Study the Immune Response of Conjunctival and Subcutaneous Vaccination of Goats with RB51 Vaccine for Controlling Brucellosis

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Summary

This study was conducted to evaluate the efficacy of RB51 vaccine in immunization of goats against brucellosis although it is a specific vaccine for cattle, fifteen pregnant and non-pregnant goats were divided into three groups, 5 goats were injected subcutaneously(S/C) with 2 mls of RB51 vaccine which contain 4×10^8 CFU , 5 goats were vaccinated through conjunctival route (C/J) with 0.2 ml of RB51 which contain 4×10^8 CFU , and the last 5 goats were injected with PBS and kept as control group. It has been ascertained that no abortion occurred in the vaccinated animals except one case of a weak kid was born in S/C vaccinated group. Humeral immune response for the vaccinated animals measured by serological test (Rose Bengal and Passive hemagglutination tests) every two weeks, while the cellular immune response measured by brucellin test at the 6th week. Passive haemaglutination test (PHA) was positive and the antibodies were increased significantly ($P<0.05$) at the 2nd week to reach 10.4±2.4 and 6.4±0.9 in S/C and C/J routes respectively, and increased significantly ($P<0.05$) at 8th week to reach 38.4±6.4 and 22.4±3.9 in S/C and C/J routes respectively, and decreased significantly at 12th week which reach 9.6±1.6 to 6.4±0.9. Brucellin from RB51 strain prepared to be used as antigen in the PHA test and also in skin test to determine delayed type hypersensitivity, serial dilutions of RB51 brucellin had been done to confer the optimal concentration in skin test that did not induce toxicity for mice 40µg was the optimal concentration that has been given to goats. In goats the results indicated that the S/C group showed a high delayed type hypersensitivity compared with C/J group and the good skin reactions was increased to reach the maximum at 48 hrs post brucellin injection (PBI), when the erythema diameter reached 7.1±0.09mm and 5.4±0.3mm in S/C and C/J groups respectively and skin thickness was2.9±0.1 mm and 2.1±0.2 mm in S/C and C/J groups respectively. These results showed that immunized animals expressed cellular and humeral immune response examined by DTH and PHA. Concluded that the RB51 vaccine gave good level of immunization in goats and it can be considered as alternative vaccine against heterogonous *Brucella* spp.

Keywords: Brucellosis, RB51 vaccine, Goats, Passive haemagglutination.

Introduction

Brucella melitensis is the main causative agent of caprine and ovine brucellosis, sporadic cases caused by *B. abortus* have been observed, but cases of natural infection are rare in sheep and goats. Infection is widespread world-wide; North America (except Mexico) is believed to be free from the agent, as are Northern and Central Europe, South-East Asia, Australia and New Zealand (1). Some developed countries were able to control brucellosis but the disease is still forming a problem in the growing countries because of the importation of noncertified animals (infected), in addition, the increment in the numbers of sheep and goats might lead to increase infection ratio with *Brucella melitensis* .Thus more patients will appear due to its zoonotic importance (2).

There are three vaccines strains used in ruminants, *B. abortus* S19, *B. abortus* RB51, and *B. melitensis* Rev.1 (3 and 4). There are many disadvantages of using S19 and Rev.1 strains in which cannot differentiate between vaccinated and infected animals and the vaccine may cause abortion and the infection may be permanent in the uterus and mammary gland (5

and 6), while the partial lack of the oligopolysaccharide (OPS) prevents RB51 vaccine from inducing antibodies detectable by most of the serological tests, routinely used for the diagnosis of brucellosis (5, 6 and 7).

Goats are not the primary hosts of *B. abortus* but it produces similar clinical and serological results in those shown in cattle (8 and 9). Many studies have been done to determine the effects of rough strains of *B. abortus* in goats. A rough strain of *B. abortus*, RB-51, was found to maintain its rough phenotype, produce significant levels of anti-*Brucella* IgG, and did not produce abortions when experimentally injected into the fetuses of goats in their last trimester of pregnancy (10). The use of the caprine model to evaluate *B. abortus* for its applicability for bovine brucellosis has been documented (11), it was stated that 30-50% of pregnant goats infected with *B. abortus* 2308 abort (12). There are many methods of vaccination against brucellosis, in addition to subcutaneous route, conjunctival vaccination which is easy, economic and practical and induced good level of protection without side effect as compared with subcutaneous route (13 and 14).

For all mentioned above and because of few studies dealing with vaccination of goats with RB51 in the world and there is no any study dealing with this vaccine in Iraq, it designed this study aiming to evaluate the efficacy of the RB51 vaccine in immunization of goats and to identify the best route of vaccination.

Materials and Methods

Fifteen pregnant and non-pregnant female goats aged between 2-3 years were obtained from local market and left for 2 weeks for adaptation, animals were randomly divided into three groups, and all groups were tested before vaccination by rose Bengal test. After preparation of bacterial suspension according to the instructions of manufacturing company, the counting was made by McFarland tubes according to procedure described by (15). First group (n=5) was vaccinated subcutaneously in axillary region with 2 ml of RB51 which containing 4 $\times 10^8$ CFU in accordance with (16), the second group (n=5) was vaccinated conjunctively with 0.2 ml of RB51 which containing 4 $\times$ 10^8 CFU, and the third group (n=5) was kept as non-vaccinated control group, 3 of the animals were inoculated subcutaneously 2 ml of pbs and 2 animals were instilled conjunctively with 0.1 ml of pbs. All the animals were stabled with adequate space and fed with concentrated diet and nutritional supplements (vitamins and minerals), in addition they grazed on the pasture for 3-6 hours daily for the entire duration of the experiment, all stages were conducted with consideration for their welfare of the animals.

Brucellin of RB51 strain was prepared according to workers (17) using Kjeldahl method for measuring the protein concentration which was obtained from RB51 strain was 0.45 gm / dl. The delayed type hypersnsivity test was carried out at the 6th week post vaccination, this test was done by injection of 40 µg in 0.1 ml of brucellin intradermally in the skin of the neck of goats after clipping and shaving the animal hair, the area is marked by stain or marker pen, reaction was showed by an increase thickness and erythema after 24, 48 hrs.

Blood samples were collected from Jugular vein in test tubes until clotting for serum preparation, then kept in refrigerator overnight in stand position, then centrifuged at 2500 rpm/ 15 minute, and then sera were collected and frozen at -20 ˚C.

Passive haemagglutination test (PHA) was performed according to researchers (18). Ten ml of sheep blood was collected by a sterile syringe, then mixed with 10ml of Alsever´s solution, which were kept at 4 c◦ for 24-72 hrs .Blood sample was centrifuged 1500 rpm for 10 minutes to discard Alsever´s solution and RBCs washed three times with PBS (pH=7.2) and centrifuged 1500 rpm/5 minutes. Then 2.5% of sheep RBCs suspension was prepared.

Results and Discussion

Growth of RB51 strain (lyophilized) appeared on tryptic soya agar (TSA) as yellow or whitish-yellow colony ,not shiny, large, rough with granulation appearance, not regular edges under light microscope, RB51 appears as coccobacilli form arranged as groups of gram negative coccobacilli. RB51 strain was positive in urea agar and catalase test , did not grow on

media which containing bile salts, not fermentative to glucose, lactose and sucrose and produced H_2S, no motile, indole negative when cultured on SIM, no liquefaction of gelatin, produces agglutination with acriflavin 0.1%, and the colony colored a violet with crystal violet. These results agree with the morphological and biological characteristics of strain RB51 (19 and 20).

No clinical signs were noticed on applied animals after vaccination with RB51 vaccine except one vaccinated animal produced one weak kid in S/C vaccinated group while the others produce normal kids, these results were in agreement with (10), who showed that three fetuses were directly inoculated (in utero) at 120 days of pregnancy with a dose 10^7 to 10^8 CFU of RB51 without subsequent abortion, and there was preliminary evidence suggesting that 4×10^{10} CFU would not induce abortion (21),while there was a contradicting study that showed vaccination of goats with1×10^{10} CFU caused abortion and stillborn kid (22).

All three groups of goats after 2, 4, 6, 8, 10 and 12 weeks post vaccination showed negative results for Rose Bengal test, this is due to partial lack of OPS of the RB51 strain (23) also it induced incomplete antibodies which cannot be revealed by the agglutination activity. The results of goats vaccinated with RB51 strain through the two routes were in agreement with (24) where they revealed that vaccination of cattle with RB51 strain induced incomplete antibodies which cannot appear in agglutination activity, due to in adequate extension of Fab regions which prevents the effective bacterial agglutination (25 and 26).

Results of Rose Bengal test supported by (6, 7 and 23) where they showed that the partial lack of the OPS prevents the RB51 vaccine from inducing antibodies detectable by most serologic tests routinely used for the diagnosis of bovine Brucellosis. All groups showed negative results before vaccination with RB51. Passive haemagglutination test (PHA) showed that there were significant difference ($P<0.05$) in the Ab titer between the routes and among the 2, 4, 6, 8, 10 and 12 weeks post vaccination (table,1). Antibodies (Ab) titer increased to reach high value with a mean (19.2±3.2) and (16±4.4) in S/C group and in C/J group respectively at the 4th week post vaccination (table,1), there was significant difference ($P<0.05$) between the groups at the same weeks and then decreased at 6th week post-vaccination to reach (16±4.4) and (11.2 ± 1.9) in S/C and C/J groups, respectively, and the highest values were reported at 8th week post vaccination reached to (38.4±6.4) and (22.4±3.9) in S/C and C/J groups ,respectively with a significantly difference ($P<0.05$) between these two groups and at the same week, this increasing in the Abs titer results suggesting an activation of memory cells those induced after vaccination as in table,1; these results were supported by other authors (17), who showed that Ab response in vaccinated heifers reached the maximum value at 13 days post brucellin inoculation (PBI) and then progressively decreased with a sensitivity 90٪ between 9-20 days PBI and 100% between 9-13 days PBI, and this decreasing in the Ab titer appeared significantly at 10th week post-vaccination to reach (9.6±1.6) and (6.4 ± 0.9) in S/C and C/J group, respectively, and at the same weeks.

Results of PHT (table,1) showing that goats after vaccination through two routes developed a serological response, Abs titer in C/J route was lower than Abs titer of S/C route, this result was supported with (13) who found that conjunctival vaccination of Rev.1 gave good levels of immunization in spite of the lower antibody titers compared with S/C vaccination ,and the immunological response was 100٪ in the 3rd week for S/C route and 80٪ in the 2nd week and 100% in the 4th week for C/J route, this variation could be due to bacteremia which rapidly occurred in S/C route which lead to higher Abs titer than in C/J route vaccinated animals, due to the vaccine strain will localize in the lymph nodes of the head and lead to limited distribution and bacteremia in C/J vaccination (27).

These results are supported also by (28) who mentioned that using CFT-RB51 as antigen with sheep vaccinated with RB51 developed peak antibody titers at 15 days and remained for more extended period to reach 30 days post-vaccination.

Delayed type hypersensitivity test of goats was carried out at 6th week post-vaccination, the erythema and thickness were increased after

24 hrs and reach to the peak after 48 hrs as in (table,2). Results showed a significant difference (P<0.05) in erythema and skin thickness during 24 ,48 hrs after injection at 6th weeks post-vaccination and between the groups ,the mean of erythema in S/C vaccinated group and C/J vaccinated group was (6.2±0.2) mm and (4.5±0.2) mm respectively in a significantly difference (P<0.05) within 24 hrs, while within 48 hrs the mean of erythema in S/C vaccinated group and C/J vaccinated group was (7.1±0.09) mm and (5.4±0.3) mm respectively in a significantly difference (P<0.05). Mean of skin thickness in S/C vaccinated group and C/J vaccinated group was (2.3±0.2) mm and (1.4±0.1) mm respectively, is significantly different (P<0.05) and mean of skin thickness in S/C vaccinated and C/J vaccinated groups was (2.9±0.1) mm and (2.1±0.2) mm, were also significantly different (P<0.05) within 48 hrs.

Cell-mediated response was evidenced by inducing positive skin thickness to RB51 antigen as a result to macrophage development or due to activation of Th1 cells by antigen presented by macrophages or skin dendritic cells, these Th1 cells become memory cells for activation of hypersensitivity reaction capable of triggering inflammatory response, secondary exposure to antigen induced activation of memory cells from previous exposure and proliferation a new effectors T cell and influx of macrophages released of cytokines (CKs) and inflammation starts 24-48 hours after contact with antigen and dermatitis reactions are DTH responses. (29). These results showed that the cell-mediated immunity was different according to intensity of DTH response which represented by skin test in goats which was in agreement with those stated by (13-30) who revealed that results of skin test value in S/C vaccinated animals were more than in C/J vaccinated animals. Skin thickness showed slight increase at 24hrs PBI and reached the maximum at 48hrs PBI of RB51 brucellin which were in agreement with (2,31and32) who evaluated the skin thickness of sheep infected with *B. melitensis*. The brucellin in this study characterized by high degree of purity (17), this agrees with (2) who showed that preparation of brucellin in a high degree of purity do not induce non-specific reaction characterized by immediate type hypersensitivity in 24hrs which was supported these results.

Generally, sensitization of vaccinated animal with the same antigen will cause expression of the antigen on antigen presenting cell and activation of memory CD4 T-cell and produce IL2, IL4, IL8 cytokines and act as chemotactic factor to macrophage, which leads to congestion, redness and skin thickness (33). In Mexico (34) showed that the effectiveness of using RB51 strain in goats resulted in 87٪ of protection and reducing 50٪ of initial seroprevalence of goats measured through conventional serologic test and elimination of 83٪ of field *B.melitensis*, and prevention 80% of serum convert by smooth *Brucella spp*. The RB51 vaccine has been tested in small ruminants and reported that it gave protection up to 93% of vaccinated goats against *B. melitensis* (35).

Martinez *et al.* (34) showed that vaccination of goats with 3×10^8 to 3×10^9 CFU does not produce abortion in pregnant females, these results disagreement with our results in spite of that the dose of vaccination is higher than the dose used by them.

Table, 1: The mean of antibody titer of goats at 2, 4, 6, 8,10and12 weeks post-vaccination with RB51.

Group	Weeks (Mean ±SE)					
	2	4	6	8	10	12
1/ S/C	A 10.4±2.4	A 19.2±3.2	A 16±4.4	A 38.4±6.4	A 25.6±3.9	A 9.6±1.6
2/ C/ J	B 6.4±0.9	B 16±4.4	B 11.2±1.9	B 22.4±3.9	B 19.2±3.2	B 6.4±0.9
3/Control	0	0	0	0	0	0

Number of goats in each group = 5; L.S.D= 3.1
The differences in capital letters vertically refer to presence of significant value at (P<0.05).

Table, 2: Mean of skin thickness and standard error of goats inoculated with brucellin at 6th week post-vaccination

Group	Weeks	Erythema (Mean ±SE)		Skin thickness (Mean ±SE)	
		24 hrs	48 hrs	24 hrs	48 hrs
1/ S/ C	6	A 6.2± 0.2	A 7.1±0.09	A 2.3±0.2	A 2.9±0.1
2/ C/J	6	B 4.5±0.2	B 5.4±0.3	B 1.4±0.1	B 2.1±0.2
3/Control	6	0	0	0	0

Number of goats in each group = 5; L.S.D=0.46
The differences in capital letters vertically refer to presence of significant value at (P<0.05).

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دراسة الاستجابة المناعية للمعز الملقحة بلقاح RB51 بطريقتي التقطير على ملتحمة العين والحقن تحت الجلد للسيطرة على داء البروسيلات

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الخلاصة

أجريت هذه الدراسة لتقييم فعالية لقاح RB51 في تمنيع المعز على الرغم من كونه مخصص للأبقار حيث اعتمدت التجربة على 15معزة من الحوامل وغير الحوامل, 5 منها عوملت بـ 2 مل تحت الجلد من لقاح RB51 الذي يحتوي على 4×10^8 مستعمرة, و5 معزات أخرى عوملت بـ 0.2 مل من لقاح RB51 الذي يحتوي على 4×10^8 مستعمرة بطريقة التقطير في ملتحمة العين والخمسة الأخرى تركت كمجموعة سيطرة وعوملت بـ PBS. وبعد إعطاء اللقاح لم يلاحظ أي حالة إجهاض ماعدا معزة واحدة ولدت جدي ضعيف كانت قد لقحت تحت الجلد. قيست الاستجابة المناعية الخلطية في الحيوانات الملقحة كل أسبوعين بواسطة فحص الروزبنجال وفحص التلازن المنفعل وقيست الاستجابة المناعية الخلوية بفحص البروسلين في الأسبوع السادس. في الأسبوع الثاني من تلقيح المعز بلغ معيار الأضداد إلى2.4±10.4و 0.9±6.4 في الحقن تحت الجلد وطريقة التقطير على ملتحمة العين بمستوى معنوية ($P<0.05$) ثم ارتفع المعيار في الأسبوع الثامن ليصل إلى 6.4±38.4 و 3.9±22.4 في الحقن تحت الجلد وطريقة التقطير على ملتحمة العين بمستوى معنوية ($P<0.05$) ثم انخفض المعيار في الأسبوع العاشر ليصل في الأسبوع الثاني عشر إلى 1.6±9.6 و 0.9±6.4في الحقن تحت الجلد وطريقة التقطير على ملتحمة العين بمستوى معنوية ($P<0.05$) . حضر البروسلين من عتره RB51 اللقاحية ليستخدم كمستضد في اختبار التلازن الدمي المنفعل وكان التركيز الأمثل هو 40µg وكانت نتائج فحص البروسلين تشير إلى أن طريقة الحقن تحت الجلد لها قيمة جيدة وواضحة أكثر من طريقة التقطير على ملتحمة العين.

الكلمات المفتاحية: داء البروسيلات، لقاح RB51، معز، التلازن الدموي.