IMMUNOPATHOLOGICAL CHANGES OF *STAPHYLOCOCCUS AUREUS* EXPERIMENTAL INFECTION IN MICE IMMUNIZED WITH FOOT AND MOUTH DISEASE VACCINE

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ABSTRACT

The present study was carried out to investigate immunopathological effect of foot and mouth disease vaccine against nosocomial infections, to achieve this goal different samples obtained from urine of hospitalized patient, the isolated bacteria were identified by cultural examination, biochemical tests PCR. Twenty-four mice were divided equally into three groups. The 1st group 8 mice were immunized I/M with 0.1 ml of FMD vaccine, two dose with 14 days intervals, 2nd group 8 mice was inoculated I/P with 0.5 from 3x10^8 CFU/ml while 3rd group was inoculated 0.2 ml of phosphate buffer saline and served as control negative. At 27-30 days post immunization, cellular immune response was done by DTH and 3 mice from each mentioned groups were sacrificed for measuring IgG titer then all the remained animals were injected I/P with 0.5 ml from infections dose3x10^8 CFU/ml. Post mortem examination was made at 3 days post infections and specimen of liver, kidney, lung and spleen were fixed with 10 % formalin for histopathological study. The result of skin test showed that the mean values of skin thickness against FMD vaccine at 24 hr. was 3.47 mm then increase to 4.66 at 48 hr. The result expressed high value of AB titer against FMD vaccine 2778.66 compared with control group recorded heavy bacterial growth recorded in the infected group with sever histological lesion characterized by suppurative granuloma in liver with lymphoid depletion in splenic tissue together with renal tubules degeneration while the main histopathogical findings of immunized groups revealed multiple MNCs infiltration with mature granuloma in liver and lung together with mild or absent bacterial growth. The result concluded that FMD vaccine reduce the infection in the immunized mice and enhance protective immune response against experimental murine bacterial infection.

Key words: PCR, AB titer, histopathological, albino mice.

التغيرات المرضية المناعية للاصابة التجريبية بالمكورات العنقودية الذهبية للفئران الممنعة بلقاح الحمى القلاعية

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المستخلص

اجريت الدراسة للتحقق من التأثير المناعي للقاح الحمى القلاعية البيطري ضد الاصابة بالمكورات العنقودية الذهبية ولتحقيق ذلك جمعت عينات الادرار من المصابين الراقدين في المستشفى وشخصت البكتريا المعزولة بزرعها واستخدمت الاختبارات الكيموحيوية لها وكذلك استخدم تفاعل البلمرة المتسلسل, 24فأر قسمت بالتساوي الى ثلاثة مجاميع, المجموعة الاولى تم تمنيعها بأستخدام 0,1ملغم من لقاحFMD بالعضلة بجرعتين بفاصل زمني14يوم, المجموعة الثانية اصيبت بجرعة 0,5مل بتركيز3x 10^{8}مستعمرة/ مل بالخلب وعدت سيطرة موجبة بينما عدت المجموعة الثالثة سيطرة سالبة حقنت0,2من المحلول الملحي المتعادل. بعد 27–30 يوما من التمنيع قيست المناعة الخلوية وقتلت3 فئران من كل مجموعة لقياس معيار الاجسام المضادة بعدها اصيبت بقية الحيوانات بنفس جرعة الاصابة واجريت الصفة التشريحية بعد 3 ايام من الاصابة واخذت عينات للكبد, النبيبات الكلوية, الرئتين والطحال لدراسة التغيرات المرضية والنسجية. أظهرت نتيجة اختبار الجلد أن متوسط سمك الجلد بالمجموعة الاولى3,47ملم بعد24 ساعة 1,35 ملم بعد 48 ساعة اماقيمة الاجسام المضادة2778,66 مقارنة بقيم مجموعات السيطرة التي سجلت عزل بكتيري كثيف مع آفة نسيجية حادة تميزت بالورم الحبيبي القيحي في الكبد مصاحبة لتنكس الأنابيب الكلوية, اما تغيرات المجموعة الممنعة تمثلت بأرتشاحات متعددة من الخلايا وحيدة النواة مع وجود افة حبيبية ناضجة في الكبد والرئة وانعدام او وجود عزل بكتيري ضعيف. استنتجت الدراسة ان لقاح الحمى القلاعية يقلل من خطرالاصابة في الفئران الممنعة ويحسن الاستجابة المناعية ضد الاصابة التجريبية بالمكورات العنقودية الذهبية في الفئران.

الكلمات المفتاحية: تفاعل البلمرة المتسلسل, معيار الاجسام المضادة, التغيرات المرضية النسيجية, الفئران البيضاء.

Received:22 /9/2022, Accepted:7/12/2022

INTRODUCTION

Nosocomial is any disease caused via nosocomial pathogens such as *Staphylococcus aureus, Streptococcus* spp., *Bacillus cereus, Acinetobacter* spp., coagulase negative *staphylococci, enterococci, Pseudomonas aeruginosa, Legionella* and members of the *Enterobacteriaceae* family such as *E.coli, Proteus mirabilis, Salmonella* spp., *Serratia marcescens* and *Klebsiella Pneumonia* (3). contracted by a patients under medical care and developed were staying in a hospital and seeming either during the hospitalization or after liberation (1, 18). S*taphylococcus aureus* is cause primary hospital- acquired infections such as lower respiratory tract, surgical site infections and the second reason of nosocomial bacteremia, pneumonia, and cardiovascular infections, *S.aureus* have extensive armamentarium of virulence factors with both structural and secreted products singing a starring role in the pathogenesis of infections (20). A predominantly humoral immune response in the vaccinated animal is stimulated by FMD vaccine, there is a good correspondence between level of Abs and safety in contrast to live virus challenge by the same strain of FMD virus from which the vaccine was created(5). This study aimed to use the veterinary FMD vaccine against experimental murine infection with the main nosocomial pathogens.

MATERIALS AND METHODS

FMD vaccine is inactive vaccine was obtained from Veterinary Clinic, the dose injected I/M $3x10^8$ according to man facture's in striations with booster dose after 2 week and eleven clinical urine specimens were collected from patients at Baghdad Teaching Hospital/Department of Medicine City, which brought to Pathology Department in the College of veterinary Medicine/Baghdad University. A total number of 24 mice from both sexes with ages ranged 4-8 weeks old which were gotten from the (National Center of Research and drug Monitor in Bagdad) were adapted for two weeks before starting the experimentation by rearing in separated pure cages, they were fed on commercial assorted pellets and clean water then divided into three groups (1^{st} immunized group, 2^{nd} control positive group and 3^{rd} control negative group). The cellular immune response was done by skin test while humeral immunity detection by using radial immune diffusion plate and the isolate molecular detection by conventional PCR also by using the biochemical tests and culture characteristics were studied depending on colony morphology on growth media (Nutrient agar, mannitol salt agar, macConkey agar) (13) and microscopically examination by gram stain. On nutrient appear very smooth, round, small with yellow color, but on mannitol salt agar media the colonies appeared round ,smooth with yellow color & large ,round ,yellow to golden in color appear on macConkey agar as in then confirmed by macroscopically examination using gram stain and in order make biochemist test to be sure, these specimen was sent to Central Health Laboratory to Confirmed their diagnosis. Determination of infective dose of selective bacteria, the infective dose was prepare by using McFarland tubes (10). For histopathological study, mice were anesthetized, scarified and pieces 1 cubic cm from liver, lung; kidney and spleen were taken and fixed in %10 formalin solution for 72 hrs. fixed tissue were dehydrated in a graded series of ethyl alcohol, cleared in xylenes. Tissues were cleared and imbedded in paraffin blocks then sectioned at 5micrones. Sections were stained with Hematoxylin & Eosin stain and examined under light microspore(14)

RESULTS AND DISCUSSION

Bacterial Isolation & Identification: The current results showed the bacterial isolates from lung, liver, spleen and kidney were detected by morphological examination on different media(Table1).

Table1. Cultural examination of bacterial samples on different media

Sample	Macconkey agar	Nutrient agar	Monnitol salt agar
1	-	-	-
2	-	-	-
3	-	-	-
4	Growth	Growth	-
5	-	-	-
6	-	-	-
7	-	-	-
8	Growth	-	Growth
9	-	-	-
10	-	-	Growth
11	-	-	-

Immunological Tests

A. DTH (Delayed Type Hypersensitivity)

The results of the current study exposed that the mean values of skin test thickness after 24 hours in immunized and control group are 3.47and 1.35while in 48 hours 4.66 and 1.35 as in (Table2) with significant difference p≤0.05.

Table2. The mean values and standard error of skin test in immunized and control group

Groups	0 day	24 hours	48 hours	LSD value
		Ab	Aa	
Immunized group	1.35±0.00	3.47±0.2	4.66±0.34	157.88
		Bb	Bb	
Control group	1.35±0.00	1.35±0.00	1.35±0.00	168.32

Means having different capital letters in same column and small letters in the same arrow P≤0.05

B. Determination Of IgG Protein, By Radial Immunodiffusion Plate

By using Radial immune diffusion plate the mean value of Ab (IgG) titer which appear as hemolysis zone in the gel of plate. Immunized group showed highest level of IgG Ab which reached to 2778.66 while control group showed lowest level of Ab titer 1180.12 as in (Table3) with significant differences p≤0.05.

Table3. Mean and standard error of IgG concentration (mg/dl)

Groups	Mean & SE of IgGA	LSD value
Immunized group	2778.66±108.4	356.71
	B	168.32
Control group	1180.12±98.16	

Means having with the different capital letters in same column with significant differences (P≤0.05).

Bacterial isolation

The present results in non-immunized infected mice showed heavy bacterial growth from internal organs during 72h. post infection accompanied with moderate clinical signs while immunized mice with FMD vaccine appear more good healthy with mild to absent bacterial isolation as compare with previous group as in (Table4).

Table4. Bacterial isolation in nutrient agar from internal organ of immunized and non-immunized infected mice with *staph. aurous* at 3day Post challenge

Groups	Liver	Lung	Kidney	spleen
Control group				
1	++++	+++	++	++++
2	+++	+++	+++	+++
3	+++	+++	+++	+++
4	+++	++++	++	+++
5	++++	++++	++	++++
Immunized group				
1	+	-	+	+
2	+	+	-	+
3	+	+	-	+
4	-	-	-	-
5	+	-	-	-

Mild: +, Moderate : ++, Heavy : +++, More heavy : ++++.

Pathological Examination

A- Gross Pathological Changes At 27days Post Immunization characterized by moderate congestion, swelling of internal viscera mainly liver and spleen (hepatosplenomegaly) of immunized group.

B- Gross Pathological Changes At 3 Days Post Infection

The main characteristic macroscopic lesions in infected control group revealed presence multiple pale necrotic foci with variable size in parenchyma of liver and spleen also in abdominal viscera accompanied with moderate congestion, While no obvious gross lesion were observed in non-immunized groups

Histopathologic Examination

A- At 27 Day Post Immunization: The main lesion in lung of mice immunized with FMD vaccine show large mononuclear cells (MNCs) aggregations mainly lymphocytes around blood vessels and airways with slight homogenous proteinaceous substance in some alveolar lumen (Figure1). In liver periportal cellular infiltration consist of lymphocytes together with PMNs accompanied with ductal and vascular dilation **(**Figure2) as well as focal lymphocytic infiltration in liver parenchyma with kupffer's cells proliferation (Figure3). The kidney section showed perivascular lymphocytic aggregation, other section showed focal interstitial and periglomerular MNCs infiltration consist of lymphocytes and macrophages (Figure4). Moderate hyperplasia of lymphoid tissue with moderate proliferation of megakaryocytes in red pulp of spleen (Figure5).

B- At 3 Days Post-Infection

Non-immunized and infected mice (control positive) expressed moderate destruction in alveolar tissue accompanied with PMNs infiltration in the inter-alveolar septa (Figure6) together with pulmonary vessels congestion, while the main liver lesion was presence of large granuloma with necrotic center containing dead neutrophils surrounded MNCs and PMNs aggregation, as well as portal vein congestion and dilation accompanied with portal infiltration with MNCs(Figure7), the kidney lesions revealed extensive vacuolar degeneration of tubular epithelium lining accompanied with focal interstitial MNCs infiltration (Figure8), also moderate to severe depletion of splenic lymphoid tissue was recorded (Figure9) ,in addition to severe red pulp congestion together with hemorrhage and hemosiderin deposition(Figure10). The predominant microscopic lesions in immunized-infected mice revealed development of granulomatous lesion in the pulmonary, hepatic and renal tissues (Figures11, 12 and 13), with evidence of apoptosis in splenic lymphoid tissue (that revealed space formulation due to apoptosis with moderate red pulp congestion **(**Figure14**).**

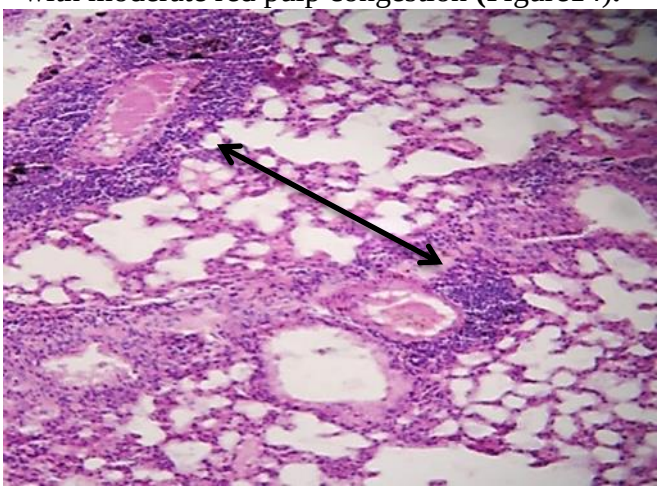

Figure1. Histopathologic section in lung of mouse at 27 day post immunization with FMD vaccine showed marked lymphocytic perivascular cuffing(⟷), (H and E,20X)

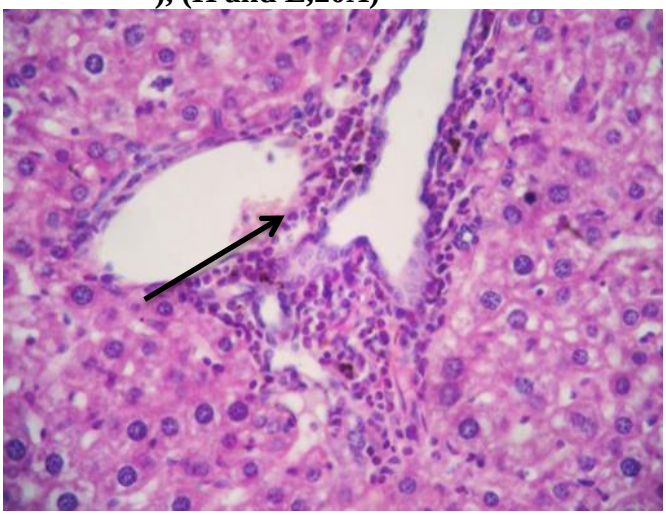

Figure2. Histopathologic section in liver of mouse at 27 day post immunization with FMD vaccine showed periductal PMNCs &MNCs infiltration with slight ductal and vessels dilation (⟶) (H and E,40X).

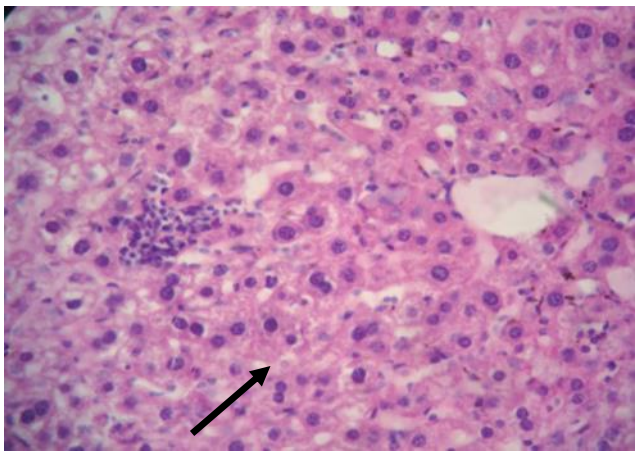

Figure3. Histopathologic section in liver of mouse at 27 day post immunization with FMD vaccine showed focal lymphocytic aggregation (⟶) in liver parenchyma (H and E ,40X)

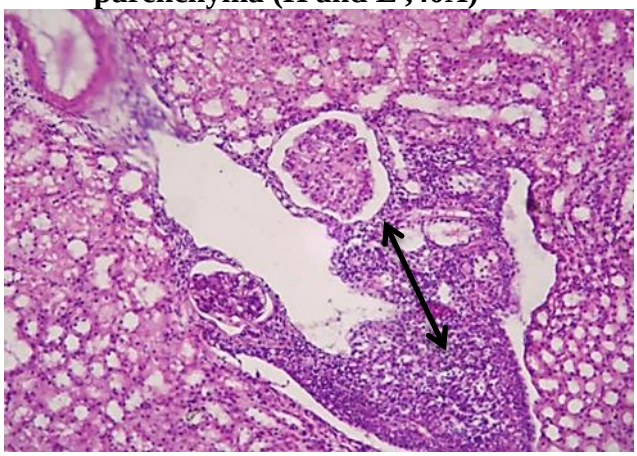

Figure4. Histopathologic section in kidney of mouse at 27 day post immunization with FMD vaccine showed peri glomerular and interstitial MNCs infiltration (⟷) (H and E,20X)

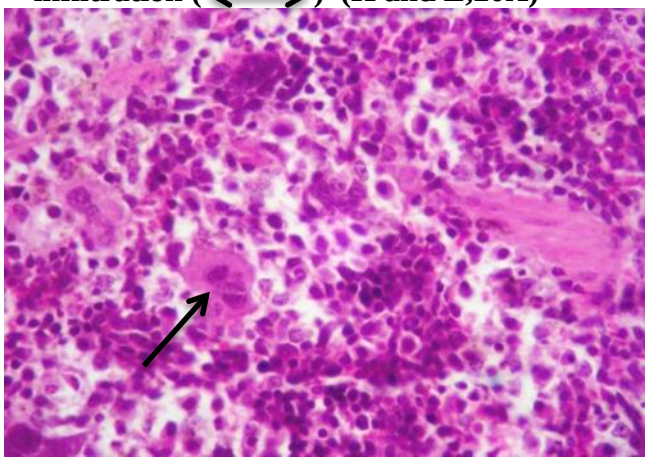

Figure5. Histopathologic section in spleen of mouse at 27 day post immunization with FMD vaccine showed proliferation of megakaryocytes (⟶)(H and E,40X)

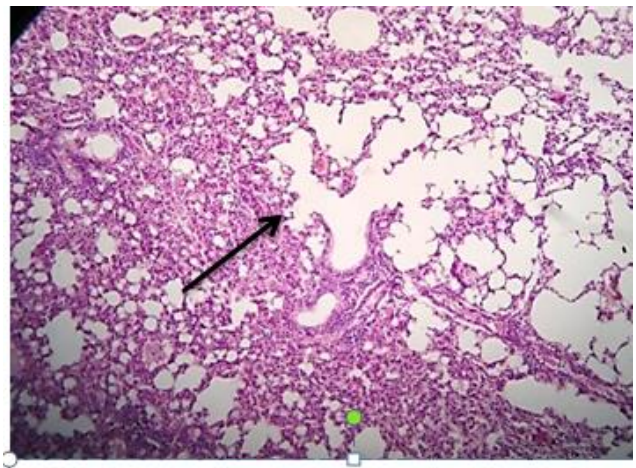

Figure6. Histopathologic section in lung of mouse at 3 days post challenge, showed moderate neutrophile infiltration in the alveolar septa with emphysema(⟶)(H

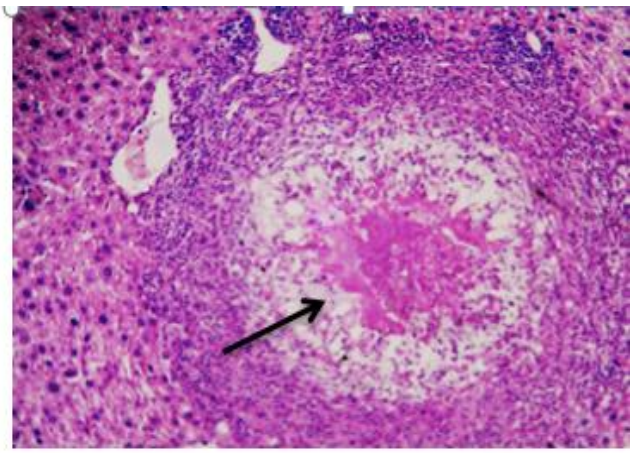

Figure7. Histopathologic section in liver of mouse at 3 days post challenge, showed large pyogranuloma- with necrotic center (⟶)(H and

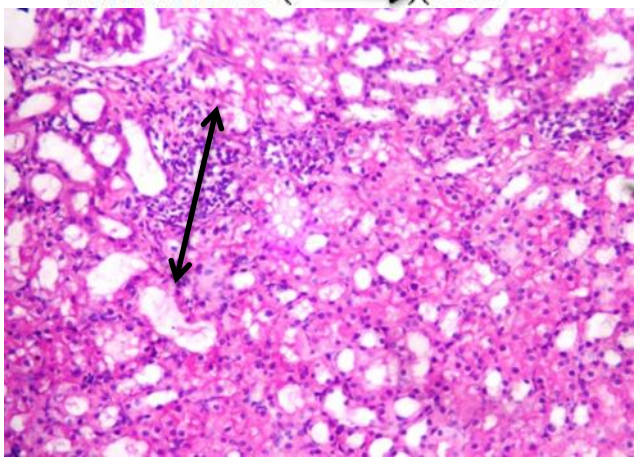

Figure8. Histopathologic section in kidney of mouse at 3 days post challenge, showed focal interstitial MNCs infiltration with marked vacuolation of tubular lining ⟷

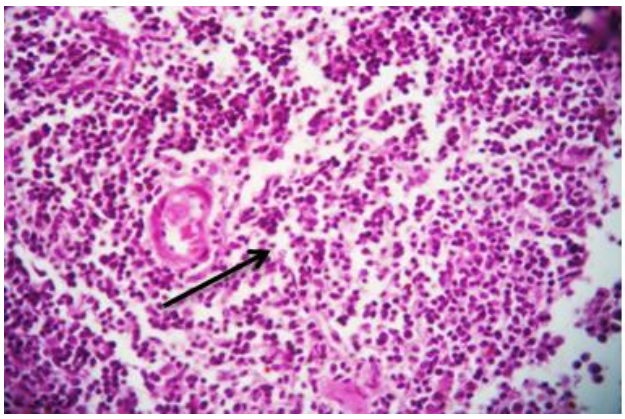

Figure9. Histopathologic section in spleen of mouse at 3 days post challenge, showed lymphoid depletion of white pulp (⟶)(H and E,40X)

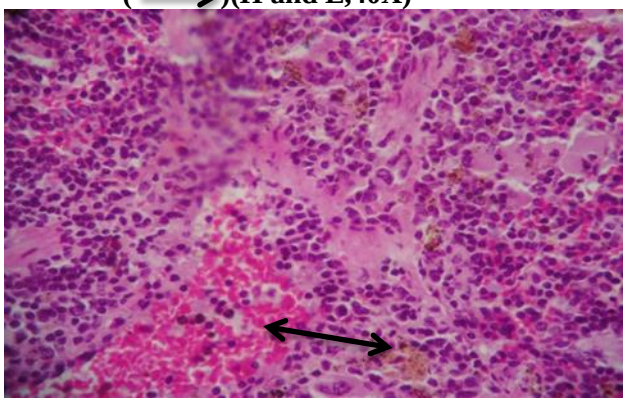

Figure10. Histopathologic section in kidney of mouse at 3 days post challenge, showed red pulp congested and hemorrhage with hemosiderin deposition (⟷) (H and E,40X)

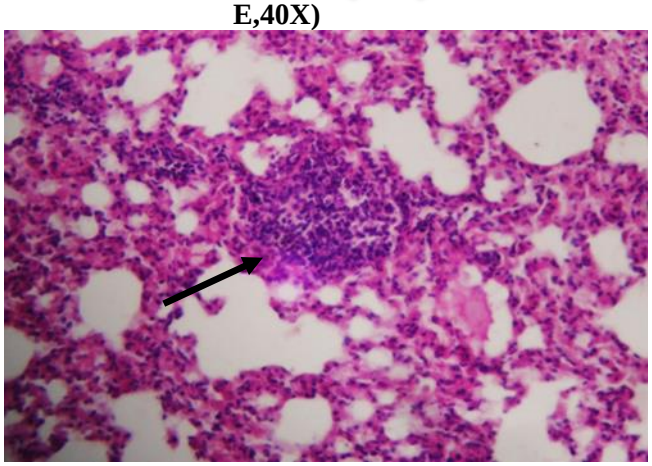

Figure11. Histopathologic section in lung of mouse at 3 day post immunization with FMD vaccine showed granulomatous like lesion (⟶)(H and E, 20x).

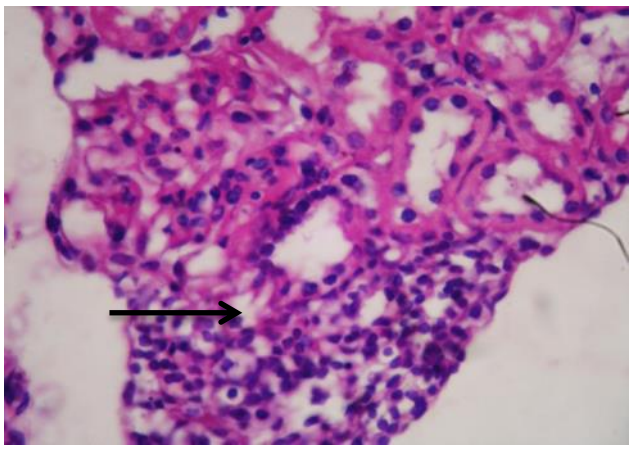

Figure12. Histopathologic section in kidney of mouse at 3 day post immunization with FMD vaccine showed interstitial MNCs aggregation,(⟶),(H and E,40X)

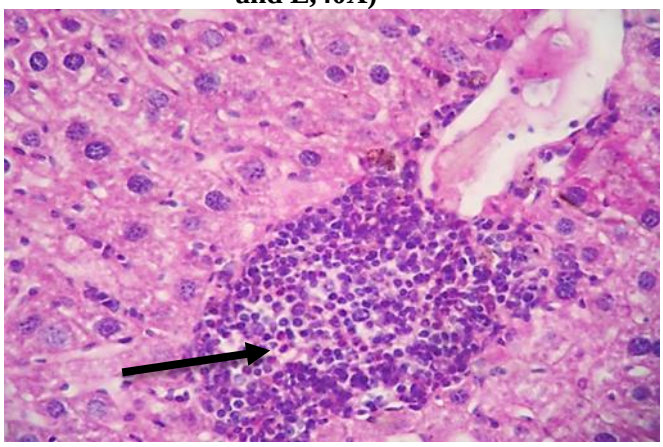

Figure13. Histopathologic section in liver of mouse at 3 day post immunization with FMD vaccine showed granulomatous like lesion with prominence of kupffer cell (⟶), (H and E,

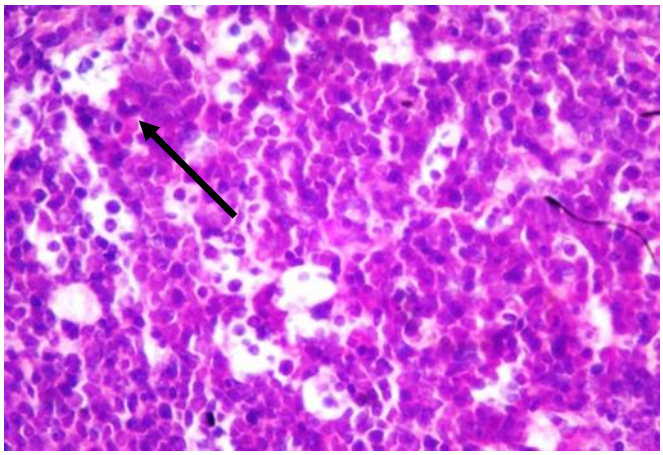

Figure 14. Histopathologic section in spleen of mouse at 3 day post immunization with FMD vaccine showed space formation due to apoptosis filled with cellular depress (⟶), (H and E, 40x).

The present work used FMD vaccines strategies and provided evidence for the central role of cellular immune response and neutralizing Abs in obtaining protection against staphylococcus infection. FMD vaccine were successful to enhance good protection and derivate the chance to develop the infection, this evidence was in consistence with present observation that revealed high IgG titers as compared with other non-immunized group. The induction DTH reaction in mice immunized with FMD vaccine may be due to CMI response mediated by cytotoxic T lymphocytes that play an important role in protecting animals from FMD viral infection after vaccination with Abs titers within gray zone (2) who revealed the concentration change of Th1 cytokines such as TNF-γ, IL-12, IL15, M1G and IL-18 suggestion that cellular immune response mediated by Th1 cytokines also in the present study investigate reduced levels of humeral immunity as may be due to type vaccine (killed in activated vaccine) which reduce lower Ab titers which persist for less time than those induce by live viral (7). Although several previous studies confirm the significance of humeral immunity in FMD murine model (17), The difference in Ab response in mice immunized with FMD vaccine may be due to structure of mice Ab and how interaction with FMD vaccine.

Pathological Examination

The current study showed severe pathological lesions in the internal organs of positive groups, these results may indicate that *S.aureus* overcome innate immune response of the host which acting an integral role in determine the outcome of the infection (8). Also demonstrated that suppurative reaction characterized by neutrophils infiltrating in all examined organs mainly in liver and kidney together with necrotic lesions reported in liver tissue may due to the enzyme and toxins produced by these pathogens, as well as lesions of lymphoid depletion in spleen and destructive lesion in the pulmonary tissue, these observations in agreement with Giampiero *et al*.(8), who recorded that pan ten-valentine leukocidin is a pore-forming toxin secreted by *S.aureus* associated with disease such as necrotizing pneumonia. The liver was the most affected organ, spleen and kidney may be due to the normal function of liver from nutritional metabolic balance in the body which include all nutrient materials and the liver play a potential role in elimination of toxins, body weight protective and other foreign materials included the pathogens(19) , also it's a major organ depends in portal circulation so PMNs were attracted from circulation to the infection or inflammation locate by CXCL8 chemokines; including IL-8 homologs keratinocyte chemo attractant (kc) and macrophage inflammatory protein- 2(MIP-2) (6).The presence of pyogranulomatous lesions in the liver consist from number of neutrophils and macrophage, the latter can also produce IL-12 which enhanced, activated and aggregation of phagocytes around invasive agents (15), According to above evidence there was correlation between pathological examination and bacterial isolation from examined organs of the control group. Immunized groups showed congestion, moderate to marked aggregations of phagocytic cells together with lymphocytic infiltration accompanied with appearance of granulomatous lesions in FMD vaccinated group which have the ability to induce cellular response that induce these cells to migrate and localized the liver, lung and kidney in order to phagocyte and eliminate the foreign proteins. A number of reasons demonstrate that cellular immune response is key factor responsible for immune protection in FMD vaccinated animals that leading to the rapidly and protective T-independent Abs response and FMD virus mediated modulation of dendritic cells which are central players in both innate and adaptive immunity are potent APCs and have ability to activate have T cell responses, also the results showed small granuloma in liver and lung of this group may indicate that vaccine activated immune cells to secret cytokines that play essential role in initiated granuloma (11).

MOLECULAR DETECTION

Figure15. PCR product the band siz . The product was electrophoresis on 1.5 % agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100)

Score	ct	Identities	Gaps	rand
1108 bits(1228)	)	617/619(99%)	0/619(0%)	lus/Plus

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Query  1    TAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGG  60
Sbjct  87   ............................................................  146
Query  61   ATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCC  120
Sbjct  147  ............................................................  206
Query  121  GACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGATTCCTACGGGAGG  180
Sbjct  207  ...........................................C................  266
Query  181  CAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGA  240
Sbjct  267  ............................................................  326
Query  241  TGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGT  300
Sbjct  327  ............................................................  386
Query  301  GCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT  360
Sbjct  387  ............................................................  446
Query  361  AAAACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT  420
Sbjct  447  ..T.........................................................  506
Query  421  TTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAAC  480
Sbjct  507  ............................................................  566
Query  481  TTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGG  540
Sbjct  567  ............................................................  626
Query  541  AGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCG  600
Sbjct  627  ............................................................  686

Query  601  TGGGGATCAAACAGGATTA  619
Sbjct  687  ..................  705
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There are numerous research on the characterization of the *Staphylococci aurous* from other species of *Staphylococci* because is most important and recurrently pathogen (4), Therefore you most distinguish *S.aureus* in clinical practice and food safety investigation (9). McClure *et al.* (16) established multiplex conventional PCR analysis because it's a powerful and higher sensitive more than microscopical examination for detecting of *Staphylococci* in general and *Staphylococci aurous* by selection of 16 *SrRNA* gene as targeted genes for detection of these pathogenic bacteria.

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