EVALUATION OF HUMORAL IMMUNE RESPONSE TO ASLIME PRODUCING *Staphylococcus aureus* MASTITIS VACCINE IN AWASSI EWES

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ABSTRACT

This study aims to evaluate the humeral immune response to a slime producing *Staphylococcus aureus* mastitis vaccine through the preparing two vaccines (Live attenuated and killed vaccines) from a slime producing *Staphylococcus aureus* bacteria isolated from Awassi ewes with mastitis. The first part of study was carried out to isolate the slime producing *Staphylococcus aureus* bacteria from many lactating Awassi ewes located around Baghdad. The second part included preparation of live attenuated and killed vaccines from isolated *S.aureus*. Safety and sterility testes were done on vaccines preparation. In the immunization study, fifteen Awassi ewes were randomly divided into three equal groups. Group-A:- Ewes were inoculated with one ml of a live attenuated vaccine (containing $1x10^{10}$ CFU) S/C near supra mammary lymph node, twice at 2 weeks intervals. at first month of lactation. Group-B:- Ewes were inoculated with killed vaccine twice, two weeks interval, at the same dose, route and time as previously described in group-A. Group-C:- Served as control. Clinical examination had been carried out in all animals pre and post vaccination. Moreover Serum and milk whey was obtained for measuring antibody by Elisa test, Animals show normal clinical signs pre vaccination. Nevertheless, moderate increase in body temperature, respiration, pulse rates in vaccinated groups, however the control group show normal systemic reaction pre and post vaccination. The vaccines induced humoral immunity, group-A revealed higher significant ($P \leq 0.05$) antibody titers in serum (0.136± 4.74) and milk whey (2.55±0.15) as compared with group-B which exhibited antibody titers (3.68± 0.153) in serum and whey attained at day 42 post- vaccination and thereafter, a decline in titers was observed towards the termination of the study. While control group showed low titers of antibody.

Key word: humoral immunity, a slime, Staphylococcus aureus, mastitis vaccine.

INTRODUCTION

Ovine mastitis is one of the main cases of alteration in milk content and has a major impact on both animal welfare and economy in the dairy industry (Chiaradia *et al.*, 2013). *Staphylococcus aureus* is a major causative agent in

dairy animals. In Sheep the *S. aureus*, may account 25-30% of total of intramammary infections (IMI). (Bonnefont, 2012). The ability of *Staphylococcus aureus* bacteria to persist in the mammary tissue as a biofilm (Slime) is one of the possible sources of chronic or persistent infections (Vaseduvan *et al*., 2003). These organisms were resistant to antibiotic due to their ability to exist both intracellular and due to their location within micro abscesses in the udder, The sources of *S. aureus* are include infected glands, contaminated milking equipment or bedding, and the skin (Kunz *et al.*, 2011). Antibiotic treatment has its own demerits including antibiotic resistance and drug residues in milk thus rendering mastitis control through antibiotic as an unacceptable approach due to public health hazards. In the absence of any mastitis control program and presence of high antibiotic resistance efforts were done to investigate the role of vaccination as an alternative mastitis control strategy for farms lacking standard mastitis control program (Kheirabadi *et al*., 2008). The recovery of intramammary infection after immunization has been explained by the development of humeral and cellular immune response in the udder, vaccination against some of the most prevalent mastitis pathogens seems logical choice to decrease losses associated with mastitis (Hadimli *et al.*,2005). The aim of this study was to evaluate the humeral immune response against staph- aureus mastitis vaccine.

MATERIAL AND METHODS

-Ewes: The experiment was conducted on fifteen clinically healthy lactating ewes of the Awassi breed aged 2 to 3 years which were given food and water *ad libitum*. The ewes were kept under observation for 2 weeks in clean building before starting the experiment. The ewes were examined clinically. Milk samples were collected in sterile test tubes aseptically after discarding the fore milk for bacterial and chemical analysis to confirm that these ewes were free from intramammary infection prior to vaccination (Bonnefont *et al.*, 2012). At day (3) before immunization. The lactating ewes were randomly divided into three groups (5) animals for each group, (live attenuated vaccine group, killed vaccine group and control group) by using different color spray.

-Rabbits: Thirty five (35) rabbits, local domestic breed were used in this study. They were obtained from the local market. Animals age ranged between 6-9 months, and their weight ranged between 1.5-2 Kg. They were housed in clean metal cages at room temperature about (22±3), they fed on commercial assorted pellet, alpha alpha hay and watered freely with tap water. Care was taken to avoid any unnecessary stress (Hrapkiewicz *et al.*, 1998). Animals were left for

10 days for adaptation before experiment start, and divided into seven groups (5 rabbits in each group), Two groups for pathogenicity test while the other for making safety test (live attenuated, killed antigens and control groups).

-Bacterium: A slime producing *S. aureus* bacteria, were isolated from many cases of clinical mastitis by culture of ovine milk specimens provided by different field in Baghdad area.

-Preparation of killed vaccine: The selected bacteria of *S. aureus* grew on nutrient agar and incubated at 37 °C. 24 hours, the bacteria were harvested by washing with sterile phosphate-buffered saline (PBS) and centrifuged by cooled centrifuge at (6000 rpm) for 60 minutes at 4 C, for three times, the bacterial suspension are inactivate with formalin (0.4%v/v), centrifuged (6000 rpm) for 60 minutes at 4 °C then washed three time with (PBS) (pH 7.2), the suspension was examined for purity by using Grams stain, the total bacterial count was calculated by McFarland Standard solution, and adjusted to10^{10} CFU ml^{-1} (Watson, 1984).

-Live attenuated vaccine: The selected *S. aureus* were passaged serially on 5% sheep blood agar until it lost its hemolytic activity and then maintained on mannitol salt agar slope at 4 °C. The organisms are culture in nutrient broth for 18 hours at 37 °C in an orbital shaker. The bacteria were harvested by centrifugation (6000 rpm) for 60 minutes at 4 °C and washed three times with sterile phosphate-buffered saline (PBS) (pH 7.2), resuspended in sterile PBS. The concentration of bacterial cells in suspension were determined by viable count and adjusted to10^{10} CFU ml^{-1} (Watson, 1984).

-Bacterial count: Counting of *S. aureus* for preparation of vaccine by using Standard (viable) Plate Count, according to Atlas (1995).

-Pathogenicity test: Pathogenicity of *S. aureus* was conducted in 5 healthy adult rabbits by subcutaneous inoculation of 0.2 ml of bacterial suspension containing 10^6 CFU ml^{-1}. The morbidity and mortality was observed for 20-40 hours (Ahmed and Muhammad, 2008). The vaccines were tested for safety and sterility prior to immunization as follows:

a-Safety test: The safety test was done by using 5 groups of rabbits (5 animal in each group), The 1st and 2nd groups were injected subcutaneously with two concentrations (0.5 ml and 0.2 ml) of live attenuated vaccine, while the 3rd and 4th groups were injected with killed vaccine in the same way and the 5th group served as control group. Rabbits were monitored for one week to record morbidity and mortality rates (Ahmed and Muhammad, 2008).

b-Sterility test: The vaccines were cultured on blood agar aerobically and anaerobically for 48 hours and check the growth of other microorganism (Ahmed and Muhammad, 2008).

-Experimental study: Fifteen lactating ewes were used for immunization, and randomly divided into three groups of (5) ewes in each group as follows:

Group-A:- Ewes were inoculated with one ml of a live attenuated vaccine (containing $1x10^{10}$ CFU ml^{-1}) S/C near supra mammary lymph node, twice at 2 weeks intervals at first month of lactation. Group- B:- Ewes were inoculated with killed vaccine twice, two weeks interval, at the same dose, route and time in group-A. Group-C:- Served as control receiving one ml of PBS .

Post immunization: All animals examined clinically (body temperature, pulse rate, respiratory, appetite and other general examination of animals) and examination of SCC in milk samples.

-Serum and milk samples: Blood samples were collected via jugular vein puncture by sterile plastic syringe (10) ml, and the serum was collected after centrifugation of blood sample and stored at -18 °C for detection and evaluation of antibodies, the serum was collected from ewes of each group at days 0, 14, 28, 42, 56 and 63 days post immunization (Watson, 1984). Milk samples were centrifuged at 3700 xg for 15 min. The fat layer was discarded, and the skim milk was stored at -18 °C until analysis for detection of antibody (Nordhaug *et al*., 1994).

Humoral immunity: Measurement of antibody was done by ELISA to detect the IgG in Serum and milk against *S.aureus* antigens.

RESULT AND DISCUSSION

- Pathogenicity of *S. aureus* isolate:

Pathogenicity of *S. aureus* was checked in five rabbits, four out of five rabbits were died within 18 hours and postmortem examination indicated septicemia, petechiation on intestinal serosal surface, straw coloured fluid in abdomen, swollen kidney, congested lungs and heart, the results of culture from different organ indicated *S. aureus* infection. These results were agreed with those reported by Butt (2006) and Athar (2007) they observed petichation in different organs with straw color in abdomen of rabbit after injected with *S.aureus* vaccine.

- Quality control of the vaccines:

- Sterility test of prepared *S. aureus* vaccines:

Culturing of killed vaccines on blood agar showed no growth after 48 hours of whereas growth of *S. aureus* was observed in live attenuated vaccine, which indicated the sterility of vaccine under trail.

- Safety test: The results of safety test of live attenuated and killed vaccine in rabbits are presented in table 1. In the 2nd group mild to moderate swelling developed in response to subcutaneous administration of the higher 0.5 ml dose of antigens and one rabbit showed clinical signs as fever, depression and anorexia while in the (1st, 3rd and 4th groups) it did not show any troublesome effect in the inoculated rabbits.

Table 1. Safety of live attenuated and killed *S. aureus* vaccines in rabbits

	Groups of rabbits	No. of rabbits	Dose and Route	Morbidity No.	Mortality No.
Live attenuated vaccine (Group-A)	1st	5	0.2 ml – S/C	0	0
	2nd	5	0.5 ml – S/C	1	0
Killed vaccine (group-B)	3rd	5	0.2 ml – S/C	0	0
	4th	5	0.5 ml – S/C	0	0

Sterility and safety evaluation is an integral part of quality assurance procedure for a test vaccine, all the standard procedures were attempted for sterility and safety conformation of the experimental vaccines. Therefore, rabbits were used and it was observed that this vaccine is safe to inject and free from any side effects when given at dose rate (0.2 mL S/C) in group A (live attenuated vaccine) and group B (killed vaccine). High dose (0.5) mL, caused a mild to moderate local swelling and increase in body temperature, only in group A. While group B didn't show any signs at this dose. The outcome is in line with those of Giraudo *et al.*, (1997) who also tested the safety of vaccine in rabbits and other laboratory animals. Soulebot *et al.*, (1997) ; Butt (2006) and Athar (2007) also reported the similar findings in their studies.

- Immunization Study

Two type of vaccines (Live attenuated and killed vaccine) in two doses were used in this study. The clinical signs after immunization result as followed:

Before challenge all groups (immunized and control) were examined clinically for body temperature, respiratory rate and pulse rate. All groups (A, B and C) showed normal body temperature (39.2±0.05) (39.4±0.1) (39.2±0.08) respectively, normal respiratory rate (26.5±2.07) (27.4±1.2) (25.2±3.4) respectively and normal pulse rate (75.2±2.7) (79±1.33) (78 ±5.39) respectively. After (12) hours the immunized groups (A and B) showed a slight increase in body temperature, respiratory and pulse rates. While the control group appeared high increase in the three parameters (Table 2). Group-C showed a significant variation (P≤0.05) as compared with immunized groups (A and B) and no significant variation (P>0.05) between immunized groups (A and B) in all clinical parameters. There was a high increase in body temperature, respiratory and pulse rates after 24−48 hours in group C and had a significance variation at (P ≤0.05) between group-C and immunized group, but non-significant variation (P >0.05) appeared between immunized groups.

Table 2. Effect of immunization on body temperature, respiratory rate and pulse rate values (M ± SE) in immunized and control groups

	Time (hours)	Groups of ewes		
		Group-A (live attenuated) Vaccine (M ± SE)	Group-B (killed vaccine) (M ± SE)	Group-C (Control) (M ± SE)
body Temperatures	0	39.2±0.05 Aa	39.4±0.1 Ab	39.2±0.08 Aa
	12	39.9±0.07 Aa	39.9±0.16 Ab	40.4±0.8 Bb
	24	39.8±0.09 Aa	39.7±0.08 Ab	40.5±0.1 Bb
	48	39.9±0.1 Aa	39.8±0.04 Ab	40.4±0.08 Bb
Respiration rate	0	26.5±2.07 Aa	27.4±1.2 Ab	26.2±3.4 Aa
	12	30.9±2.5 Ab	33.2 ± 4.3 Ac	49.6±4.4 Bb
	24	31.7±1.1 Ab	32.3±5.2 Ac	48.1±8.3 Bb
	48	30.5±1.1 Ab	31.3±2.7 Ac	48 ±4.23 Bb
Pulse rate	0	75.2±2.7 Aa	79±1.33 Aa	78 ±5.39 Aa
	12	84.3±7.1 Ab	89.7±8.2 Ab	109.4 ±3.5 Bb
	24	83.2±1.8 Ab	87.4±2.9 Ab	111.4 ±2.4 Bb
	48	82.6±2.4 Ab	86.6±6.3 Ab	113.6 ±4.6 Bb

Small letters= differences inside the same group, capital letters= differences between the different groups, The same letters = no significant differences (P ≥0.05), The different letters= significant differences (P ≤0.05).

Clinical signs were observed in ewes prior and during three days post immunization in immunized and control groups of ewes. Ewes in group A which received live attenuated vaccine showed local clinical signs after immunization included hot, painful swelling at the site of injection of vaccines also there was a decrease in appetite during 24 hours and all clinical signs disappeared after 72 hours. Group B which received killed vaccine showed moderate clinical signs

while group C did not show any clinical signs. This result is in agreement with leitner *et al.,* (2003) and Athar (2007) who used Staphylococcal vaccine for prevention of mastitis and this vaccine did not show systemic symptom and only swelling for short time at site of injection. These results was in agreement with study of Caroline *et al.*, (2011) who recorded an elevation of body temperature in the majority of inoculated animals after three administration of killed *S. aureus* vaccine and the fever was monitored for the purpose of verification of the inflammatory reaction to killed bacteria and fever determined by increase in body temperature > 0.7 C from that before vaccination. Also Rotta (1999) did not record any change in body temperature after repeated inoculation of rabbits with killed *S. aureus* vaccine. Our results is contrary to Athar (2007) who recorded that the cause of increased body temperature post immunization were mediated due to the action of endogenous pyrogens, which are protein in nature and produced by granulocytes, monocytes and macrophages. The present study revealed an increase in body temperature, respiratory rate and pulse rate these were in compatible with finding of Farooq *et al.,* (2007) who referred that elevation of body temperature lead to increase the respiration and pulse rates at same time post immunization of animals with different vaccines. Increase in body temperature might considered as one of the methods for detection of mastitis, as in study of Schutz *et al.,* (2000) made evaluation for detection of experimental *S.aureus* mastitis in cows by using infrared thermography and recorded elevation in rectal temperature in 5 out of 6 cows. The live vaccine induce greater up regulation of CD40, CD86, interlukin-6, interlukin-12, and granulocytes- macrophages colony- stimulating factor than do killed vaccine, these chemotaxis increased of macrophage and monocyte at site of injection in live vaccine compared with killed vaccine (Tizard, 2009).

- Humoral immune response of ewes

Antibody titers of Sera and whey from milk were detected by ELISA test at day 0, 14, 28, 42, 56 and 63 in immunized groups and control group, the results were recorded as followed:

- Antibody Titer in serum samples:

Before immunization, all experimental animals (group A, B and C) revealed low level of antibodies titers (0.024±0.005, 0.022±0.005 and 0.026±0.005) respectively. Post immunization, group-A revealed an increase in serum antibody titer (0.91±0.02) at day 14 and continued to increase till the peak antibody titer reaching (4.74±0.13) at day 42 post-immunization. In this group, a decline in antibody titer was recorded towards the termination of the study. In

Group-B, serum antibody titer (0.47±0.02) was observed at day 14 post-immunization as compared to that at zero day. Peak of antibody titer was observed (3.68±0.15) in this group at day 42 post- immunization subsequently after that a decline in titer was recorded towards the termination of the study. While the control group showed low antibody titer throughout the study period that did not differ from the baseline titer at zero day. A significantly increase of antibody titer at (P≤0.05) was observed in group-A as compared to that recorded in groups-B and C, also group-B showed higher significance at (P≤0.05) as compared with group-C during the study period at (14, 28, 42, 56 and 63) days. The overall results indicated that group-A behaved best throughout the experiment which showed the highest titer of serum antibody as shown in table 3.

Table 3. Serum antibody titers in immunized and control groups of ewes as determined by ELISA test at different times

Time (days)	Groups of ewes		
	Group-A (live attenuated) (M ± SE)	Group-B (killed vaccine) (M ± SE)	Group-C (Control) (M ± SE)
0	0.024±0.005 Aa	0.022±0.005 Aa	0.026±0.005 Aa
14	0.91±0.02 Ab	0.47±0.02 Bb	0.03 ±0.07 Ca
28	3.69±0.15 Ac	2.6±0.2 Bc	0.032±0.09 Ca
42	4.74±0.13 Ad	3.68±0.15 Bd	0.018±0.003 Ca
56	4.24±0.34 Aed	3.46±0.17 Bed	0.018±0.007 Ca
63	3.74±0.14 Afc	2.54±0.18 Bfc	0.024±0.005 CBa

Small letters = differences inside the same group, capital letters = differences between the different groups, The same letters = no significant differences (P ≥0.05), The different letters = significant differences (P ≤0.05).

Our study showed that both live attenuated and killed *S. aureus* vaccines stimulated a considerable serum antibody response, a better response was elicited by ewes given live attenuated *S. aureus* vaccine more than the killed vaccines. The similar findings correspond to those of (Watson and Kennedy, 1981 ; Watson and Colditz, 1982 ; Amorena *et al*., 1994 ; Tollersrud *et al*., 2002; Watson, 2002 and Farooq *et al.*, 2007) who showed higher antibody response in vaccinates animals. But Davidson (1987) reported a higher efficacy

of live attenuated vaccine in triggering production of specific antibody and enhanced neutrophil ability to destroy *S. aureus* as compared to killed vaccine and found that killed vaccine did not stimulate production of specific antibodies. Other studies are compatible with our study, Nickerson (1991) showed that 4.7 fold increases in serum antibody titer against *S. aureus* bacterin in vaccinated animals compared to non-vaccinated and the elevation of titer was maintained throughout 10 weeks of trial period, Hadilmi (2005) determined efficacy of live attenuated *Staphylococcus aureus* vaccine against staphylococcal mastitis in sheep. Marisa *et al*., (2002) reported high efficacy of intramammary immunization with a live attenuated *S. aureus* mutant to produce specific antibodies and protect the mouse mammary gland from infection. Watson and Lee (1978) recorded a significant differences in antibody titer between vaccinated ewes with live attenuated and killed vaccines of *S.aureus* and control groups and also between the two vaccinated groups. The results of humoral immune responses to the prepared vaccines in this study revealed that the vaccines were affective in their action and gave sera antibody at different time of the study Mellenberge (1977) recorded that vaccination against mastitis is considered one of the useful procedures to increase the resistance of animal against bacterial infection of the mammary gland. Administration of the vaccine to the draining area of the supramammary lymph node has been reported to stimulate the changes in the local lymph node and thus enhance the immunity response (kerlin and Watson, 1986).

Antibody Titer in whey samples

The level of antibodies to Staphylococcal antigens in milk whey of the immunized (live attenuated and killed groups) and non-immunized group were determined by ELIZA test at different days (0, 14, 28, 42, 56 and 63) post-immunization. Before immunization (at zero time), all ewes in three groups showed very low antibody titers against *S. aureus* antigens. Immunization with live attenuated *S. aureus* antigens in group-A resulted in increase in whey antibody titer (0.54±0.02) at day (14) post- immunization as compared to antibody titer at day (zero). Peak antibody titers (2.55 ±0.15) were attained at day (42) post-immunization in this group and after that there was a declining pattern in antibody titer toward the end of study. In group-B, antibody titer (0.26 ±0.03) was observed at day (14) post immunization as compared to antibody titer at zero day the Peak of antibody titers (1.74±0.06) attained at day 42 post-immunization and thereafter, a decline in titers was observed towards the termination of the study. The control group did not show any increase in

antibody titers from baseline values throughout the study period. The highest mean whey antibody titers was observed in group-A followed by that in group-B. The two groups (A and B) showed a significant difference at ($p \leq 0.05$) as compared with the control group. At 14, 28, 42, 56 and 63 days post immunization, group-A showed a significant increase ($p \leq 0.05$) of milk antibody titers as compared with groups-B and C (Table 4).

Table 4. Milk whey antibody titers in immunized and control groups of ewes as determined through ELISA test at different days

Time (days)	Groups of ewes		
	Group-A (live attenuated) (M ± SE)	Group-B (killed vaccine) (M ± SE)	Group-C (Control) (M ± SE)
0	0.002±0.003 Aa	0.003 ±0.007 Aa	0.003±0.003 Aa
14	0.54±0.02 Ab	0.26 ±0.03 Bb	0.003±0.002 Ca
28	1.53±0.15 Ac	0.89 ±0.028 Bc	0.002±0.006 Ca
42	2.55 ±0.15 Ad	1.74±0.062 Bd	0.003 ±0.004 Ca
56	2.16±0.11 Aed	1.27±0.04 Bed	0.002±0.005 Ca
63	1.9±0.08 Af	1.01±0.04 Bfc	0.004±0.004 Ca

Small letters= differences inside the same group, capital letters= differences between the different groups, The same letters = no significant differences ($P \geq 0.05$), The different letters= significant differences ($P \leq 0.05$).

Ideally an effective vaccine should raise antibody titers to a protective level in milk whey, In the present study, immunized ewes (A and B groups) showed significantly higher milk whey antibody titers as compared to their respective control groups. Although, the whey antibody titers were significantly higher in immunized groups than in control, these titers were lower as compared to serum antibody titers in respective vaccinated groups. One of the possible reasons for low antibody titers in whey is the inability of immunoglobulins to enter freely from blood into mammary glands unless there is inflammation. A similar finding was mentioned by Tollersrud *et. al*., (2002) Watson and Colditz (1982) ; Shakoor (2006) ; and Butt (2006) ; Athar (2007) evaluated formalin inactivated vaccine in lactating buffaloes. The vaccinated buffaloes showed significantly higher serum and whey antibody titers, higher milk yield, and lower prevalence and incidence of subclinical mastitis as compared to unvaccinated control. Also

our study is compatible with Leitner *et al.*, (2003) who recorded that the vaccinated groups exhibited specific antibodies to *S. aureus* in milk whey and these antibodies persist throughout the whole period of experiment, these antibodies may play a significant role in the acquisition of immunity against subsequent *S. aureus* infection. Watson and Kennedy (1981) found that the ewes immunized with live attenuated vaccine developed a significantly greater level of opsoninsin serum and milk, than did those immunized with killed vaccine or non-immunized control. Post immunization, most of the IgG antibodies in milk was derived from serum, The mechanisms by which IgG antibodies enter the udder differ, however, are according to the isotype, most of the IgG antibodies enter by passive diffusion through the epithelial cells of the udder, or with neutrophil leucocytes bound to the Fc -receptor (Poutrel *et al*., 1988).

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تقييم الاستجابة المناعية الخلطية للقاح المحضر من بكتريا المكورات العنقودية المنتجة للغراء في النعاج العواسي

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المستخلص

هدفت هذه الدراسة الى تقييم الاستجابة المناعية الخلطية للقاح المحضر من جراثيم المكورات العنقودية الذهبية المنتجة للغراء *Staphylococcus aureus*، والمعزوله من ضرع الاغنام المصابة بالتهاب الضرع، إذ تم تحضير نوعين من اللقاح (اللقاح الحي المضعف واللقاح المقتول)، صمم الجزء الاول لعزل جراثيم المكورات العنقودية الذهبية المنتجة للغراء من عدد من النعاج الحلوبة في مناطق حول بغداد، وشمل الجزء الثاني تحضير اللقاح الحي المضعف واللقاح المقتول من بكتريا المكورات العنقوديه الذهبية، وخضعت اللقاحات المحضرة لاختبارات السلامة والنقاوة. في الدراسة التمنيعية، تم استخدام خمسة عشر من النعاج العواسي قسمت عشوائياً الى ثلاث مجاميع متساوية: المجموعة (أ): حقنت بـ 1 مل من القاح الحي المضعف يحوي على $1x10^{10}$ خلية حية مل$^{-1}$ تحت الجلد قرب الغدد اللمفية فوق الضرع مرتين كل اسبوعين في الشهر الاول للحلب، المجموعة (ب): حقنت باللقاح المقتول بنفس جرعة ووقت وطريقة الاعطاء في المجموعة (أ)، المجموعة (ج): استخدمت كمجموعة سيطرة. فحصت جميع النعاج سريريا قبل وبعد اعطاء اللقاح، واستخدم مصل الدم ومصل الحليب لغرض قياس مستوى الاجسام المضادة بواسطة فحص الاليزا. لم تظهر الحيوانات قبل التلقيح علامات سريرية، وبعد التمنيع لوحظ ارتفاع في درجة الحرارة والتنفس والنبض في الحيوانات الممنعة بينما لم يظهر اي تغير في هذه المعايير بالنسبة لحيوانات السيطرة. حفز اللقاح المناعة الخلطية فأظهرت المجموعة (أ) ارتفاعا ملحوظا ($P\leq0.05$) في مستوى الاجسام المضادة في مصل الدم (4.74 ± 0.136) ومصل الحليب (2.55 ± 0.15) بالمقارنة مع المجموعة ب والتي كان فيها مستوى الاجسام المضادة (3.68 ± 0.153) في مصل الدم ومصل الحليب على التوالي في اليوم 42 بعد التمنيع، بعد ذلك اظهرت هاتين المجموعتين انخفاضا في مستوى الاجسام المضادة حتى نهاية التجربة. بينما اظهرت مجموعة السيطرة انخفاضا في مستوى الاجسام المضادة.

الكلمات المفتاحية: الاستجابة المناعية الخلطية، المكورات العنقودية الذهبية، التهاب الضرع، النعاج.