM, Rood JI, Bryant AE. Clostridial gas gangrene: Evidence that α and θ toxins differentially modulate the immune response and induce acute tissue necrosis. J Infect Dis. 1997;176(1):189-195. DOI: 10.1086/514505
26. Nuccitelli A, Cozzi R, Gourlay LJ, Donnarumma D, Necchi F, Norais N, Telford JL, Rappuoli R, Bolognesi M, Maione D, Grandi G. Structure-based approach to rationally design a chimeric protein for an effective vaccine against Group B Streptococcus infections. Proc Nat Acad Sci. 2011;108(25):10278-83. DOI: 10.1073/pnas.1015634108
27. Al-Ubaidy Y, Alsultan A. Using of integrons as biomarker to assess dissemination and diversity of antimicrobial resistance genes in farm animal manure. J Pure Appl Microbiol. 2023;17(3):1708-14. DOI: 10.22207/JPAM.17.3.35
28. Al-Sallami D, Alsultan A, Abbas KH, Clarke SR. Evaluation of efflux pump inhibitory activity of some plant extracts and using them as adjuvants to potentiate the inhibitory activity of some antibiotics against *Staphylococcus aureus*. Open Vet J. 2023;13(1):42-7. DOI: 10.5455/OVJ.2023.v13.i1.5
29. Wang S, Gao Y, Jin Q, Ji J. Emerging antibacterial nanomedicine for enhanced antibiotic therapy. Biomater Sci. 2020;8(24):682. DOI: 10.1039/d0bm00974a
30. Buckland B, Sanyal G, Ranheim T, Pollard D, Searles JA, Behrens S, Pluschkell S, Josefsberg J, Roberts CJ. Vaccine process technology—A decade of progress. Biotechnol Bioeng. 2024;121(9):2604-35. DOI: 10.1002/bit.28703
31. Wang J, Ma Q, Tian S. Against Clostridioides difficile Infection: An Update on Vaccine Development. Toxins. 2025;17(5):222. DOI: 10.3390/toxins17050222
32. Eloe-Fadrosh EA, McArthur MA, Seekatz AM, Drabek EF, Rasko DA, Sztein MB, Fraser CM. Impact of Oral Typhoid Vaccination on the Human Gut Microbiota and Correlations with *S. typhi*-Specific Immunological Responses. PLoS One. 2013;8:e62026. DOI: 10.1371/journal.pone.0062026
33. Zhang Y, Wu Q, Zhou M, Luo Z, Lv L, Pei J, Wang C, Chai B, Sui B, Huang F, Fu ZF. Composition of the murine gut microbiome impacts humoral immunity induced by rabies vaccines. Clin Transl Med. 2020;10(4):e161. DOI: 10.1002/ctm2.161
34. Borey M, Blanc F, Lemonnier G, Leplat JJ, Jardet D, Rossignol MN, Ravon L, Billon Y, Bernard M, Estellé J, Rogel-Gaillard C. Links between fecal microbiota and the response to vaccination against influenza A virus in pigs. npj Vaccines. 2021;22;6(1):92. DOI: 10.1038/s41541-021-00351-2
35. Hanoush N. Honey as antibacterial agent used against *Bacillus* spp. Isolated from locally produced juice. Mesopotamia J Agric. 2023;51(4):72-0. DOI: 10.33899/mja.2023.143201.1273
36. Khayoon TH, Abba RJ, Abdullah FA. Effects of feeding various levels of postbiotics produced by lactic acid bacteria on growth performance, gastrointestinal microbiota count, and digestibility of some nutrients in broiler chickens. Mesopotamia J Agric. 2024;52(2). DOI: 10.33899/mja.2024.145531.132019
37. Hussain KJ, Ali SH, Abdulla OA, Muwafaq OM, Alsarhan QT. Prevalence of bovine viral diarrhea virus in sheep in nineveh province. Egypt J Vet Sci. 2025;56(2):417-25. DOI: 10.33899/ijvs.2025.157630.4135

تطوير لقاح فموي مٍعتمد على الحمض النووي المعدل ضد بكتيريا الكلوستريديوم بيرفرينجنز باستخدام بكتيريا اللاكتوباسيليس المعبره جينيا عن سموم بيتا المحورة

ظمأ السلامي[١]، تقى عماد الساعدي[١]، أمجد السلطان[١]، رواف العنزي[٣]، نورا الميموني[٤]، عبد الله المنتظري[٤]، علي الغامدي[٤] و بيريسا تهمازي[٥]

[١]فرع الطب الباطني والوقائي، [٢]فرع الفسلجة والأدوية والكيمياء الحيوية، كلية الطب البيطري جامعة القادسية، الديوانية، العراق، [٣]فرع المختبرات الطبية، جامعة الشقراء، الشقراء، [٤]قسم البايولوجي، كلية العلوم، جامعة الأبهاء، الأبهاء، المملكة العربية السعودية، [٥]قسم البايولوجي، كلية العلوم، جامعة إيلام، إيلام، الجمهورية الإسلامية في إيران

الخلاصة

تعتبر العدوى الناتجة عن الكلوستريديوم بيرفرنجنز من المشكلات الخطيرة التي تؤثر على صحة العجول، حيث يمكن أن تؤدي إلى الوفاة. يُعزى السبب الرئيسي للمرض إلى السم المعروف باسم بيتا، والذي يُعتبر عامل الخطر الرئيسي. في هذه الدراسة، تم تطوير لقاح جديد عن طريق حذف أجزاء معينة من بروتين السم بيتا، واختيار الجزء C (CPB-C (311-143 كمرشح مثالي للقاح، حيث يحتوي على ١٧٠ حمضًا أمينيًا. تم اختبار السم المعدل لمعرفة خصائصه السمية والمناعية والتميز الفيزيائي والكيميائي. أظهرت النتائج أن السم المعدل قد يكون مضادًا، غير سام، وغير مسبب للحساسية، كما أنه مستقر. تم إنتاج البروتين المعدل واستنساخه في بلازميد التعبير pUC وتحويله إلى سلالة الإيشريكيا القولونية المعروفة، بالإضافة إلى تحويله إلى سلالة لاكتوباسيليوس المعزولة سريرياً. في التجارب الداخلية، تم تحصين ثلاث مجموعات من الفئران عن طريق الفم باستخدام معلقات من بكتيريا الإيشريكيا القولونية ولاكتوباسيليوس، وكذلك المحلول الفسلجي. وقد أظهرت الفئران المحصنة سلوكًا ونشاطًا طبيعيين، ولم تظهر أي إصابات تشريحية بعد ١٤ يومًا، مما يشير إلى سلامة البروتين المعدل. علاوة على ذلك، أدت الإدارة عن طريق الفم لمعلق البكتيريا في الأيام ٤ و ٧ و ١٤ من التجربة إلى تحفيز استجابة مناعية قوية في أمعاء الفئران التجريبية، وزيادة ملحوظة في نسبة اللمفاويات وإجمالي الكلوبولين المناعي ج. هذا يشير إلى أن البروتين المعدل قد يشكل خطوة واعدة في الحماية ضد المطثيات لدى الفئران.