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Preliminary Immunization Study of *Hyalomma* Spp . In Local BreedRabbits (*Oryctolagus cuniculus*)

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Abstract

Ticks blood-sucking arthropods are of great importance in the fields of veterinary and medical worldwide. This study was aimed to investigate the immune response of *Hyalomma* spp. ticks (eggs and adults-females) antigens by using fifteen local breed rabbits **(*Oryctolagus cuniculus*)** which **divided** to three groups; first and second groups were immunized by 500µg s/c of sonicated eggs and adults antigens respectively and the third group (control negative) was injected 0.1 ml phosphate buffer saline (PBS) s/c. The first showed good immune responses through the skin tests compared to the second group and both of them than the third group that showed didn't any immune response. Also for the histopathological changes of the vital organs (spleen, liver and kidneys), the first group showed high mononuclear cellular proliferation and infiltration compared to the other remain groups. This is the preliminary study by using sonicated *Hyalomma* spp. eggs antigens in local breed rabbit's which showed higher immune response than sonicated adult's females antigen and infiltration ,activation and proliferation of the mononuclear cells in the vital organs.

Keywords: *Hyalomma*, Skin Test, rabbits, immune response, sonicated antigen

Introduction

Hard ticks are obligatorily blood-sucking ectoparasites belong to the family Ixodid which served as vectors for pathogenic microorganisms transmission that causes serious infection disorders in animal and human (1, 2, 3, 4) and have a great impact on human and veterinary health (5). Ticks are important vectors of pathogens causing a large list of diseases (3).There are more than 900 tick's species known worldwide and about 10% of

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them are responsible for the transmission of pathogens among domestic animals and humans (6). Also, ticks borne diseases and infestation constitute a growing burden for animal and human health through the world, which considered a second vector for pathogenic microorganisms tomosquitoes (5), such as, nematodes, protozoans, viruses, and bacteria, that causing life-threatening diseases to humans and animals such as livestock, pets, or wildlife (6), through a huge economic burden, the negatively impacts productivity of livestock worldwide (7). Most ticks preferred target different animal hosts and they are may be taking two or three years to complete the life cycle and larvae and nymphs species, after feeding , drop off from their host to molt to the next stage (5).

In Iraq many studies were available that dealing with the prevalence and distribution of some species of hard ticks (8, 9, 10, 11, 12, 13, 14) and the main genera of *Ixodid* that infesting livestock are *Hyalomma, Rhipicephalus* and *Haemaphysalis* (13).

Tick immune system is more rudimentary from the vertebrates immune system and only presenting immune response (innate) and an evidences a large species showed an immune system efficiency ; Also, ticks saliva are contains modulate molecules bioactive to their host immune reactions and hemostasis(15), as well as (16) was demonstrated the intravenous transfer immune serum from tick infested animal with *Amblyomma americanum* larvae conferred a significant levels resistance to tick in naïve animals, and the acquired tick resistance (ATR) against ticks to the naive syngeneic animals can be transferred by sera or leukocytes that isolated from animals previously infested (16,17,18,19). It was referred to factors in each organ of tick immune system interact with pathogens develop a new control strategies for potentially targets ticks and tick-borne diseases (15).

Due to the importance of *Hyalomma* tick in the transmission of diseases to the domesticated animals this study was prepared for the first time in Iraq to compare the immune responses of sonicated eggs and adults females of *Hyalomma* spp. antigens in local breed rabbits.

Methodology

Tick isolate

Adult *Hyalomma* spp. females were collected from domesticated animals (sheep and cattle) from different areas at Baghdad city and transferred to Department of Parasitology Laboratory, Veterinary Medicine College, University of Baghdad. They were divided into two parts; the first one for morphological diagnosis according to bases on main characteristic features including mouthparts, basic capitulum, color, scutum, and festoons (20), the second part was kept for eggs culture for larval production by using engorged females propagated in glass bags at room temperature.

Protein extract

Eggs and adult female tick's crud antigens (Protein) were prepared by used ceramic mortar and ultrasound waves system (US SOLD Company), centrifuged at 5000 rpm for 15 min., after that supernatant was collected, filtered by 0.2 µ Millipore filter and divided into two parts, the first part for

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protein estimation and the second one was kept at -18°C until used for immunization the experimental animals (Rabbits).

Determine protein concentration

The protein concentration was estimated by using a special kit (LP87016/BIOLABO) and according to manufacture instructions which used in immunological analysis.

Experimental Animals

Fifteen local breed rabbits of both sexes (about 1000-1500 g) were kept and land rearing in a special room of the Animal House / College of Veterinary Medicine /University of Baghdad. Animals were divided into equal three groups randomly; the first group was injected by sonicated eggs extract 500 µg /animal s/c; the second group was injected by sonicated adult females ticks extract 500 µg/animal s/c and the last one was injected 0.1 ml PBS s/c as a control negative group.After 14 days of all immunized animals gave the same doses as booster doses of immunization.

Histopathological examination

All rabbits were sacrificed, anatomized and the vital organs (Spleen, liver and kidneys) were used for histopathological examination by using paraffin blocks and slide preparation according to (21).

Data Analysis

Statistical data analyzed by Chi-Square Test(22).

Results

Tick isolate

Adult female ticks were diagnosed as *Hyalomma* spp. according to their morphological characteristics of the mouth parts, triangular basic capitulum, color, scutum, legs , and Festoons (Figure, 1)

Skin test

The results of the skin test were shown a significant ($P \leq 0.01$) increased in the diameters of the indurations of the immunized groups by sonicated eggs and female adult ticks antigens compared to control that injected PBS(Tables,1, 2 ; Figure, 2) .

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Figure (1): *Hyalomma* spp. females tick.

Table (1).Skin reaction (Indurations) of sonicated eggs and adult female of *Hyalomma* spp. ticks in the immunize rabbits.

Eggs / Mean ± SE (mm)				Adults (Females)/ Mean ± SE (mm)			
Hours	24	48	72	Hours	24	48	72
Eggs	6±0.316	8±0.316	7±0.447	Adults	4±0.316	6±0.316	4.2±1.113
PBS	1.8±0.489	4±0.316	3±0.316	PBS	2.2±0.2	3.2±0.2	2.2±0.2
χ^2	5.217**	6.329**	5.242**		3.488**	5.426**	1.523 NS
**P≤0.01 NS: Nonsignificant							

Table (2). Skin reaction (Indurations) of sonicated eggs and adult female of *Hyalomma* spp. ticks of the immunized rabbits.

Mean ± SE (mm)			
Hours	24	48	72
Eggs	6±0.316	8±0.316	7±0.447
Adults	4±0.316	6±0.316	4.2±1.113
χ2	3.164**	3.164**	1.794 NS
**P≤0.01 NS: Nonsignificant			

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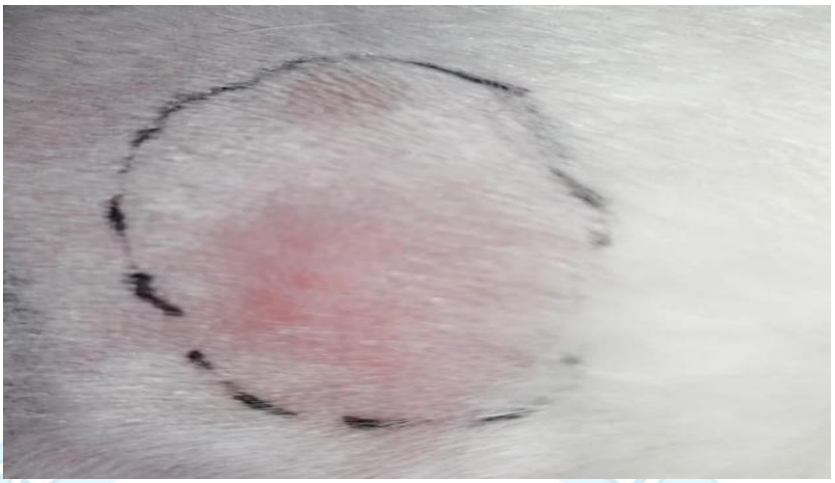
Figure (2).Skin induration of sonicated *Hyalomma* spp. eggs antigen of immunized rabbits.



Histopathological lesions

The immunized group of sonicated eggs antigen showed a high mononuclear cells infiltration in the vital organs liver, spleen and kidneys (Figures,4,5,6) compared to the adult female ticks which showed a slightly mononuclear cellular infiltration (Figures,7,8,9) and also to the control negative group that injected PBS (Figures,10,11,12).

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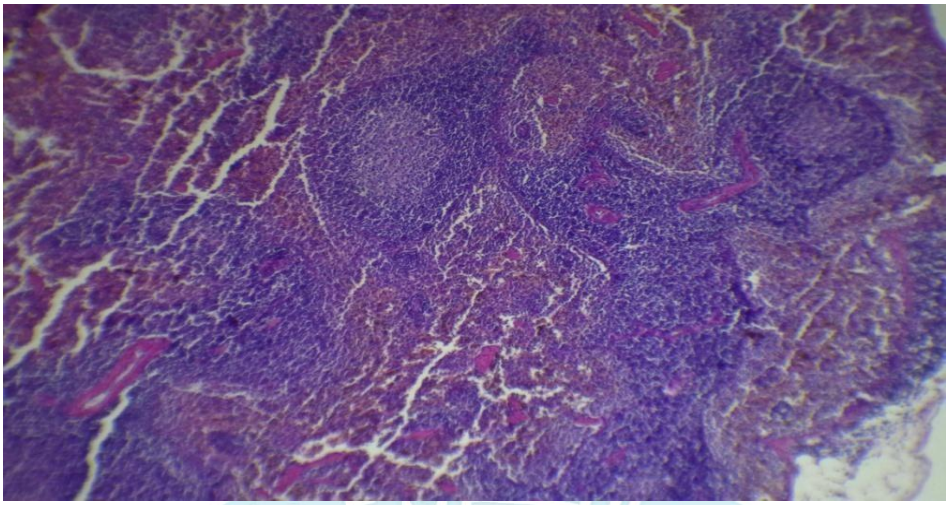

Figure (3). Spleen of immunized rabbits with sonicated *Hyalomma* spp. eggs antigen shows hyper-cellular proliferation of white pulp (H&E 10X).

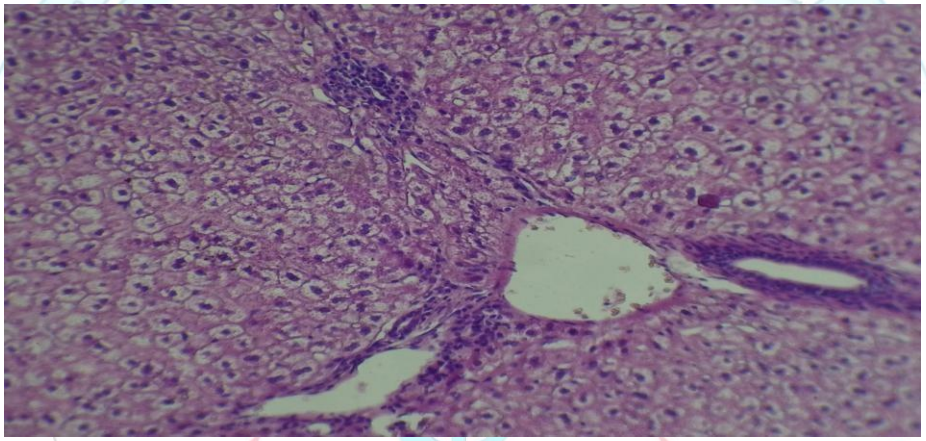

Figure (4).Liver of immunized rabbits with sonicated *Hyalomma* spp. eggs antigen shows perivascular cuffing of lymphocytes (H&E 20X).

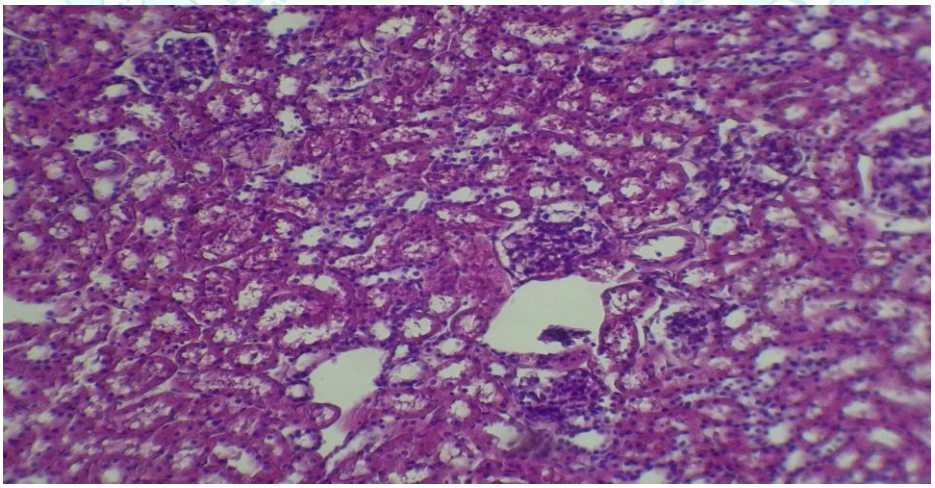

Figure (5).Kidney of immunized rabbits with sonicated *Hyalomma* spp. eggs antigen shows normal glomeruli and normal tubules (H&E 20X).

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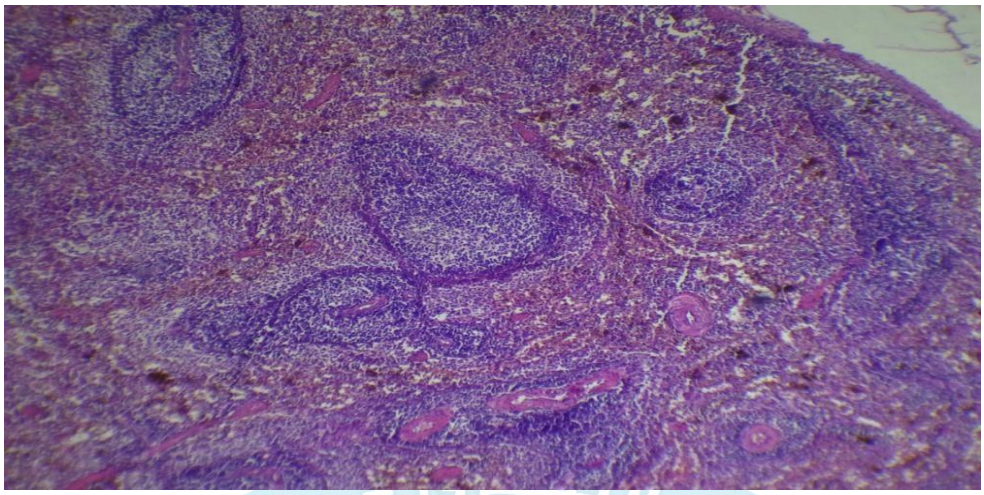

Figure (6). Spleen of immunized rabbits with sonicated *Hyalomma* spp. adult females antigen shows slight depletion of lymphocytes (H&E 10X).

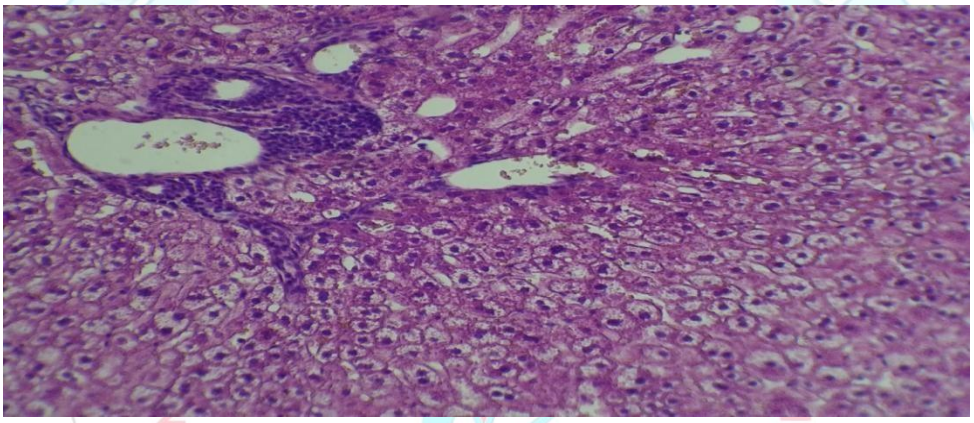

Figure (7). Liver of immunized rabbits with sonicated *Hyalomma* spp. adult females shows severe lymphocytic cuffing infiltration (H&E 20X).

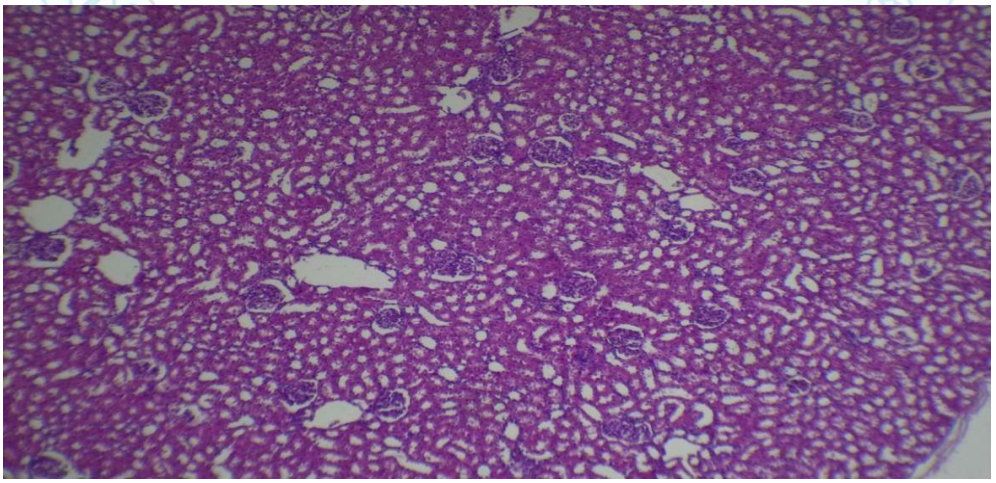

Figure (8). Kidney of immunized rabbits with sonicated *Hyalomma* spp. adult females antigen shows no histopathological changes only distention of tubules (H&E 10X).

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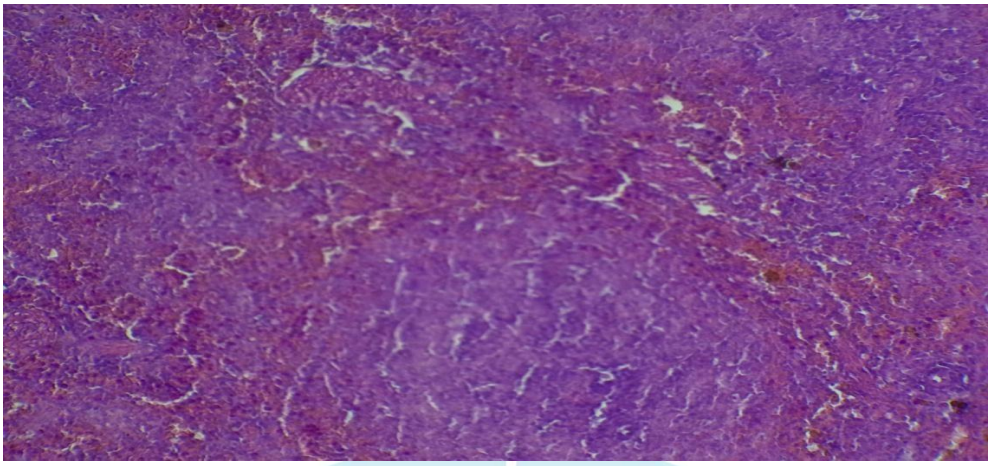

Figure (9) .Spleen of the control negative rabbit injected phosphate buffer saline (PBS) shows no histopathological changes (H&E 10X).

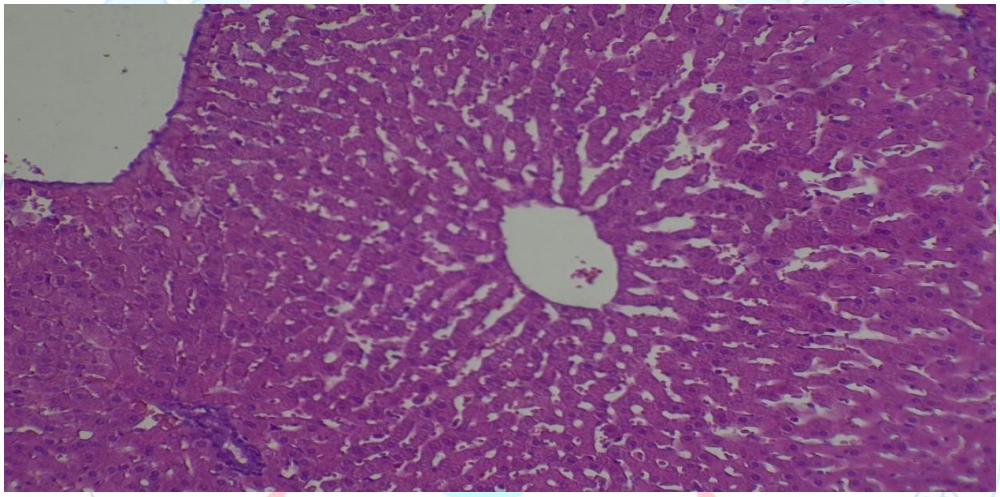

Figure (10).Liver of the control negative rabbit injected phosphate buffer saline shows normal architecture hepatic cord and sinusoid with normal central vein (H&E 10X).

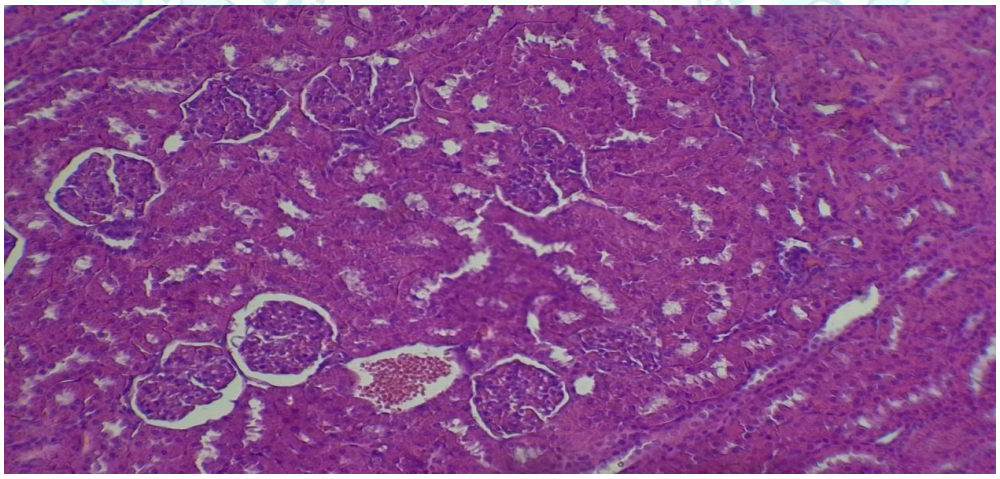

Figure (11). Kidney of the control negative rabbit injected phosphate buffer saline shows no histopathological changes, normal glomeruli and renal tubules (H&E 10X).

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Discussion

The immune signaling has a direct correlation with effector specificity for ticks that may be result in the absence of important and new mechanisms of tick immune system and current works have been suggested an express different types for effectors from the activation of immune signaling pathway such as phagocytosis, antimicrobial peptides (AMPs), complement like molecules and reactive oxygen species-ROS production which are considered an important factors for protecting ticks from the infection (15). Secret saliva into the host which returns the excess water and ions to host that concentrating the blood meal (23),also contains several histamine binding proteins (24, 25, 26) .On the same hand, it was found that cattle treatment with antihistamine leads to higher numbers of ticks (27), while injected histamine into cattle skin promoted detachment of ticks (28).Similar an observations were reported in guinea pigs that infested with *Dermacentor andersoni* (29) and infested rabbits with *Ixodes ricinus* (30), that explain the implying histamine produced by host could be threat the successful ticks blood feeding by ticks (15). To counteract the bioactive substances (24, 25, 26) of ticks; host activate a various defense pathways including innate and acquired immunity against the tick infestation (5). Strictly hard ticks are hematophagous feeding on their host blood of for several days, blood acquisition that depends on the types of injected and properties of pharmacological substances of tick saliva during their feeding, and the acquired tick resistance(ATR),which manifested by reducing ticks weight and prolonged duration of feeding, reduced engorgement numbers, molting inhibition, death , diminished production
,and reducing viability of ova and; also, it is not restricted to the skin (tick bites) of sensitized animals, that may be suggesting the systemic involvement fairly than the localized response (15), that explanations were closely agreement with our results of the present study, the immunized host by sonicated tick antigens give a systemic immune response rather than localized which characterized by the mononuclear proliferation and infiltration in the vital organs (Spleen ,liver, kidneys) and the immunization by sonicated *Hyalomma* spp. eggs give a high immune response than adult females in the skin test (Increase induration) . On the same way, the complement system as an important branch for both cellular and humoral immunity in vertebrate and invertebrate (metazoan), which recognized the foreign cells (microbes), specifically tag them via opsonization, and eliminate them via cell lysis or phagocytosis (31).
(32)was reported , amount of histamine in the skin lesions was correlates with resistance degree to tick infestation. On the same way, some species of animals can be acquired resistance to ticks blood feeding after single or repeated infestation resulting in decreases weight and number of engorged ticks or death during the subsequent infestations, that is basis for vaccines development (tick antigen-target) for prevent tick infestation and tick borne diseases; On one way, basophils accumulation that were detected in the skin lesions shows ATR (5). Other animals species (cattle, rabbits, guinea pigs and mice), have been developed after a single or repeated infestations resistance to tick feeding and it is depending on the ticks and animal species (33; 34; 35).

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