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Humoral immune response of Salmonella *typhimurium and Salmonella enteritidis* sonicated antigens in rabbits.

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Summary

Salmonella typhimurium and *salmonella enteritidis* were isolated from infected goat and prepared an antigens of whole cell sonicated antigen of *S.typhimurium* (WCS.Ag.*S.typhimurium*),whole cell sonicated antigen of *S.enteritidis (*WCS.Ag.*S. entertidis)* and combination of whole cell sonicated antigen (*Salmonella typhimurium and Salmonella enteritidis*) (CWS.Ag) . Their efficacy was evaluated by using tube agglutination test and enzyme linked immune sorbent assay (ELISA). Twenty rabbits were randomly divided into four groups; the 1st group was immunized by WCS. Ag - *Salmonella enteritidis,* 2nd *group* immunized by (WCS Ag*s .typhimurium*), 3rd group immunized by CWCS.Ag compound and 4th left as control group which injected by physiological buffer saline (pH 7.2). The antibody titer was increased in after the day 12, first, second and third months of immunization by agglutination test. IgG concentration was done by ELISA at the same time ;which were recorded a higher significant differences ($p< 0.01$) at the first month in the group immunized by CWS Ag (449.65 ±38.6 1ng/ml IgG and 952± 20.85 antibodies titer) compared with other immunized groups (WCS – Ag – *S. enteritidis* and WCS.Ag.*S.typhimurium).* Also, the IgG concentration and antibodies titer are still higher in the second and the third months in the immunized group by CWCS.Ag. 218.90± 6.69ng/ml, 528± 68.58 and 89.55± 2.63ng/ml, 280± 49.98 respectively with significant differences ($p <0.01$) compared with the immunized groups (WCS.Ag.S. *entertidis* and WCS. Ag. *S.typhimurium)* and also, they are significant ($p< 0.01$) when compared with the control group Research

الاستجابة المناعية الخلطية للمستضدات المكسرة لجرثومتي *S.typhimurim* و *S.enteritidis* في الارانب.

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الخلاصة

عزلت جرثومتي *S.typhimurium* و*S. entertidis* من الماعز ؛وحضر مستضد السالمونيلا الكلى المكسر للنمط المصلى *S.typhimurium* و*S. entertidis* والمستضد المركب (كلا النمطين) وتم تقييم فاعلية كل مستضد بأستعمال فحص التلازن فى الانابيب و المقايسة المناعيةالممتزة بالانزيم (ELISA) بأستعمال عشرين ارنب قسمت عشوائيا الى اربعة مجاميع متساوية , منعت الاولى بمستضد WCS.Ag.*S. entertidis* ،الثانية بالمستضد WCS.Ag.*S.typhimurium* ، الثالثة WCS.Ag compound وتركت الرابعة كمجموعة سيطرة اعطيت المحلول الملحى الوظيفي (pH7.2) تمثلت الاستجابة المناعية الخلطية بأرتفاع مستوى الضدات وتركيز الكلوبيولين المناعى IgG فى المجاميع الممنعة بعد اليوم 12 ، الشهر الاول ،الثانى والثالث من التمنيع وقد بلغ اعلى مستو لهما فى الشهـــر الاول وكان معنويـــــا ($p < 0.01$) عند التمنيـــــع بالمستضـــد WCS.Ag (449.65 ±38.61 ng/ml,952±20.85) compound مقارنة مع المجاميع الممنعة الاخرى بالمستضدين (WCS.Ag.*S. entertidis* and WCS. Ag. S.typhimurium). اظهرت المجاميع الممنعـــــــــة بالمستضـــد المركب بقـــاء زيادة مستوى تركيـــز الكلوبيولين المناعى IgG والضـــــــدات خـــــــلال الشهر الثانى والثالث ، (218.90± 6.69 ng/ml,528± 49.98 ±2.63ng,280± 89.55 and 68.58على التوالي وكان معنويا ($p< 0.01$) عند التمنيـــــــع بالمستضديـــــــــن الاخريـــن (WCS.Ag.*S. entertidis* وWCS.Ag.S.typhimurium) وكانت معدلات المجاميع الممنعة معنوية ($p< 0.01$) مقارنة بمجموعة السيطرة.

Introduction

Salmonella typhimurium and *Salmonella enteritidis are* infectious agents cause disease in human and animals; both *Salmonella* spp. are associated with acute and chronic diarrhea, but

they are also found in animals that show no signs of clinical illness (1). Immune responses to Salmonella depend on the host species and the bacterial serotype. A significant feature of *Salmonella* pathogenesis is the requirement of both innate and adaptive immune system for the clearance of infection (2). Salmonella strains of different serotypes have common antigenic determinants in their outer membrane proteins and in the LPS core (3) which elicit a protective response and cross reaction between serogroup (4). Protective response against infections of *Salmonella typhimurium* and *Salmonella enteritidis* is largely serogroup specific (5).

Janeway et al. (6) referred to B cell play a large role in the humoral immune response which make antibodies identify and neutralized invading pathogens; anti –*Salmonella* IgM usually appear in serum earlier than IgG but the titer mediates the majority of humoral immunity and has got long life period than IgM (7)

The aim of the study is to evaluate the humoral immune response of *Salmonella typhimurium and Salmonella enteritidis* sonicated antigens in rabbits.

Materials and Methods

Salmonella typhimurium and *Salmonella enteritidis* were isolated from infected goats and *Salmonella* whole cell sonicated antigens (WCAg) were prepared according to (8). The protein concentration of both antigens *(Salmonella typhimurium and salmonella enteritidis)* was measured according to (9).by using biuret method (9mg/ml and 11mg/ml respectively).

Twenty local bread rabbits of both sexes were divided randomly into four equal groups (5 animals each) as follow:

A. First group was immunized by 1ml (11mg/ml) of WCS-Ag *Salmonella enteritidis* subcutaneously.

B. Second group was immunized by 1ml (9mg/ml) of WCS-Ag *Salmonella typhimurium* subcutaneously.

C. Third group was immunized by 1ml of mixed equal volume of both antigens compound whole cell sonicated antigen of(C WCS-Ag) above subcutaneously .

D. Fourth group (control negative group) was injected by 1ml PBS (pH7.2) subcutaneously.

At day 14, first, second and third groups were given a booster dose at the same doses of antigens that immunized above.

Blood samples were collected at day 12, first ,second and third months from all groups to estimate the antibody titers by the tube agglutination test (10) and ELISA –IgG Kit (ANTI-Rabbit IgG.ELISA kit. USA.). The results statistically analyzed by using the program of SAS -2000.

Results

1. Tube agglutination test

The first group, all rabbits had significant higher($p< 0.01$) anti- *S. enteritidis* titer at day 12(64.00± 9.79) compared to control group; On the first month , *S. enteritidis* –specific antibodies raised to 448± 117.57 ,and remaining significantly higher($p< 0.01$) than in the control group .On the second and third months mean antibody titers of rabbit decline to 320± 40 and 144± 16.

Anti –*S .typhimurium* antibodies in the rabbits of the second group reached 48± 8; elevated on the first month to 248± 68.58, then decline on the second and third months to 144± 16and 72 ±8; also was higher significant ($p< 0.01$) than the mean titer of the control group.

The sera of the third group (C WCS-Ag) reacted strongly (128± 19.59) with somatic *S. enteritidis* antigen on day 12;on the first month there was a higher titer that reached to 952 ±20.85 , decline on the second and third months to 528± 68.58 and 280± 49.98 respectively remaining higher significant ($p< 0.01$) than control group which recorded a negative reaction with *S . enteritidis* antigen from the beginning of the experiment. (Table -1-) .

Table -1: Mean of antibodies titers of rabbits vaccinated with WCS –Ags by tube agglutination test:-

Time / The group	Day12	First month	Second month	Third month
	Mean ± SE			
First group (WCS-Ag *S.enteritidis).*	64.00 ±9.79 aA	448± 117.57 bA	320± 40 cA	144±16 dA
Second group (WCS-Ag. *S.typhimurium).*	48± 8.00 aB	248± 68± 58 bB	144± 16.00 cB	72.00 ±8.00 dB
Third group (CWCS-Ag)	128± 19.59 aC	952± 20.85 bC	528± 68.58 cD	280± 49.98 dC
Fourth group (control)	O	O	O	O

Different small and capital letters shows significant differences (p< 0.01) within and between groups respectively.

2. Enzym linked immunosorbent assay (ELISA):

The results of the first group showed significant differences (p< 0.01) in their IgG concentration means at day 12, first, second and third months post immunization .The IgG concentration reached 89.80± 4.77ng/ml then elevated to 288. 95± 14.92ng/ml; subsequently declined after the second and third months to 138± 6.8ng/ml and 68.40± 3.7ng/ml respectively.

The second group reached to the higher concentration after the first month 111.73± 5.25 ng/ml and was significant (p< 0.01)as compared with their value after day12, first ,second and third months which reached to 56± 5.72 ng/ml ; 93.5± 4.04ng/ml and 60.35± 0.82ng/ml respectively, showed a significant differences(p< 0.01) as compared with other groups.

The higher IgG concentration was induced in the third group and showed a significant differences in the means(p< 0.01) at day 12 ,second and third months which reached to 139.60± 6.265ng/ml;449.65 ±38.61ng/ml ;218± 6.69ng/ml and 89.55± 2.63ng/ml respectively .The IgG concentration remain low in the control group during the beginning of the experiment, compared with other groups at day 12 ;first; second and third months that reached to 22.96± 5.00ng/ml ;13.77±2.74ng/ml ;28.89± 5.93 ng/ml and 10.034 ±0.008 ng/ml respectively.

Table -2: Means of IgG concentration of immunized rabbits with WCS Ags by ELISA.

Time / Groups	**IgG concentrations (ng/ml) Mean ± SE**			
	Day 12	First month	Second month	Third month
First group (WCS-Ag *S. enteritidis)*	89.80±4.77 aA	288.95±14.92 bA	138± 6.81 cA	86.4± 3.71 aA
Second group (WCS-Ag *S. typhimurium).*	56± 5.72 aB	111.73±5.25 bB	93.5 ± 4.04 cB	60.35± 0.82 aB
Third group (WCS-Ag)	139.60±6.26 aB	449.65±38.61* bC	218.90± 6.6 cC	89.55 ± 2.63 dA
Fourth group (control)	22.96±5.00 aB	13.77±2.74 bD	28.89±5.93 aD	10.034± 0.008 bC

Different small and capital letters showed significant differences (p<0.01) within and between groups respectively.

Discussion

The recorded differences in the antibody titers and IgG concentration represent the variation of humoral immune response between the groups as a result of stimulation of different numbers of Th2 that secret IL- 5 and IL- 4 that play an important role in the processing activation and differentiation of B- cell to the plasma cells that will produce the neutralizing antibodies (11, 12) and this variation may be resulted due to the injection of WCA .Ags prepared from different serogroups ; each one have different numbers of antigenic determinants that stimulated different numbers of T.cells (13); Also immunization with WCS – Ags was able to induce immune response due to release all antigenic structures of bacteria which agreed with (14,15) who explained that the crude outer membrane proteins of *S .typhimurium*__ evoke antibody response to both LPS and porin ; As well as , the IgG concentration and antibody titer that induced after immunization with WCS-Ags compound in the third group was supported the idea of cross reaction between two serotypes , this observation was supported by (16) who recorded that the levels of sera IgG was assessed protection in the chicken that were immunized with *S.gallinarum* and *S. abortusovis* and was challenge by *S.gallinarum*

Cell mediated immune responses are induced against killed vaccine and subunit peptide vaccines .These vaccines are taken up by APCs complexes to MHC class Ⅱ molecules and presented to CD 4 T cells, which in turn help mainly the B cells ; Hence , these vaccines induce strong humoral immune response . In contrast, attenuated live vaccine enter into the host cells, multiply and produce antigen peptides; these antigenic peptides are present a long with MHC class1 molecules to CD8 + T cell; consequently, strong cell mediated immune responses are also induced (17).

ELISA was shown to be more sensitive than the passive haemagglutination test .(18) ; 10_100 folds sensitive than the older methods like direct agglutination , passive heamagglutinaton test and immunoelectrophoresis (19) .Our conclusion of this study is that the compound whole cell sonicated antigens of *S. typhimurium* and *S.enteritidis* more efficient for stimulating humoral immune response than the single whole cell sonicated antigens of each one and can be give a good protection against both bacteria .

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