The immune response of rabbits immunized by *Salmonella typhimurium* and *Lactobacillus acidophilus*

Ikram A. A. Al-Samarraae and Alaa A. Kareem
Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Iraq.
E-mail: dr.ikram_abbas2014@yahoo.com

Received: 12/10/2017
Accepted: 9/1/2018
Publishing: 28/6/2018

Summary

Antigens prepared from sonicated *Salmonella typhimurium* (KWCSA-*ST*) and *Lactobacillus acidophilus* (KWCSA-*LBA*) were used to evaluate synergistic effect on interlukine production (IL-2, IL-4) and Immunoglobulin-G (IgG) which were evaluated by using Enzyme Linked Immunosorbent Assay and Delayed Type Hypersensitivity-skin test at day 20 post immunization. Twenty five rabbits of both sexes, 2-3 Kg body weight, were divided into five equal groups; the first group immunized by KWCSA-*ST* (1000 µg/ml) and KWCSA-*LBA* (1000 µg/ml), the second group by KWCSA-*ST* (1000 µg/ml) and KWCSA-*LBA* (500 µg/ml), the third group by KWCSA-*ST* (1000 µg/ml) as positive control, the fourth group by KWCSA-*LBA* (1000 µg/ml) as positive control and the fifth group was injected by P.B.S. (pH7.2) as negative control group subcutaneously. The result of delayed type hypersensitivity showed an increase in the means of erythema and induration in the 1st group after 24hrs and 48hrs while the 4th group recorded the lowest mean after 24hrs and 48hrs. These means showed significant differences ($P<0.05$) compared with injection by 1:2 and 1:4 diluted Ag. While the results of IgG showed that the highest concentration was at 35th day in the first group while the lowest concentration at 35th day in the fourth group with significant differences ($P<0.05$). Also the high concentration of IL-2, IL-4 was recorded in the first group at 35th day and the lowest concentration was in the fourth group at 35th day with significant differences ($P<0.05$); also the results showed significant differences ($P<0.05$) between the 1st, 2nd, 3rd compared with 4th and 5th groups.

Keywords: *Salmonella typhimurium*, Rabbits, *Lactobacillus acidophilus*, Immunoglobulin-G.

Introduction

Salmonella typhimurium is a common facultative intracellular pathogen that causes Salmonellosis. Intracellular survival and replication are important virulence determinants and bacteria can infect phagocytic and non – phagocytic cells (1). Symptoms were ranging from systemic infection to gastroenteritis, depending on the particular bacterial serovar and the infected host species (2 and 3). The host defense mechanism against *S. typhimurium*, is mainly cell mediated immunity and requires the cooperation of various effector cells, such as T lymphocytes, granulocytes, and monocytes/ macrophages. The functional activities of these cells are regulated by a complex network of cytokines (4). The host defense mechanism against *Salmonella* species occurs through the neutrophils, followed by mononuclear cells. These inflammatory cells produce cytokines as TNF-α, IFN- γ, IL-1, IL-2, IL-6, IL-8 (5). IL-2 is the major growth factor of T lymphocytes (6). Interleukin 4 (IL4) is a cytokine that induces differentiation of naive helper T cells (Th_0 cells) to Th2 cells (7).

Lactobacillus acidophilus are commensally constant in the intestine causing interaction with host cells and stimulated the immune system (8); induce different cytokine profiles by mononuclear cells in vitro (9). The consumption of Lactic acid-producing bacteria strengthens the non-immunological anti-infective defenses of the gastrointestinal tract because of the production of antimicrobial substances, such as mucin production, which cause stabilization of the gut mucosal barrier and improvement of gut motility (10). Enhance the expression of Toll-like receptor (TLR), and T cell-related mRNA expression levels in the gut, increase the number of intestinal epithelial lymphocytes (IELs) expression CD_3, CD_4, CD_8, and T cell receptor (TCR) α_β and improve systemic antibody response (11). The current study had been

conducted to explore the interaction effect of *S. typhimurium* and *L. acidophilus* in enhancing the immune response in immunized rabbit, by stimulating some interleukines (IL-2 and IL-4).

Materials and Methods

Salmonella typhimurium which was obtained from Pathology Department /College of Veterinary Medicine/ University of Baghdad and the diagnosis was confirmed in the Central Public Health Laboratory according to AP1-20E system and *L. acidophilus* was identified and characterized by Veterinary public Health Department/ College of Veterinary Medicine/ University of Baghdad.

Sonication was used to kill whole *S. typhimurium* cell according to (12) procedure and kill whole *L. acidophilus* cell according to (12 and 13) procedures to obtain antigens. The proteins concentration of *S. typhimurium* and *L. acidophilus* were measured by using Biuret method (14). Twenty five albino rabbits of both genders (6-7 month of age) were used and randomly divided into five equal groups, as follows: The first group was immunized by1 ml (1000 µg/ ml) of killed whole cell sonicated antigen- *S. typhimurium* (KWCSA-*ST*) and 1ml (1000 µg/ ml) of killed whole cell sonicated antigen- *L. acidophilus* (KWCSA-*LBA*) each antigen given single in the neck at the same time subcutaneously. The second group was immunized by 1ml (1000 µg/ ml) of KWCSA-ST and 1ml (500 µg/ ml) of KWCSA-LBA each antigen given single in the nick at the same time antigen subcutaneously.

The third group was immunized by 1 ml (1000µg / ml) KWCSA-ST antigen only in the neck subcutaneously. The forth group (positive control group) was immunized by 1ml (1000 µg/ ml) of KWCSA-LBA antigen only in the neck subcutaneously. And the fifth group (negative control group) was injected with 1ml of PBS (PH 7.2) subcutaneously. Blood samples were collected by the direct puncture of the heart with sterile syringes (15) at day 10, 21, 35 and 49 post immunization and sera were separated for estimating interleukines (IL-2, IL-4) and IgG concentration by ELISA kits. A booster dose was given of 1 ml KWCSA-*ST* subcutaneously for 1st, 2nd and 3rd groups at day 14 post immunization. Delayed type hypersensitivity-skin test was done for all immunized rabbits at day 20 post immunization (16); at the site of flank after prepared aseptically in the four sites of injection for using dilution of whole cell bacterial sonicated antigen at different concentrations: concentrated Ag, 1:2, 1:4 and PBS (PH7.2) as a control by intradermal injection of all immunized groups. The erythema and induration of the skin of the injected sites were measured after 24, 48 and 72 hours post injection by using stander vernier caliper.

Immunologic tests: Delayed type hypersensitivity (DTH)-skin test for immunized groups were carried out on 1, 2, 3 and 4 at 20th day. This test was done according to method described by (16) and ELISA kits (IL-4, IL-2). Shanghai (China), and IgG. Cusabio (China).

Results and Discussion

Delayed Type Hypersensitivity (DTH) – skin test: A significant ($P<0.05$) increase in size of erythema on skin occurs at 24 and 72 hours, this was in agreement with (17), later author reported that higher size was observed in animal vaccinated with combined vaccines; killed whole cell sonicated antigen- *S. typhimurium* (KWCSA-*ST* 1000µg/ml) and *B. mellitensis* (Rev-1) antigen ($2x10^9$cfu/ml) compared with control group. Also other researchers used whole cell sonicated antigen- *S. typhimurium* and killed whole cell sonicated antigen- *Cryptococcus neoformans* (18). And (19) observed that *Trichinella spiralis*-specific DTH responses were significantly augmented more in mice fed *L. casei* than in control mice and significantly enhanced *T. spiralis*-specific antibody IgG2b in both types of rats; DTH response is considered to be a manifestation of Th1 cell-mediated immunity (Table, 1 and 2).

Table, 1: Mean of diameter erythema in rabbits immunized by KWCSA-ST and KWCSA- LBA.

Means + E S (mm)					
Hours	**Group Ag**	**G 1**	**G 2**	**G 3**	**G 4**
24	Crude	9.40 ± 0.40 A a	8.00 ± 0.71 A a	7.20 ± 0.37 A a	6.60 ± 0.51 A a
	1:2	8.60 ± 0.40 A ab	7.00 ± 0.54 A ab	6.20 ± 0.37 A ab	5.60 ± 0.51 A ab
	1:4	7.80 ± 0.49 A b	6.00 ± 0.63 A b	5.80 ± 0.37 A b	4.40 ± 0.40 A b
	PBS	2.40 ± 0.24 A c	1.80 ± 0.37 A c	1.80 ± 0.20 A c	1.20 ± 0.20 A c
48	Crude	7.40 ± 0.51 B a	6.80 ± 0.37 A a	6.60 ± 0.40 A a	5.40± 0.37 A a
	1:2	6.40 ± 0.51 B ab	5.80 ± 0.58 A ab	5.60 ± 0.40 A ab	4.20± 0.39 A ab
	1:4	5.60 ± 0.60 B b	4.80 ± 0.20 A b	4.60 ± 0.40 B b	3.20± 0.39 A b
	PBS	2.00 ± 0.44 AB c	1.40 ± 0.24 AB c	1.40 ± 0.24 AB c	1.40 ± 0.24 A c
72	Crude	4.20 ± 0.37 C a	3.20 ± 0.20 B a	3.00 ± 0.31 B a	2.60 ± 0.24 B a
	1:2	2.60 ± 0.24 C b	2.00 ± 0.00 B b	2.00 ± 0.31 B b	1.80 ± 0.20 B b
	1:4	2.00 ± 0.00 C b	1.60 ± 0.24 B b	1.20 ± 0.20 C c	1.20 ± 0.20 B b
	PBS	1.20 ± 0.20 B c	1.00 ± 0.00 B c	1.00 ± 0.00 B c	1.30 ± 0.30 A b

1st group-KWCSA-*ST* 1000 µg/ml & KWCSA-*LBA* 1000 µg/ml , 2nd group-KWCSA-*ST* 1000 µg/ml &KWCSA-*LBA* 500 µg/ml, 3rd group-KWCSA-*ST* 1000 µg/ml (positive control), 4th group-KWCSA-*LBA*1000 µg/ml (positive control).
*(P<0.05). Means having with the different small letters (between Groups) in same column and capital letters (between days) in same row differed significantly.

Table, 2: Mean of thickness in rabbits immunized by KWCSA-ST and KWCSA-LBA.

Means + E S (mm)					
Hours	**Group Ag**	**G 1**	**G 2**	**G 3**	**G 4**
24	Crude	3.60 ± 0.24 B a	3.20 ± 0.20 B a	3.10 ± 0.20 B a	2.52 ± 0.22 B a
	1:2	3.00 ± 0.31 B ab	2.60 ± 0.24 B ab	2.80 ± 0.49 A a	2.34 ± 0.17 B a
	1:4	2.20 ± 0.37 AB b	2.40 ± 0.24 A b	2.30 ± 0.24 B a	2.20 ± 0.16 B a
	PBS	0.800 ± 0.12 A c	0.760 ± 0.04 A c	0.700 ± 0.07 A b	0.700 ± 0.07 A b
48	Crude	5.40 ± 0.20 A a	5.20 ± 0.24 A a	4.40 ± 0.24 A a	3.50 ± 0.27 A a
	1:2	4.00 ± 0.32 A b	3.80 ± 0.20 A b	3.80 ± 0.34 A ab	3.20 ± 0.32 A ab
	1:4	2.40 ± 0.24 A c	3.00 ± 0.32 A c	3.50 ± 0.22 A b	3.05 ± 0.20 A b
	PBS	0.800 ± 0.12 A d	0.740 ± 0.16 A d	0.760 ± 0.09 A c	0.760 ± 0.09 A c
72	Crude	3.20 ± 0.20 B a	2.60 ± 0.24 B a	2.34 ± 0.27 C a	1.50 ± 0.22 C a
	1:2	2.00 ± 0.31 C b	1.60 ± 0.24 C b	1.66 ± 0.18 B b	1.32 ± 0.19 C a
	1:4	1.40 ± 0.24 B b	1.20 ± 0.12 B bc	1.52 ± 0.15 C b	1.22 ± 0.13 C ab
	PBS	0.680 ± 0.10 A c	0.700 ± 0.12 A c	0.740 ± 0.08 A c	0.720 ± 0.07 A b

1st group-KWCSA-ST 1000 µg/ml & KWCSA-LBA 1000 µg/ml , 2nd group-KWCSA-ST 1000 µg/ml &KWCSA-LBA 500 µg/ml, 3rd group-KWCSA-ST 1000 µg/ml (positive control), 4th group-KWCSA-LBA1000 µg/ml (positive control).

The serum concentration of IgG at 35 day was significantly (P<0.05) higher than 10, 21 and 49 days post injection in group immunized by KWCSA-*ST* (1000 µg/mL) and KWCSA-*LBA* (1000 µg/mL) (Table, 3), similar finding was reported proved that Immunoglobulin-G antibody response were stimulated to both the heterologous antigen (recombinant pneumococcal antigen) rPspA and *Salmonella* lipopolysaccharide (LPS) and outer membrane proteins (OMPs), after a single oral immunization in BALB/c mice (20); also other author reported that IgG in mice during *S. typhimurium* infection at 7 to 35 days raised on day 15 and continued to increase slightly until day 35 (21); (22) mentioned that IgG titer from sera of mice at 2 weeks after infection with the *typhimurium*. Auther (23) used lactic acid bacteria as adjuvant, when they assayed the levels of antibody responses after either oral or systemic administration of antigen, they found the levels of antibody to be higher in mice given the bacteria than in control mice. Their results suggested that lactic acid bacteria as feed supplements in mice was able to enhance several factors of both humoral and cellular immune responses, also (24) investigated the differential impacts of *L. johnsonii* and *L. paracasei* on the development of mucosal and systemic antibody responses in mice.

Table, 3: Mean of IgG concentration in the immunized rabbits with different antigens by ELISA.

Groups \ Days	IgG concentration(µg/L) -Means± S E			
	10	21	35	49
1st group KWCSA-*ST* 1000 µg/ml and KWCSA-*LBA* 1000 µg/ml	4.93 ± 0.11 B a	5.70 ± 0.14 A a	5.98 ± 0.45 A a	5.42 ± 0.11 AB a
2nd group KWCSA-*ST* 1000 µg/ml and KWCSA-*LBA* 500 µg/ml	4.81 ± 0.09 B a	5.67 ± 0.56 A a	5.34 ± 0.05 A a	5.17 ± 0.26 AB a
3rd group KWCSA-*ST* 1000 µg/ml (positive control).	4.73 ± 0.14 B a	5.56 ± 0.54 A a	5.30 ± 0.32 A a	5.07 ± 0.79 AB a
4th group KWCSA-*LBA*1000 µg/ml (positive control).	4.61 ± 0.08 B a	5.40 ± 0.51 A a	5.20 ± 0.18 AB a	5.24 ± 0.74 A a
5th group PBS. (pH7.2) (negative control).	3.36 ± 0.06 A b	3.37 ± 0.09 A b	3.38 ± 0.09 A b	3.39 ± 0.7 A b

*(P<0.05). Small letters means significant different between groups, capital letters means significant different between days.

Interleukin-2 concentration at 35 day was significantly (P<0.05) higher (152.96 ± 4.84) than other, in the first group these results (25) it showed that *S. typhimurium* was capable of eliciting significant levels of IL-2 production in immunized mice, whereas no significant variation in the levels of IL-2 induced by porin of *Enteritidis* or *Escherichia coli* (Table, 4). Also it was recorded significant increase of IL-2 concentration in rats immunized by killed whole cell sonicated antigen- *typhimurium* and *neoformans* (18). *L. acidophilus* showed negative correlation with *L. acidophilus* with Th1 cytokine (IL-2), T regulatory cytokine (IL-10) but positive correlation with Th2 cytokine (IL-4) in patients group compared with healthy control group who had the reverse correlation. *L. acidophilus* influence the inflammatory cytokines towards normal balance with suppression of augmented Th1,T-regulatory responses and stimulation of suppressed Th2 response, T-reg cells play an important role in regulating the Th1_Th2 imbalance underlying the immunomodulative effects of lactic acid bacteria (LAB) (26).

A significant highest concentration of IL-4 was recorded at 35 day, in the first group (596.77±22.16) (Table, 5), similar finding noted by others (17), the later reported significant increase of IL-4 concentration in immunized rabbits with killed whole cell sonicated antigen-*S. typhimurium* (1000µg/ml) and *B. mellitensis* (Rev-1) antigen $2x10^9$cfu/ml compared with control group. Oral administration of heat-killed *acidophilus* strain L-92 (L-92) which significantly suppressed serum ova lbumin (OVA)-specific IgE levels for a long period. Cytokines such as interferon (IFN)-γ, interleukin (IL-4) and IL-10 and Igs such as total IgE and OVA-specific IgE were produced at significantly lower levels by splenocytes of *acidophilus* strain L-92 (L-92) treated mice, compared with those of control mice. Other author (27) it was suggested that

acidophilus strain L-92 (L-92) may modulate immunity through a mechanism that does not involve polarization of the immune response toward Th1. T-reg cells play an important role in regulating the Th1_Th2 imbalance underlying the immunomodulative effects of lactic acid bacteria (LAB) causing significant decrease in IL-4 and IL-10, which were both Th2 cytokines. It was observed that mice given the bacterium intranasally had greater expression of IL-12, IFN-γ, and TNF-α in their mediastinal lymph node cells (27 and 28).

Table, 4: Mean of IL-2 concentration in the immunized rabbits with different antigens by ELISA.

Days / Groups	IL-2 concentration(ng/L) -Means± S E			
	10	21	35	49
1st group KWCSA-*ST* 1000 µg/ml and KWCSA-*LBA* 1000 µg/ml	147.63 ± 1.92 A a	147.33 ± 1.89 A a	152.96 ± 4.84 A a	148.00 ± 1.51 A a
2nd group KWCSA-*ST* 1000 µg/ml and KWCSA-*LBA* 500 µg/ml	141.75 ± 2.69 A a	140.36 ± 6.68 A a	147.82 ± 2.67 A a	146.36 ± 1.28 A a
3rd group KWCSA-*ST* 1000 µg/ml (Positive control).	142.18 ± 2.47 A a	139.33 ± 4.96 A a	146.78 ± 2.83 A a	143.52 ± 3.67 A a
4th group KWCSA-*LBA*1000 µg/ml (positive control).	140.67 ± 2.28 A a	133.28 ± 6.49 A a	144.96 ± 2.25 A a	142.06 ± 2.39 A a
5th group PBS. (pH7.2) (Negative control).	100.00 ± 2.84 A b	105.36 ± 3.06 A b	103.09 ± 2.71 A b	107.15 ± 3.66 A b

*(P<0.05). Small letters means significant different between groups, capital letters means significant different between days.

Table, 5: Mean of IL-4 concentration in the immunized rabbits with different antigens by ELISA.

Days / Groups	IL-4 concentration(pg/L)-Means± S.E			
	10	21	35	49
1st group KWCSA-ST1000 µg/ml and KWCSA-LBA 1000 µg/ml	592.34 ± 10.72 A a	568.14 ± 10.96 A a	596.77 ± 22.16 A a	589.11 ± 9.68 A a
2nd group KWCSA-ST1000 µg/ml and KWCSA-LBA 500 µg/ml	581.93 ± 13.89 A a	560.96 ± 12.63 A a	591.61 ± 14.47 A a	584.19 ± 14.05 A a
3rd group KWCSA-ST1000 µg/ml (positive control).	578.39 ± 13.26 A a	557.23 ± 21.80 A a	580.64 ± 6.01 A a	583.54 ± 20.42 A a
4th group KWCSA-LBA1000 µg/ml (positive control).	572.26 ± 7.22 A a	544.51 ± 25.57 A a	555.16 ± 6.31 A a	567.74 ± 9.87 A a
5th group PBS.(pH7.2) (negative control).	300.00 ± 6.92 A b	303.87 ± 8.31 A b	307.96 ± 8.22 A b	309.96 ± 7.52 A b

*(P<0.05). Small letters means significant different between groups, capital letters means significant different between days.

References

1. Antonio, I. and Olivia-Steel, M. (2009). *Salmonella*: The ultimate insider *Salmonella* virulence factors that modulate intracellular survival. Cellular Microbiol., 11(11):1579-1586.
2. Ravindran, R. and McSorley, S.J. (2005). Traking the dynamics of T-cell activation in response to *Salmonella* infection. Immunol., 114(4):450–458.
3. Majowicz, S.E.; Musto, J.; Scallan, E.; Angulo, F.J.; Kirk, M.; O'Brien, S.J.; Jones, T.F.; Fazil, A. and Hoekstra, R.M. (2010). The global burden of nontyphoidal *Salmonella gastroenteritis*. Clin. Infect. Dis., 50(6):882–889.
4. Langermans, J.A.M. and Furth, R.V. (1994). Cytokines and the host defense against *Listeria monocytogens* and *Salmonella typhimurium*. Biotherapy. 7:169-178.
5. Kaur, J. and Jain, S.K. (2011). Role of antigens and virulence factors of *Salmonella enterica* serovar *typhi* in its pathogenesis. Microbiol. Res., 167:199-210.
6. Khan, M.M. (2008). Role of Cytokines. Immunopharm. Springer Science + Business Media, LLC. Pp:33-53.
7. Sokol, C.L.; Barton, G.M.; Farr, A.G. and Medzhitov, R. (2008). A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat. Immunol., 9(3):310–318.

8. Lei, W.; Mingjian, F.; Yanping, H.U.; Yuxin, Y.; Mingming, Y. and Yulin, C. (2014). Characterization of the most abundant *Lactobacillus* Spp in chicken gastrointestinal tract and potential use as probiotics for genetic engineering. Acta. Biochim. Biophys. Sin., 46:612-619.
9. Ticiana, S.R.; Ana, A.A.S. and Tais, C. (2014). Identification and adhesion profile of *Lactobacillus* spp. strains isolated from poultry. Braz. J. Microbiol. Sao Paula. 45(3): 1065-1073.
10. Sarker, S.A. and Gyr, k. (1992). Non-Immunological defense mechanisms of the gut. Gut, 33:987-993. DOI: 10.1136/ gut. 33.7.987.
11. Karimi, M.A.; Moghaddam, A.R. and Mojgani, N. (2010). Assessing the effect administering probiotics in water or as feed supplement on broiler performance and immune response. Br. Poult. Sci., 51:178-184.
12. Motive, I.; Denchen, V. and Linde, K. (1992). Humoral and cell mediated immunity in mice after immunization with live oral vaccines of *S. typhimurium*: auxotrophic mutants with two attenuating markers. Vac., 10:61-66.
13. Zedan, Z.K. (2012). Immune effect of viable and heat killed *Lactobacillus acidophilus* in mice infected with *Salmonella typhimurium*. J. Al-Nahrain Uni., 15(4):156-160.
14. Henry, R.J.; Cannon, D.C. and Winkelman, J.W. (1974). Clinical Chemistry, Principles and techniques. 2nd Eds. Harber and Row Company. England.
15. Weiss, D.J. and Wardrop, K.J. (2010). Schalm's Veterinary Hematology. 6th Ed. Wiley-Blackwell. USA.
16. Hudson, L. and Hay, F.C. (1980). Practical Immunology. 3rd Ed. Black-Well Scientific Publication, Oxford London.
17. Mohammed, N.T. (2015). The synergetic effect of sonicated *Salmonella typhimurium* and *Brucella mellitensis* antigens on some immunological parameters in rabbits. MSc. Thesis. Collage of Veterinary Medicine. University of Baghdad.
18. Al-Maadhidi, R.N.A. (2014). Study the immunopathological effect of sonicated *Cryptococcus neoformans* antigen and killed whole cell antigen of *S. typhimurium* on some interlukins in rats. MSc. Thesis. Collage of Veterinary Medicine. University of Baghdad.
19. Waard, De, R.; Garssen, J.; Snel, J.; Bokken, G.C.A.; Sako, T.; Huisin T.; Veld, J.H.J. and Vos, J.G. (2001). Enhanced antigen –specific delayed type hypersensitivity and immunoglobulin G2b response after oral administration of viable *Lactobacillus casei* YIT9029 in Wister and Brown Norway Rats. Clin. Diagn. Lab. Immunol., 8:762-767.
20. Kang, H.Y.; Srinivasan, J. and Curtiss, R. (2002). Immune response to recombinant pneumococcal PspA antigen delivered by live attenuated *S. enterica* serovar *typhimurium* Vaccine. Infec. Immun., 70(4): 1739-1749.
21. Matsiota-Bernard, P.; Mahana, W.; Avramast, S. and Naucel, C. (1993). Specific and natural antibody production during *Salmonella typhimurium* infection in genetically susceptible and resistant mice. Laboratoire de Microbiologie,. Immunol., 79:375-380.
22. Kusumawatil, I.D.; Harmayani, E. and Asmara, W. (2006). Effect of probiotic *Lactobacillus* Spp. Dad 13 on humoral immune response of Balb/ C Mice infected with *S. typhimurium*. Indonesian J. Biotech., 11(1):870-877.
23. Gill, H.S.; Ritherfurd, K.J.; Prasad, J. and Gopal P.K. (2000). Enhancement of natural and acquired immunity by *L. rhamnosus* (HN001), *L. acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). Br. J. Nutr. 83:167-176.
24. Ibnou-Zekri, Blum, S.; Schiffrin, E.J. and Von der Weid, T. (2003). Divergent Patterns of colonization and immune response elicited from two intestinal *Lactobacillus* strains that display similar properties in vitro. Infect. Immun., 71(1):428-436.
25. Matsui, K. and Arai, T. (1989). Specificity of *Salmonella* Porin as an eliciting antigen for Cell-Mediated Immunity (CMI) reaction in murine Salmonellosis. Microbiol. Immunol. 33(12):1063-1067.
26. El-Gaaly, S.A.; Radwan, H.H. and Yousef Attiha, M. (2016). Immunomodulatory factor of *Lactobacillus acidophilus* in pathogenesis of chronic HCV. Egyptian J. Hosp. Med., 63:229- 237.
27. Torii, A.; Torii, S.; Fujiwara, S.; Tanaka, H.; Inagaki, N.; and Nagai, H. (2007). *L.*

acidophilus strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. Allergol. Int., 56(3):293-301.

28. Hori, T.; Kiyoshima, J.; Shida, K. and Yasui, H. (2001). Effect of intranasal administration of *Lactobacillus casei* Shirota on influenza virus infection of upper respiratory tract in mice. Clin. Diagn. Lab. Immunol. 8:593-597.

الاستجابة المناعية للأرانب الممنعة بالسالمونيلا تايفموريم و بكتريا حامض اللبنيك

إكرام عباس عبود و آلاء عادل كريم

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة بغداد،العراق.

E-mail: dr.ikram_abbas2014@yahoo.com

الخلاصة

صممت الدراسة لتحضيرمستضدات السالمونيلا تايفيموريم ومستضدات بكتريا حامض اللبنيك الكاملة المقتولة المكسرة وتقييم تأثيرها التآزري في إنتاج بعض المدورات الخلوية (انترلوكين-2 وانترلوكين-4) والكلوبيولين المناعي -G والتي قُيمت باستعمال فحص الألايزا وفحص الحساسية المتاخر في الجلد في يوم 20 من التمنيع .واستعمل لهذا الغرض 25 أرنب قسمت عشوائيا إلى خمس مجاميع متساوية ومنعت المجموعة الأولى بمستضدي السالمونيلا تايفيموريم الكاملة المقتولة المكسرة (1000مايكروغرام/مل) وبكتريا حامض اللبنيك الكاملة المقتولة المكسرة (1000مايكروغرام/مل)، المجموعة الثانية بمستضدي السالمونيلا تايفيموريم الكاملة المقتولة المكسرة (1000مايكروغرام/مل) وبكتريا حامض اللبنيك الكاملة المقتولة المكسرة (500مايكروغرام/مل)، المجموعة الثالثة بمستضد السالمونيلا تايفيموريم الكاملة المقتولة المكسرة (1000مايكروغرام/مل)، المجموعة الرابعة بمستضد بكتريا حامض اللبنيك الكاملة المقتولة المكسرة (1000مايكروغرام/مل)، المجموعة الخامسة (مجموعة سيطرة) أعطيت المحلول الملحي الفسلجي 1 مل تحت الجلد. أظهرت نتائج فحص الحساسية المتأخرزيادة في قطر الاحمرار والتصلد للأرانب الممنعة بمستضدي السالمونيلا تايفيموريم الكاملة المقتولة المكسرة (1000مايكروغرام/مل) وبكتريا حامض اللبنيك الكاملة المقتولة المكسرة (1000مايكروغرام/مل) بعد 24 ساعة من الحقن، أما المجموعة التي منعت بمستضد بكتريا حامض اللبنيك الكاملة المقتولة المكسرة (1000مايكروغرام/مل) أظهرت أقل معدلات بعد 24 و48 ساعة على التوالي وكانت هذه الفروقات معنوية مقارنة بالتخافيف 1:2 و 1:4. أما بالنسبة لتركيز الكلوبيولين المناعي -G أعلى تركيز باليوم 35 والتي كانت في المجموعة الأولى واقل تركيز بنفس الحقبة والتي كانت في المجموعة الرابعة. أما بالنسبة لمستويات IL-2 وIL-4 فأظهرت المجموعة الأولى أعلى تراكيز باليوم 35 اما التراكيز الواطئة في اليوم نفسه والتي كانت في المجموعة الرابعة. كما أظهرت النتائج وجود فروقات معنوية ($P<0.05$) بين المجموعة الأولى والثانية والثالثة مقارنة بالمجموعة الرابعة والخامسة.

الكلمات المفتاحية: السالمونيلا تايفيميوريم، الأرانب، بكتريا حامض اللبنيك، الكلوبيولين المناعي - G.