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IL-6 and INF-γ Mediated Immune Responses in Mice Induced by *Campylobacter jejuni* Bacterial Ghosts

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Bacterial ghosts (BGs) a growing interest in their potential medical and pharmacological uses in recent years which is just. The empty bacterial envelopes Absent its internal substance .in this study using BGs for *campylobacter jejuni* which is curved, non- spore -forming, gram -negative rods that measure approximately 0.2 to 0.9µm×0.5 to 5.0 µm. Enteric *campylobacter* may appear as long spirals or S-or seagull -wing shapes. these organisms may appear as coccobacilli in smears prepared older cultures.
Methods: On gram stained smears, these organisms stain poorly for better visualization carbol-fuchsin is recommended as a counterstain, if safranin is used, counterstaining should be extended to 2 to 3 minutes *campylobacter spp*. is motil. There are two methods for preparation of (BGs) either by E-lysis gene creating a lysis tunnel structure inside the active bacteria's envelope. Or by using minimum inhibition concentration and minimum growth concentration for chemical compounds (MIC), (MGC) in a row. The ideal circumstances for the generation of BGs were mapped using the Plackett-Burman experimental design using two experimental.

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Results: A spectrophotometer was used to measure the amount of DNA and protein released. The whole experiment was divided into three steps: in the first step all the chemical reagents except H2O2 were added to bacterial suspension, in the second step only H2O2, and in the third step, the cell pellets reconstituted in 60% ethanol and kept at room temperature for thirty minutes with gentle swirl of the cells for thirty seconds every five minutes. Cell pellets were gathered and cleaned again using the same procedure as mentioned. It was assured that no living cells were left behind in the bacterial spoon by cultivation using N.B. after the experiment. The lab animal Mic injected with BG from Campylobacter jejuni as a vaccine 28 days from the last immunization, which used the qPCR technique for the immune test, has been chosen. IL-6 and INF-G for this test.
Conclusion: The immune responses accorded as a result of giving the vaccine was obtained from this the results of this research.

Keywords: IL-6; INF-γ; Campylobacter jejuni; bacterial ghosts.

1. INTRODUCTION

*Campylobacter jejun*i is a significant pathogen found in food. The intricate workings of the immune system involve the release of mediators from many tissues, which work together to mediate [1]. Proteins, chemokines, and cytokines are some of these mediators. They have a role in other homeostatic pathways in addition to the inflammatory response [2]. Proinflammatory cytokine production, such as Strong stimulation of interleukin 1β (IL-1β), IL-8, IL-6, and gamma interferon (IFN-γ) occurs during Campylobacter infection [3].

The antigen's chemical and physical properties and the immune systems was described by Dobrovolskaia [4]. BGs can strengthen the host's inherent immunity response to antigens used as adjuvants or vaccinations themselves. Due to their lack of internal nucleic acids and their tus as Gram-negative bacterial shells, BGs have PAMPs [5]. As a result, the effects of Gram-negative bacterial PAMPs on the immune system, including flagella, LPS, and PGN, are the main topic of this section [6].

IL-6 is one of family of cytokines involved in immunological responses, both innate and adaptive [7]. Initially recognized as a B-cell differentiation factor, IL-6 is a multipurpose cytokine that controls inflammation, hemopoiesis, the acute phase response, and the immune system. Different cell types generate IL-6, which impacts different cell types and has a variety of biological functions due to its distinct receptor system [1]. The innate immune system produces IL-6, a tiny glycoprotein (21 KDa) that is also generated by B cells, certain CD4 effector Th cells, Macrophages, dendritic cells, mast cells, and neutrophils. Furthermore, lL-6 is released by non-leukocytes like fibroblasts, astrocytes, endothelial cells, epithelial cells, and a variety of cancerous cells [8].

At the site of infection, IL-6 promotes inflammation by causing hepatocytes to produce acute phase proteins [9]. Immunization with a vaccine candidate not only produces specific antibodies but also promotes the generation of memory B cells upon subsequent exposure to the antigen. Mucosal immune cells produce interleukin-6 (IL-6), which is implicated in inflammatory responses, gastric homeostasis, and intestinal regeneration following damage. Nevertheless, little is known about how IL-6 and the dynamic balance of gut microbiota (GM) interact (Wu, 2022).

Immune IFN-γ functions to protect the body from external infections through macrophage-mediated cellular immunity. It is a byproduct of Th1 (T-helper 1) cells, which have the ability to control Th2 lymphocyte activity. When a virus is present, the cytokines generally boost the body's cellular immunity. INF-γ may have antibacterial, antiviral, and anticancer, antiproliferative, and antiallergic. [10]. An essential cytokine in the host's defense against infection by microbial and viral pathogens is interferon-γ (IFN-γ). Numerous physiologically important reactions that are induced by IFN-γ are beneficial to immunity. When bacterial infection of macrophages stimulates production of interferon gamma, IL-18 may function as a trigger to induce Th1 T cell development. In light of this, IL-18 may cause host resistance by producing interferon gamma [11]. Animal cells infected with viral or microbial pathogens, or IFN-γ-treated, differentially express a number of target genes. DNA T-cells and NK cells produce IFN-g in response to various stimulation conditions. In the case of viral infection, Th1 CD4+ cells and CD8+ T-cells produce IFN-g. The alpha chain of the IFN-g receptor, sometimes referred to as IFN-g RI,

binds IFN-g, and the accompanying beta chain, also known as IFN-g RII, is necessary for biologic activity. When IFN-g binds to its receptor, tyrosine kinases are activated. Th1 type cytokines (IFN-γ) inhibits the action of Th2 cells which acknowledged as a pleiotropic cytokine with the ability to function as an intracellular nuclear factor in inflammatory illnesses [12] and the Th2 type cytokines (IL-10) inhibits the action of Th1 cells [13].

2. MATERIALS AND METHODS

2.1 Preparation of Bacterial Ghost as Vaccine

In this study, the vaccine is prepared, which is the bacterial ghost of *Campylobacter jejuni* according to Amara et al. [14]. Fifty laboratory male mice at 4 months age, were used to conduct this experiment ,and they were divided into two group, Group 1 included of fifteen control she was not given anything Group 2 included of thirty five samples were given 200 µl of the vaccine subcutaneously and after 14 days she was given the second dose and then after 14 days blood samples were taken for both groups in order to conduct immunological tests and know the effect of the vaccine on the immune system [15].

Gene amplification was measured by quantitative PCR, which calculates DNA amplification at the cycle index number on the fluorescence index (SYBR green) [16]. The initial step in deciphering intricate biological processes within human cells involves examining the expression of target genes. Real-Time Polymerase Chain Reaction (qRT-PCR) is a crucial yet reasonably easy test [17].

After collected the blood from the animals it was inserted with a centrifuge device to obtain the serum, after that the RNA was extracted using AccuZolTM kit, and then converted to cDNA using EasyScript one according to the instructions of the manufacturer at last take the test QPCR by using BrightGreen qPCR MasterMix [18]. For the test using IL-6 and INF-γ and housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) It is recommended to use a reference gene with steady expression under various settings to standardize the data. Finding the greatest housekeeping genes (HKG) is a crucial first step in researching a target gene's mRNA expression. According to Lemma et al. [19], HKG shouldn't be affected by the experimental setup or co-regulated with the target gene [20] and design all primers in the primer-blast web application, available from the NCBI (National Center for Biotechnology and Information) site at https://www.ncbi.nlm.nih.gov/tools/primer-blast/ as the following:

IL-6:
Forward Primer: 5- GGCGGATCGGATGTTGTGAT-3
Reverse Primer: 5- GGACCCCAGACAATCGGTTG-3

IFN-γ:

Forward Primer: 5 -TCTCCAGAAACCCTCACTGGT -3
Reverse Primer: 5- TCAGCGGATTCATCTGCTTCG -3

2.2 Statistical Analysis

According to Table 1, the findings demonstrated that, when compared to the control group (1±0), the statical indices rose significantly ($p< 0.05$) in the immunized group injected by BGs (9.01± 1.22) in the case of IL-6 and (8.13±0.97) in the case of INF-γ.

3. RESULTS

The results of present study (Table 1) is evident that there is an increase in the percentage of IL-6 in the sample (A01,A02,A03,A04) which were obtained vaccine compare to its percentage in the control samples (A03,A04,A05,A06).

3.1 Gene Expression

The findings of qRT- PCR revealed that there were significant variations in fold change which were (1, 9.01 and 8.13) for control, IL-6 and INF-γ respectively as shown in Figs. 5 and 6.

4. DISCUSSION

In case of IL-6 increase reached 9.01476841 as clear in the results. This research also notice a significant increase in the percentage of INF-gamma in the vaccinated samples (B05, B06, B07, and B08) reaching 8.133227439 compared to the control samples and this meaning (eight times) the control. These increase in interleukin and interferon is evidence that the goal of the study has been achieved, which is to stimulate the immune system using bacterial ghosts through the control of immune response and immunogenicity are important functions of chemically produced BGs. The structural elements and surface antigens of the original bacterial cells, including LPS, outer membrane proteins, and other surface chemicals, are retained [21].

Table 1. Gene expression results if IL-6 and INF-G genes

		IL-6	HKG					
CTRL	AO3	32.33	23.33	-9				
CTRL	AO4	32.21	22.73	-9.48				
CTRL	A05	32.33	23.33	-9	-9.24	0	1	
CTRL	A06	32.21	22.73	-9.48			1	1
IL-6	A01	30.012	22.11	-7.902		1.338	2.528006	9.01476841
IL-6	A02	29.21	23.12	-6.09		3.15	8.876556	
IL-6	A03	29.61	22.99	-6.62		2.62	6.147501	
IL-6	A04	28.59	23.56	-5.03		4.21	18.50701	
		INF-G	HKG					
CTRL	AO3	32.33	23.33	-9.16				
CTRL	AO4	32.21	22.73	-10.58				
CTRL	A05	32.49	23.33	-9.16	-9.87	0	1	1
CTRL	A06	33.31	22.73	-10.58				8.133227439
INF-G	B05	30.77	21.58	-9.19		0.68	1.60214	
INF-G	B06	30.43	25.26	-5.17		4.7	25.99208	
INF-G	B07	31.76	21.92	-9.84		0.03	1.021012	
INF-G	B08	30.53	22.63	-7.9		1.97	3.917681	

Table 2. IL-6 and INF-γ levels in the vaccination and control groups

Groups	**IL-6**	**INF-Gamma**
Control	1±0	1±0
Vaccination group	9.01±1.22	8.13±5.98
Test	4.37	4.89
P value	0.012(NS)	0.008 (S)

S:Significant difference at $p < 0.05$

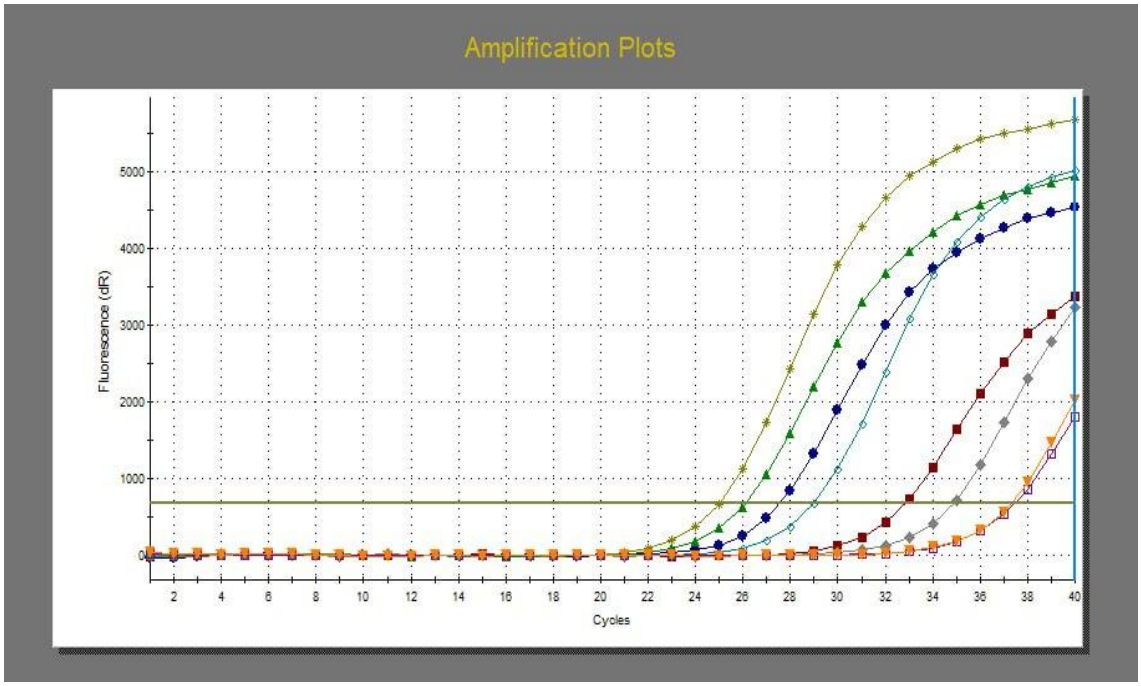

Fig. 1. Amplification curve of IL-6 for samples by qPCR

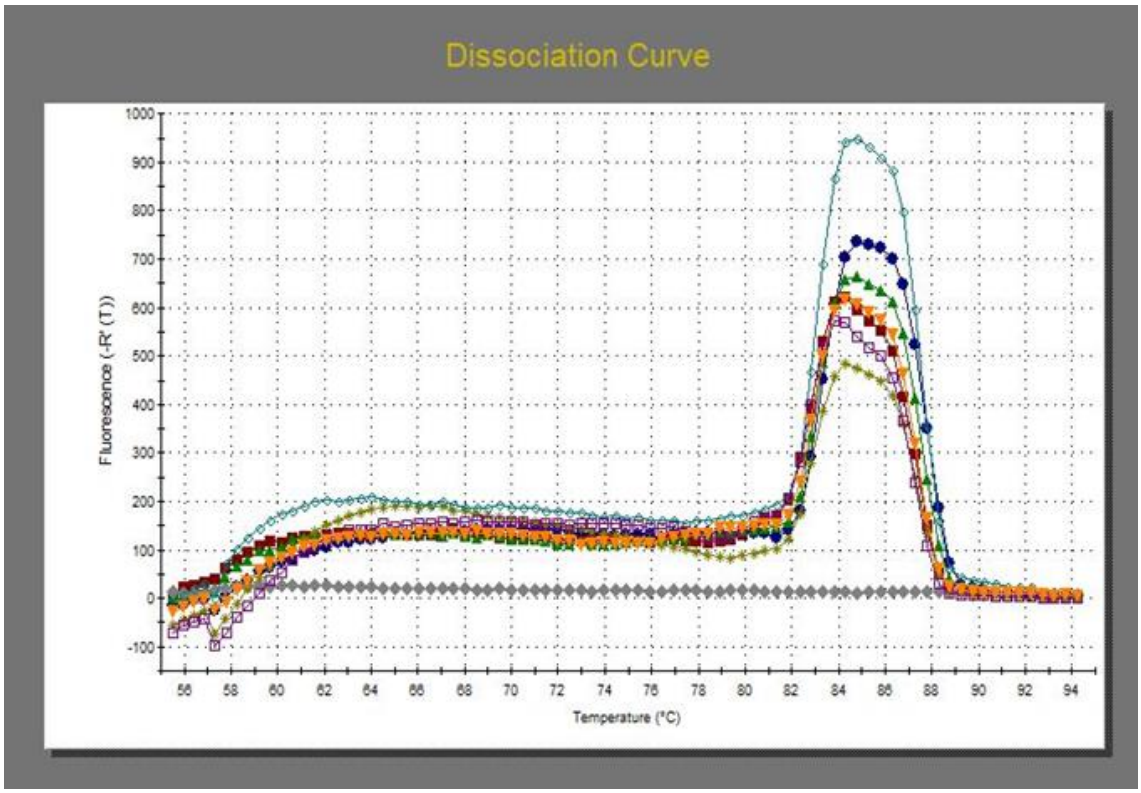

Fig. 2. Melting curve for IL-6 using qPCR

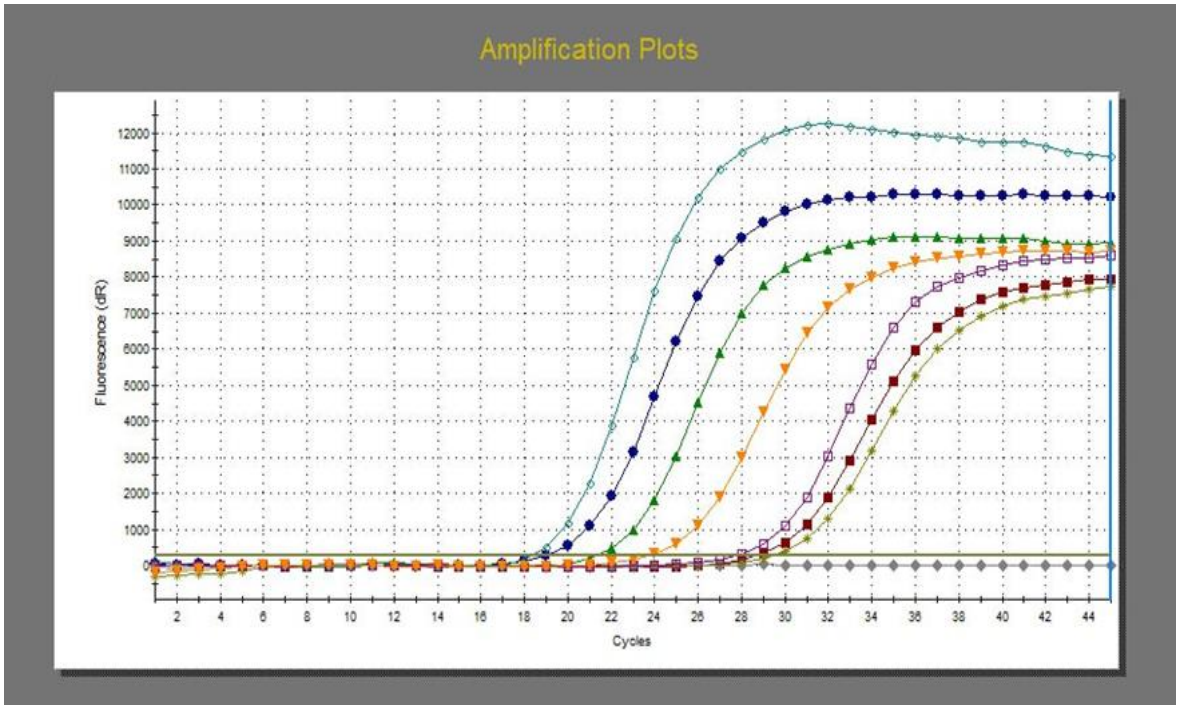

Fig. 3. Amplification of INF-γ by QPCR for samples were dosed in bacterial ghosts for *Camopylobacter jejuni*

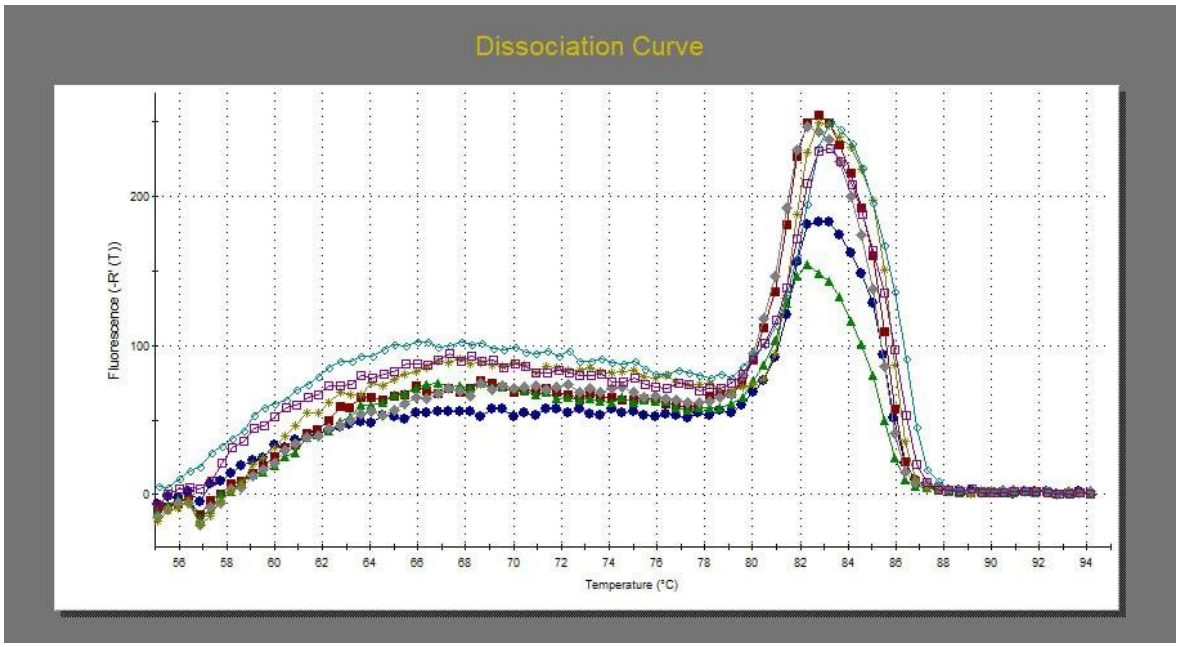

Fig. 4. Melting curve of INF-γ using QPCR

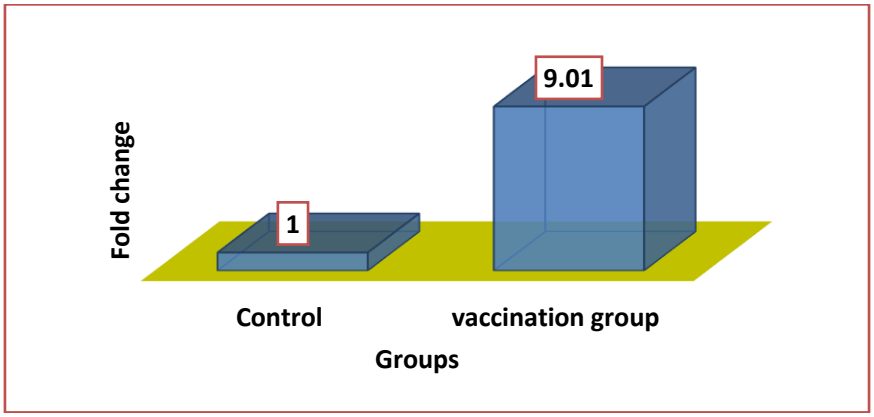

Fig. 5. Gene expression of IL-6 for samples

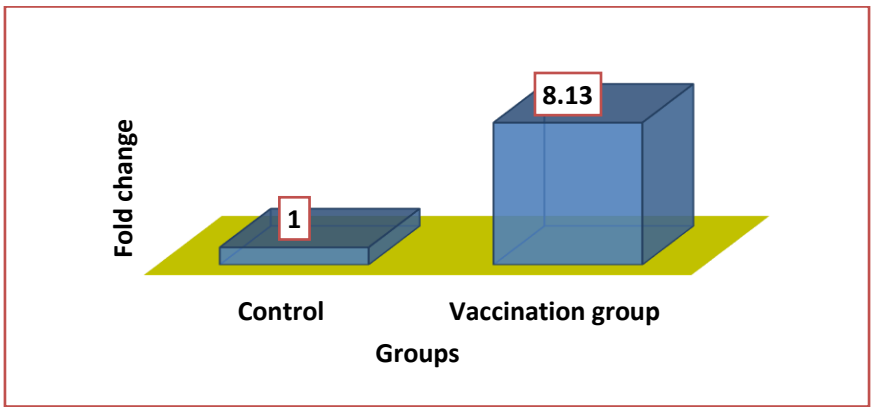

Fig. 6. Gene expression of INF- γ

Moreover, in support of this, a study conducted by Haldar et al. [22] confirmed that the above-mentioned findings are in agreement with the results of other studies reviewed by Hajam et al. [23] and also by Soleymani et al. [24]. BG vaccinations may stimulate cellular and humoral immune responses. The magnitude of the immunological response is determined by the host immune systems and physical and chemical properties of the antigen. BGs as adjuvant for vaccines or as antigens in vaccinations can enhance the host's innate immune response. BGs possess PAMPs due to the fact that their bacterial shells are Gram-negative and lack internal nucleic acids. Therefore, the focus of this section is on how Gram-negative bacterial PAMPs, such as flagella, LPS, and PGN, affect the immune system [6]. Most studies carried out in the use of bacterial ghosts as a vaccine have demonstrated an immune response, high levels of intricacies and interferons including IL-6 and INF-Gamma and different methods used for immune testing, such as ELISA and Agglutination testing, where antibodies are high, and different laboratory animals used, such as rats, chickens and mice that was used in this study, as well as the method used for ghost breeding. The 2024 study, which was carried out using the Elysis gene by Zhang et al. [25] and an increase in the level of immune response at 28 days from vaccination by Rabea et al. [26], appeared different in their results, which agree with this study.

5. CONCLUSION

This study was found increase significant in levels of IL-6 and INF-G in the serum of blood comparison to control (group one) where it was valuable of IL-6 (9 times) its value in control and (8 times) value control for INF-γ and this indicate the immune responses accorded as a result of giving the vaccine.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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