



Investigating the Effect of Three Antigens of *Citrobacter freundii* on Rabbit's Immune Response

Roua J Mohammed* , and Ikram Abbas A Al-Samarrae 

Department. of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq

***Correspondence:**

Rouajassim0@gmail.com

Received: 12 January 2021

Accepted: 26 April 2021

Published: 28 June 2021

DOI:

<https://doi.org/10.30539/ijvm.v45i1.1043>



This article is an open access distributed under the terms and conditions of the Creative Commons Attribution License (CC BY 4.0)

Cite:

Mohammed RJ, Al-Samarrae IAA. Investigating the effect of three antigens of *Citrobacter freundii* on rabbit's immune response. Iraqi J. Vet. Med. 28 June. 2021; 45(1): 56-62.

A B S T R A C T

Citrobacter freundii (*C. freundii*) is responsible for a number of significant opportunistic infections. The present research was aimed to estimate the immune response of rabbits immunized with whole cell sonicated antigen (WCSA), lipopolysaccharide (LPS), and DNA antigens (Ag) extracted from *C. freundii*. Twenty-four Albino rabbits of both sexes, with 2-3 kg body weight, were divided randomly into four groups (6 rabbits for each). Two types of tests were performed including ELISA and skin test (delayed type hypersensitivity, DTH). The 1st group was immunized with WCSA (1000 µg/mL). The 2nd group was immunized with LPS Ag at the same dose. The 3rd group was immunized with DNA extracted Ag (0.083 µg/mL). The 4th group (negative control) was injected with 1 mL PBS (pH 7.2) subcutaneously. After 14 days, rabbits were given booster doses of same Ag. The immunized animals showed significant increase of IgG and IL-6 concentration (P<0.05) following 28, 32, 46, 50 and 60 days of immunization in comparison with the negative control group. Concerning DTH, it showed an increase in the means of induration and erythema, with significant differences (P<0.05) exerted by the concentrated antigens in all immunized groups after 24 h and 48 h compared with diluted Ag and negative control group. In conclusion, WCSA and LPS Ag, in comparison to DNA Ag, were observed to promote stronger humoral (IgG) and cellular (DTH and IL-6) immune responses. DNA Ag, on the other hand, elicited a weaker humoral and cellular immune response than other Ag.

Keywords: *Citrobacter*, antigen, immunoglobulin, IL-6

INTRODUCTION

Citrobacter spp. are potential zoonotic pathogens and opportunistic nosocomial bacteria that may lead to infection of the respiratory tract, septicemia and encephalitis in sheep (1, 2). The antigenic structure of *Citrobacter* spp. is closely related to the antigens of many species such as *Salmonella* and *E. coli*, where the three antigenic species possess the flagellum H, somatic O, and capsular antigen K (3). They produce potent toxins (Shiga like toxin), lipopolysaccharide (LPS), and outer membrane

protein (4-6), cause pediatric diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (7). Infection with this bacterium leads to stimulating the systemic and local immune response as it leads to stimulating the production of CD4+ T cells, which play an important role in stimulating the humoral immune response and production of immunoglobulins from B cells including both IgM and IgG. These immunoglobulins help the opsonization process of this bacterium to help eliminating it by phagocytosis (8). T cells also play a role in the production of cytokines, including: IFN γ, αTNF-, IL-12, IL-17, and IL-6, whose role is

evident through several studies that have been conducted to demonstrate their importance in the response of innate and systemic immunity to eliminate infection with this bacterium (9). IL-6 is an endogenous pyrogen and has been shown to regulate T- and B-cell functions and to induce acute-phase response proteins and maturation of megakaryocytes (8).

The objective of the present study was to evaluate the immune response of rabbits immunized with killed whole cell sonicated antigen, LPS, and DNA, and to investigate the effect of these antigens on the humoral and cellular immune response.

MATERIALS AND METHODS

All procedures carried out in this study were reviewed and accepted in compliance with the ethical principles of animal welfare by the Scientific Committee at the College of Veterinary Medicine, University of Baghdad.

C. freundii was isolated from fecal sheep samples in Baghdad, each sample was inoculated onto the Salmonella shigella (SS) agar, MacConkey agar, and lysine deoxycholate (XLD) agar, and then incubated at 37 °C for 24-48 h (9). The *Citrobacter* isolates were identified at the level of species using the traditional morphological and biochemical tests (10). The WCSA antigen of this bacterium was prepared according to (11). They were cultivated on Nutrient agar and incubated for 48 h at 37 °C to collect bacteria, which were then microscopically checked for complete bacterial morphology after staining with Gram's stain. Afterwards, they were washed three times with PBS (pH 7.2) and centrifuged at 3000 rpm for 20 min, precipitated with 0.3% formalinized PBS (pH 7.2), incubated at 37 °C for 1-2 h and kept in the refrigerator at 4 °C overnight. Later, the bacterial suspension was washed three times with PBS (pH 7.2) and centrifuged at 3000 rpm for 20 min. The suspension was sonicated on ice by ultrasonicator at 15 KHZ/sec rate for 30 min. (intermittent intervals of 1 min of sonication followed by 1 min of rest). The sonicated suspension of bacteria was centrifuged at 3000 rpm/20 min; the supernatant was filtered by Millipore filter (0.45 µ); and the suspension was kept frozen (-20 °C) till estimating the protein concentration.

While, the LPS antigen was prepared according to (12), in which the bacteria (5 g) were placed in 50 mL of 50 mM Sodium phosphate buffer (pH 7) containing 5 mM ethylenediaminetetraacetic acid (EDTA), and 0.05% (w/v) sodiumazide (buffer L) and lysozyme (0.1 g; Worthington Biochemical Corp., 15 500 units/mg) were added, and the suspension was stirred at 4 °C for 16 h. The suspension was placed at 37 °C for 20 min, and then stirred at top speed in a mixer vessel (250 mL) for 3 min. The volume of the suspension was adjusted to 100 mL with 20 mM MgCl₂ and bovine pancreatic enzyme. Ribonuclease and deoxyribonuclease (Calbiochem) were added at final

concentrations of 1 pg/mL. The suspension was incubated for 10 min at 37 °C and then for 10 min at 60 °C. Then, partial purification by ammonium sulfate precipitation followed by purification by gel filtration chromatography were done. The precipitation was achieved by adding ammonium sulfate to the crude enzyme gradually with continuous mixing on ice at saturation ratio of 60%, the mixture was centrifuged at 6000 rpm for 20 min at 4 °C. The resulted pellet was dissolved in 50 mL of 0.05 M Tris-base buffer (pH 7) (12), and the obtained ammonium sulfate precipitates (in solution) were dialyzed in dialysis tube with 0.05 MW cutoffs against 0.05 M Tris-base buffer pH 7, for 3 h., followed by dialysis against the same buffer overnight. The obtained protease enzyme preparation was kept at 4 °C for further purification steps.

For the purification by Gel filtration chromatography, partial purification of crude LPS was done by using sephadex G-200 column, and the partially purified LPS was applied to a sephadex G-200 column (3 by 50 cm) previously equilibrated with 0.025 M phosphate buffer. the fractions were collected, and the endotoxin was recovered by detecting the carbohydrate contents for each fraction according to the method of 13 at 490 nm.

The DNA antigen of *C. freundii* isolate was extracted by using Presto™ Mini g DNA Bacteria Kit (Geneaid, KOBA) according to the manufacturer's instructions. Protein estimations of sonicated and DNA extracted antigens were measurement by NANODROP-2000 spectrophotometer (Thermo Scientific Inc., USA). While determination of LPS protein and carbohydrate was done according to (13) and (1314), respectively. For measuring protein concentration, a standard curve of bovine serum albumin was carried out by using different concentrations from BSA stock solution, then 2.5 mL of Coomassie brilliant blue G-250 dye was added, mixed and left to stand for 2 min at room temperature. The absorbance at 595 nm was measured, and the blank was prepared from 0.1 mL of Tris-HCl buffer and 2.5 mL of the dye reagent. A standard curve was plotted between the BSA concentrations against the corresponding absorbance of the bovine serum albumin. and the protein concentration of LPS sample was estimated by taking 0.1 mL of 1 mg/mL LPS solution (dissolved in Tris-HCl buffer), subjected to the same previous addition and read the absorbance at 595 nm, and the protein concentration was calculated from the standard curve.

Carbohydrate concentration was determined by using phenol-H₂SO₄ method originally described by (13) as follows: different concentrations (10, 20, 40, 60, 80, 100 µg/mL) were prepared from glucose stock solution with final volume of 1 mL. Then, 1 mL of phenol solution (5%) was added to each tube with mixing. A volume of 5 mL from H₂SO₄ was added to the mixture with vigorous mixing, then left to cool at room temperature; the absorbance at 490 nm was measured; and the blank was prepared from 1 mL of distilled water. After that, 1 mL phenol solution (5%) and 5

mL of H₂SO₄ were added, and a standard curve was plotted between the glucose amounts against the corresponding absorbance of the glucose concentrations. The carbohydrate concentration of LPS sample was estimated by taking 1 mL of 1 mg/mL LPS solution (dissolved in distilled water), subjected to the same previous addition and read the absorbance at 490 nm. The protein concentration was calculated from the standard curve.

Twenty-four Albino rabbits of both sexes 2-3 kg were divided randomly to four groups (6 rabbit for each), as follows: The 1st group was immunized with WCSA (1000 µg/mL, S/C). The 2nd group was immunized with 1000 µg/mL S/C of LPS Ag. The 3rd group was immunized with DNA extracted Ag (0.083 µg/mL, S/C). The 4th group (negative control) was injected with 1 mL PBS (pH 7.2, S/C). Blood samples were collected at day 28, 32, 46, 50 and 60 post immunization, and sera were separated for estimating IL-6 and IgG concentration by ELISA kits (Elabscience, China, for rabbits).

Antigen booster dose of 1 mL was given for 1st, 2nd and 3rd groups on day 14 post immunization. Delayed type hypersensitivity test was done for all immunized rabbits on day 18 post immunization. On rabbit flank, four sites were aseptically prepared to be injected with 3 diluents of WCSA (concentrated Ag, 1:2, 1:4) and one as a control negative (PBS pH 7.2) by intradermal injection of all immunized groups. The erythema and induration of the skin of the injected sites were measured after 24 h, 48 h, and 72 h post injection by using standard vernier metric caliper

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors on study parameters. Least significant difference test (LSD, Analysis of Variation-ANOVA) was used to compare between means (15).

RESULTS

The results of IL-6 concentration of all immunized groups (WCSA, LPS and DNA) showed a significant increase of IL-6 concentration with significant differences ($P < 0.05$) at 28, 32-, 46-, 50- and 60-days post immunization in comparison with the control group. The WCSA group showed the highest IL-6 concentration (665.17±31.62 pg/mL) at 60 day, and LPS groups showed the highest concentration of IL-6 (530.83±17.56 and 587.35±15.93 pg/mL) on day 46 and 50. While on day 60, the

concentration was 660.83 ± 22.58 pg/mL. The DNA group showed a significant increase in the IL-6 level at ($P < 0.05$) within days, but the increase in IL-6 level was less than the other immunized group (473.43±14.39, 516.04±17.52 and 583.43±22.07 pg/mL) on days 46, 50 and 60 compared with the control group (13.43± 0.47 pg/mL) on day 60 with a significant difference ($P < 0.05$) (Figure 1).

The IgG concentration results of all immunized groups showed a significant increase ($P < 0.05$) of antibody concentration on 28, 32-, 46-, 50- and 60-days post immunization in comparison with the control group. The WCSA group showed the highest antibody concentration (51.45±2.58 pg/mL) with a significant difference ($P < 0.05$) on the day 60 post immunization, while on days 28, 32, 46 and 50 the concentration was 21.12±1.03, 26.27±1.72, 34.08±0.96, and 37.08±1.47 pg/mL). LPS group showed a significant difference ($P < 0.05$) in all days of post immunization in comparison with other immunized group (sonicated and DNA group) (33.81±2.27, 37.97±2.49, 43.93±2.53, 43.87±2.40, and 44.64±2.84 pg/mL), respectively. The DNA group showed a significant difference ($P < 0.05$) in the antibody concentration within days, but the increase of IgG concentration was less than the other immunized groups (WCSA and LPS) (19.69±0.84, 31.35±1.55, 31.35±1.55, 37.07±1.61, 39.42±1.79 and 43.96± 1.93 pg/mL), respectively (Figure 2).

The results of DTH test showed an increase in the induration and erythema in the skin of the flank region of rabbits in all immunized groups in first three sites (3 conc.) in comparison with the control site (PBS). The WCSA group showed an increase in diameter of induration at 24 h and 48 h, then decrease at 72 h (3.575±0.025 mm, 5.875±0.025 mm, and 4.725±0.025 mm, respectively in concentrated antigens. In addition, the results of 1:2 Ag at 24 h, 48 h, and 72 h were 2.325±0.025 mm, 3.625±0.025 mm, and 2.475±0.025 mm, respectively. While the results of 1:4 Ag at 24 h was 1.175±0.025 mm and 48 h were 2.425±0.025 mm, and for the 72 h was 1.275±0.025 mm with a significant difference ($P < 0.05$), as shown in Figure 3.

The results of erythema of the 1st group showed a significant ($P < 0.05$) increase in size of erythema on skin after 24 h and 48 h, then decreased at 72 h (7.850±0.028 mm, 9.700±0.04 mm, and 6.225±0.025 mm, respectively) in concentrated Ag. The results of 1:2 Ag were 6.625±0.025, 7.400±0.041, and 5.100±0.041 through hours, while the results were 4.400±0.041, 5.175±0.062, and 3.800±0.041 through hours of 1:4 Ag with a significant difference ($P < 0.05$), as shown in Figure 3.

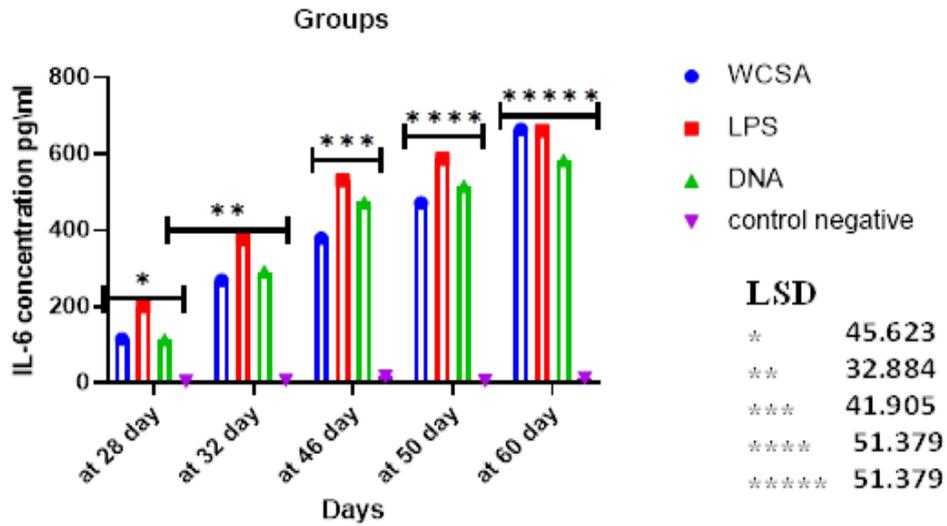


Figure 1. IL-6 concentration in the immunized groups with different antigens

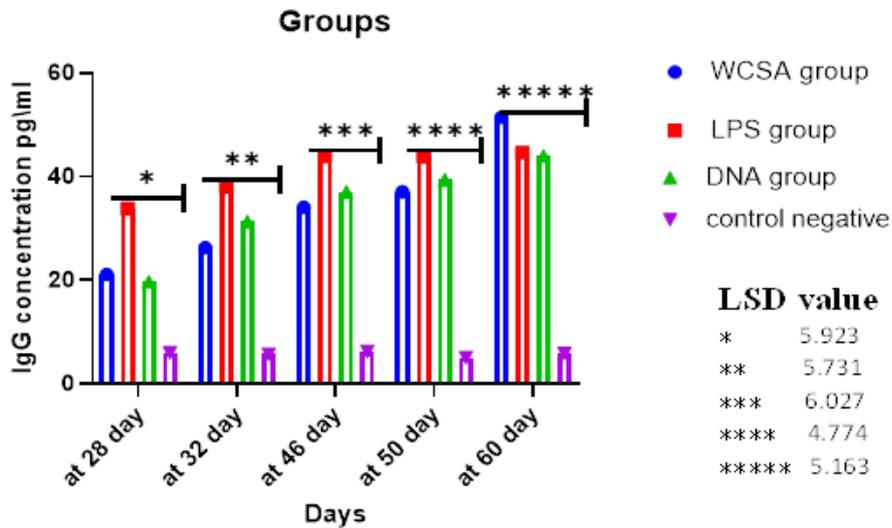


Figure 2. IgG concentration in the immunized groups with different antigens

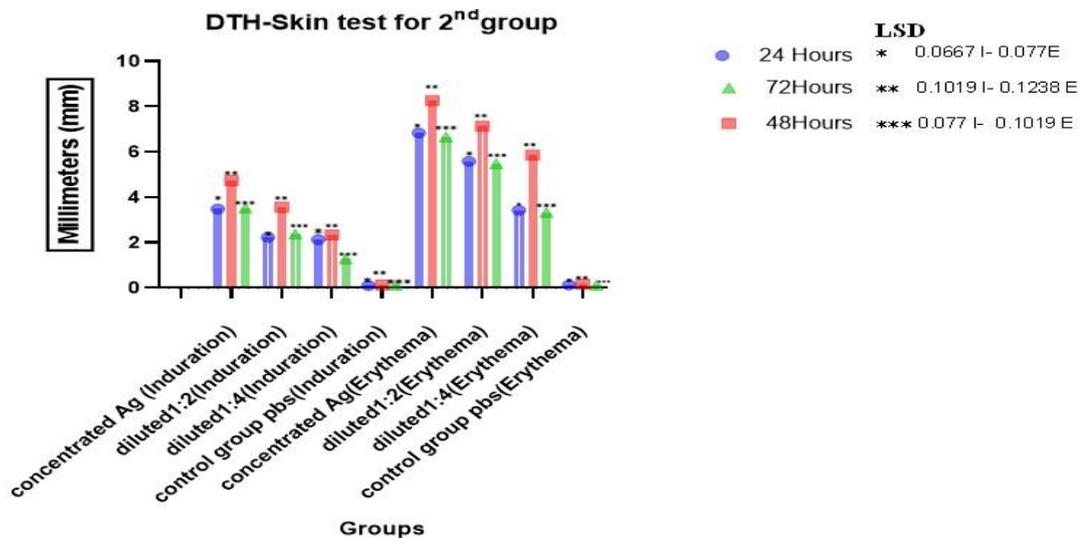


Figure 3. DTH test induration and erythema of rabbits immunized by WCSA

The indurations of the 2nd LPS group at 24 h were 3.475±0.025 mm, 4.725±0.025 mm after 48 h, and at 3.525±0.025 mm after 72 h in concentrated Ag. In addition, the results of 1:2 Ag at 24, 48 and 72 h were 2.225±0.025 mm, 3.550±0.050 mm, and 2.375±0.025 mm, respectively. While the result of 1:4 Ag at 24 h was 2.125±0.025 mm, at 48 h was 2.325±0.025 mm, and then at 72 h was 1.275±0.025 mm with a significant difference (P<0.05).

Regarding the erythema of the 2nd group, it showed a significant (P<0.05) increase in size of erythema on skin after 24 h and 48 h, then decreased at 72 h (6.825±0.025 mm, 8.250±0.050 mm, and 6.650±0.050 mm) respectively in concentrated Ag. The results of 1:2 Ag were 5.575±0.025, 7.125±0.025 and 5.475±0.025) through hours, while the results were 3.425±0.025, 5.850±0.050 and 3.325±0.025 though hours of giving 1:4 Ag with a significant difference (P<0.05) as shown in Figure 4.

The indurations of the 3rd group (DNA group) at 24 h, 48 h, and 27 h were 3.575±0.025 mm, 4.675±0.025 mm and 2.425±0.025 mm, respectively in concentrated Ag. In addition, the result of 1:2 Ag at 24 h, 48 h, and 72 h were 3.325±0.025 mm, 4.475±0.025 mm, and 2.325±0.025 mm, respectively. While the result of 1:4 Ag at 24 h was 2.125±0.025 mm), at 48 h was 3.325±0.025 mm, and then at 72 h reached 1.125±0.025 mm with a significant difference (P<0.05). The results of erythema of the 3rd group showed a significant (P<0.05) increase in size of erythema on skin at 24 h and 48 h, then decreased at 72 h (6.675±0.025 mm, 7.950±0.050 mm, and 5.625±0.025 mm) respectively in concentrated Ag. The results of 1:2 Ag were 4.450±0.028 mm, 6.725±0.025 mm, and 4.475±0.025 mm through hours, while the results were 3.325±0.025mm, 4.525±0.025 mm, and 3.325±0.025 mm though hours of the 1:4 Ag with a significant difference (P<0.05), as shown in Figure 5.

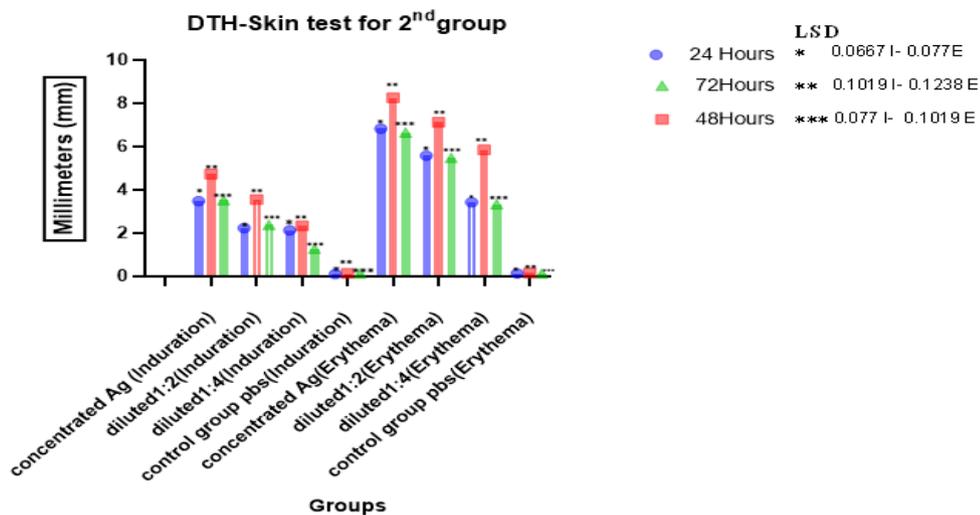


Figure 4. DTH induration and erythema of rabbits immunized by LPS antigen

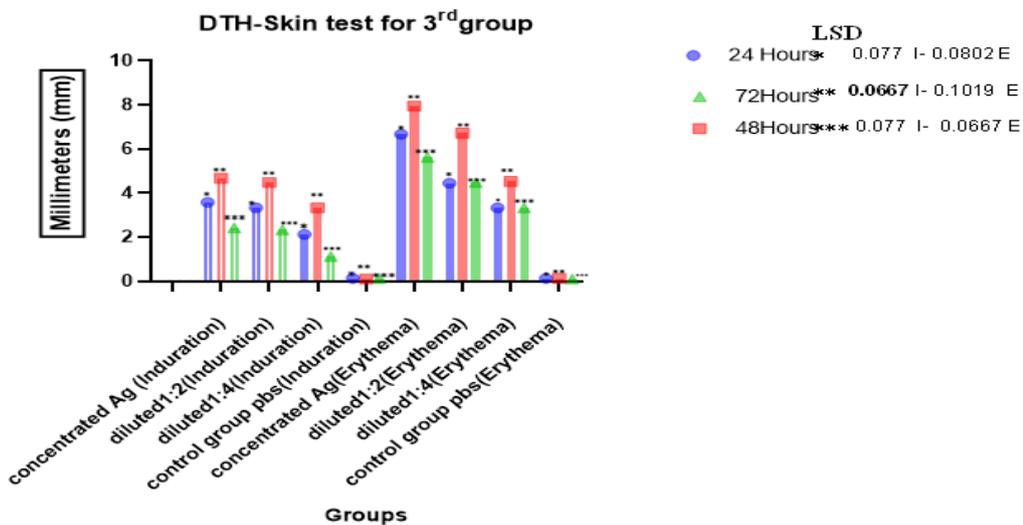


Figure 5. DTH test induration and erythema of rabbits immunized by DNA antigen

DISCUSSION

The results of IL-6 were elevated in all immunized groups that may be due to the production of inflammatory cytokines and the inhibition of microbicidal activities of macrophages, neutrophils, and neutralizing IL-6 in infected laboratory animals which may decrease mortality (16). Dann et al. (2008) (17) recorded an increase of colonic IL-6 expression after *Citrobacter rodentium* infection to investigate the role of IL-6 in mucosal host defense against *C. rodentium*, and the expression of the cytokine during the course of infection. IL-6 is required for effective host defense against *C. rodentium*; the strong colonic IL-6 response to *C. rodentium* infection suggested a potential role of this cytokine in host defense against the bacteria. Our result is in an agreement with (18) who reported that CD34+ stem cells and their cultured cells up-regulated expression of pro-inflammatory cytokines, such as iIL-1, IL-6, IL-8 and tumor necrosis factor (TNF) after infection with *E. coli*. Their expression in *E. coli* O157 can cause up-regulated expression of the pro-inflammatory cytokines including elevated IL-6 that agree with this study. The results of the present study agreed also with 19 and 20 who showed that IL-6 is a critical mediator of survival after *K. pneumoniae* infection and suggested that IL-6 protects from death by augmenting neutrophil killing of bacteria.

While the results of IgG concentration in all immunized groups showed a significant increase of antibody concentration ($P < 0.05$) during all days post immunization. The antibodies concentration elevated in all immunized groups after 14 days of booster dose administration compared with the control group, especially in the 2nd group, which had the highest antibodies concentration. These findings agreed with (21) who showed that the antibody titration against *E. coli* O157 antigen as first dose elevated then reached to the peak after booster dose post immunization. Whereas (22) found that IgG highly and significantly increased ($P < 0.05$) at 35-day post immunization with sonicated *Salmonella* Typhimurium (KWCSA-ST) and *Lactobacillus acidophilus* (KWCSA-LBA).

Skin test of all groups represented by the mean of skin erythema and induration reaction occurred due to the role of memory cells, which may modulate the Th-1 to secrete Interferon- γ (INF- γ), the potent mediator that stimulates the migration of macrophage to the site of reacted area of skin (23). While the macrophages secrete IL-1 that enhance proliferation and differentiation of other T cells into T helper-1 (Th-1) cells, which secrete IL-2 that is considered as a chemotactic factor, it caused attraction of macrophages around areas of activated T cells (23). The present study observation is consistent with another research that described the skin test depending on the ability and activity of Th-cells to recognize antigen and secrete IL-1, which enhanced proliferation and differentiation of other T-cell into Th-cells. The latter may secrete IL-2 as a

chemoattracted factor to attract macrophages around the area of activated T-cells that also secrete INF- that enhance the cytolysis activity of accumulated macrophages leading to skin thickness (23, 24). Results of the current study agree with (25) who estimated DTH test after 18 days from immunization of rabbits with KWCSA-*E. coli* O157, the result of induration for different concentration were significantly higher at $P \leq 0.05$ following 48 h post injection. The above finding is also in agreement with (22) who estimated the cellular immune response through DTH test after immunizing rabbit with sonicated *S. Typhimurium* (KWCSA-ST) and *L. acidophilus* (KWCSA-LBA) after 20-day post immunization, the results showed an increase in the means of erythema and induration in concentrated Ag.

Through ELISA test (IgG and IL-6) and DTH-skin test, LPS and sonicated were found to be potent antigens that induced higher humoral and cellular immune responses. However, LPS Ag stimulated the highest concentration of IgG and IL-6 in all day's post immunization than sonicated Ag, which also induced high concentration of IgG and IL-6, but especially on day 60 post immunization when compared to LPS and sonicated Ag. The DNA Ag caused similar levels of IgG and IL-6 throughout the days after immunization. While the skin test revealed that the sonicated Ag group had the most induration and erythema compared to the other immunized groups.

ACKNOWLEDGEMENTS

Authors sincerely wish to acknowledge the members of the Institute of Genetic Engineering, University of Baghdad, for assisting with antigen preparation, and the members of the Laboratory of the College of Veterinary Medicine, University of Baghdad for granting permission for this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Yimer N, Asseged B. Aerobic bacterial flora of the respiratory tract of healthy sheep slaughtered in Dessie municipal abattoir, northeastern Ethiopia. *Revue de Médecine Vétérinaire*. 2018;10(10):473-78.
2. Liu H, Zhao Z, Xue Y, Ding K, Xue Q. Fatal cases of *Citrobacter freundii* septicemia and encephalitis in sheep. *Journal of Veterinary Diagnostic Investigation*. 2018; 30(2):245-248.
3. Brooks GF, Carroll KC, Butel JS, Stephen A, Morse SA. *Lange Medical Microbiology*. 24th ed. McGraw Hill Medica: Jawetz M, Adelberg E A; 2007. Chapter 1, Immunology; p.4:832.
4. Lee N, Lee J, Park S, Song C, Choi I, Lee J. A review of vaccine development and research for industry animals in Korea. *Clin. Exp. Vaccine Res*. 2012; 1(1): 18-34.
5. Mermin J, Hutwagner D, Vugia S, Shallow and Daily P. Reptiles, amphibians, and human *Salmonella* infection: A population-based, case-control study. *Clin. Infec. Dis*. 2004;38: S253-S261.

6. Knirel YA, Kocharova NA, Bystrova OV, Katzenellenbogen E and Gamian A. Structures and serology of the O-specific polysaccharides of bacteria of the genus *Citrobacter*. Arch. Immunol. Ther. Exp. 2002;50:379-391.
7. Kaper J B, Nataro J P, Mobley H L. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2004; 2:123-40.
8. Bry L, Brenner M B. Critical role of T cell-dependent serum antibody, but not the gut-associated lymphoid tissue, for surviving acute mucosal infection with *Citrobacter rodentium*, an attaching and effacing pathogen. J. Immunol. 2004; 172:433.
9. Shiomi H, Masuda A, Nishiumi S, Nishida M, Takagawa T, Shiomi Y, Kutsumi H, Blumberg RS, Azuma T, Yoshida M. Gamma interferon produced by antigen-specific CD4+ T cells regulates the mucosal immune responses to *Citrobacter rodentium* infection. Infect. Immun. 2010; 78: 2653-2666.
10. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitz PE. Veterinary microbiology and Microbial Disease. 2nd ed. UK: Wiley-Blackwell West Sussex. 2011; Chapter 1, section 2, Introductory Bacteriology; p.115-165.
11. Mahmoud N M. Comparative study of antigens effect prepared from *Pseudomonas aeruginosa* from human skin burn in immunized mice. [Thesis]. Baghdad, Iraq: University of Baghdad; 2009.
12. Johnson KG, Perry MB. Improved techniques for the preparation of bacterial lipopolysaccharide. Can. J. Microbiol. 1976; 22: 29-34.
13. Dubois N, Cilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric methods for detection of sugars and related substances. Anal. Chem. 1956; 28(3): 350-6.
14. Bradford MM. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72: 248-254.
15. SAS. SAS/STAT. Users Guide for Personal Computer. Release 9.13. SAS Institute, Inc., Cary, N.C., USA. 2010.
16. Ulich TR, Yin S, Guo K, Yi ES, Remick D, del Castillo J. Intratracheal injection of endotoxin and cytokines. II. Interleukin-6 and transforming growth factor β inhibit acute inflammation. Am J Pathol. 1991; 138:1097-1101.
17. Dann SM, Spehlmann ME, Hammond DC, Iimura M, Hase K, Choi LJ, Hanson E, Eckmann L. IL-6-Dependent mucosal protection prevents establishment of a microbial niche for attaching/effacing lesion-forming enteric bacterial pathogens. J Immunol. 2008; 180(10): 6816-6826.
18. Kim JM, Oh YK, Kim YJ, Youn J, Ahn MJ. *Escherichia coli* up-regulates proinflammatory cytokine expression in granulocyte/ macrophage lineages of CD34+ stem cells via p50 homodimeric NF κ B. Clinical Experimental Immunology. 2014; 137:341-350.
19. Greenberger MJ, Strieter RM, Kunkel SL, Danforth JM, Goodman RE, Standiford TJ. Neutralization of IL-10 increases survival in a murine model of *Klebsiella pneumoniae*. Journal of Immunology. 1995; 155(2):722-729.
20. Sutherland RE, Olsen JS, McKinstry A, Villalta SA, Wolters PJ. Mast cell IL-6 improves survival from *Klebsiella pneumoniae* and sepsis by enhancing neutrophil killing. Journal of Immunology. 2008; 181:5598-5605.
21. Oladejo BO, Adebolu TT. Mechanisms of immunity to *Escherichia coli* O157:H7 in Albino rats: Role of homologous antibodies. European Journal of Biology and Medical Science Research. 2013; 1(2):31-39.
22. Al-Samarrae IAA, Kareem AA. The immune response of rabbits immunized by *Salmonella typhimurium* and *Lactobacillus acidophilus*. Iraqi J. Vet. Med. 2018. 42(1):28-34.
23. Tizard, IR. Veterinary Immunology, an introduction. 9th ed. Saunders Elsevier. Chapter 1, Type IV Hypersensitivity: Delayed Hypersensitivity; 2013, p.255-380.
24. Yousif AA, Al-Taai NA, Mahmood NM. Humoral and cellular immune response induced by *E. coli* [O157:H7 and O157:H7: K99] Vaccines in Mice. International Journal of Immunology Research. 2013; 3(1):17-20.
25. Sadeq ZE, Al-Samarrae IAA. Immune response of rabbits immunized by killed whole cell sonicated *E. coli* O157 antigen and crude Klebocin. JEZS. 2017; 5(5): 82-87.

التحري عن تأثير ثلاثة مستضدات لجرثومة *Citrobacter freundii* على الاستجابة المناعية في الارانب

رؤى جاسم محمد وإكرام عباس عبيد

فرع الاحياء المجهرية كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة

صممت هذه الدراسة لتحضير ثلاثة مستضدات (المكسرة ومتعدد السكريد الدهني والحمض النووي المنقوص الاوكسجين) من جرثومة *Citrobacter freundii* وتأثيرها على إنتاج interleukin-6 والغلوبيولين المناعي (IgG) والتي تم فحصها بواسطة فحص الإنزيم المرتبط بالامتصاص المناعي واختبار فرط الحساسية من النوع المتأخر. ولتحقيق هذا الهدف تم تقسيم أربعة وعشرين أرنبًا بصورة عشوائية من كلا الجنسين وكان وزنها 3-2 كجم لأربع مجاميع (6 أرانب لكل مجموعة). تم تمنيع المجموعة الأولى بمستضد المكسر (1000 ميكروغرام ميكروغرام) المجموعة الثانية تم تمنيعها بمستضد متعدد السكريد الشحمي (1000 ميكروغرام / مل). المجموعة الثالثة تم تمنيعها بمستضد الحمض النووي المنقوص الاوكسجين (0.083 ميكروغرام ميكروغرام). المجموعة الرابعة (مجموعة السيطرة السالبة) تم حقنها بـ 1 مل من محلول دوائر الفوسفيت (درجة الحموضة 7.2) تحت الجلد. وبعد 14 يومًا، تم إعطاء الأرانب جرعة ثانية منشطة من نفس المستضدات. وقد أظهرت نتائج تركيز الغلوبيولين المناعي IgG والانترولوكين IL-6 لجميع المجاميع المنعفة زيادة معنوية في تركيز IgG و IL-6 عند (P<0.05) في الأيام 28 و 32 و 46 و 50 و 60 بعد التمنيع، مقارنة مع (مجموعة السيطرة السالبة). وكذلك قد أظهرت نتائج فرط الحساسية من النوع المتأخر زيادة معنوية في قطر التصلد والاحمرار مع وجود فروق معنوية (P<0.05) في المستضد المركز في جميع المجاميع المنعفة بعد 24 ساعة و 48 ساعة بالمقارنة مع المستضدات المخفف ومجموعة السيطرة السالبة (.). وتنتج من ذلك، ان المستضدات المكسرة ومتعدد السكريد الشحمي على عمل على تعزيز الاستجابة المناعية الخلطية (IgG) والخلوية (DTH) و (IL-6) بنسبة أعلى من مستضدات الحمض النووي المنقوص الاوكسجين، ومع ذلك، فإن مستضد الحمض النووي مقوص الاوكسجين يحفز استجابة مناعية خلطية وخلوية ايضا بمستوى عالي مقارنة بمجموعة السيطرة ولكن بنسبة أقل من المستضدات المذكورة اعلاه.

الكلمات المفتاحية: *Citrobacter*، المستضد، الغلوبيولين المناعي، الانترولوكين 6