A STUDY ON EPIDEMIOLOGY OF HARD TICK (IXODIDAE) IN SHEEP AND A TRIAL OF IMMUNIZATION OF RABBITS AGAINST (*Hyalomma anatolicum anatolicum*) IN SULAIMANI GOVERNORATE-KURDISTAN REDION-IRAQ.

A DISSERTATION
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(ENTOMOLOGY)

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ

الْعَلِيمُ الْحَكِيمُ {32}

صدق الله العظيم

سورة البقرة

I certify that this thesis entitled " **Study on some epidemiological of hard tick Ixodidae) in sheep in Sulaimani province/Kurdistan region-Iraq with trail of mmunize rabbits with larval extract of *Hyalomma anatolicum anatolicum*** " was repared under my supervision at the University of Sulaimani, College of Agriculture, as partial requirement for obtaining **Ph.D**. Degree in **Animal Production/ Entomology.**

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In view of the available recommendation, I forward this thesis for debate.

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As the examining committee, we certify that we have read this dissertation and examined the student in its contents. In our opinion, it is adequate with ***pass*** standing as a dissertation for the degree of ***Ph.D***. of Science in Agriculture/ Animal production (**Entomology**).

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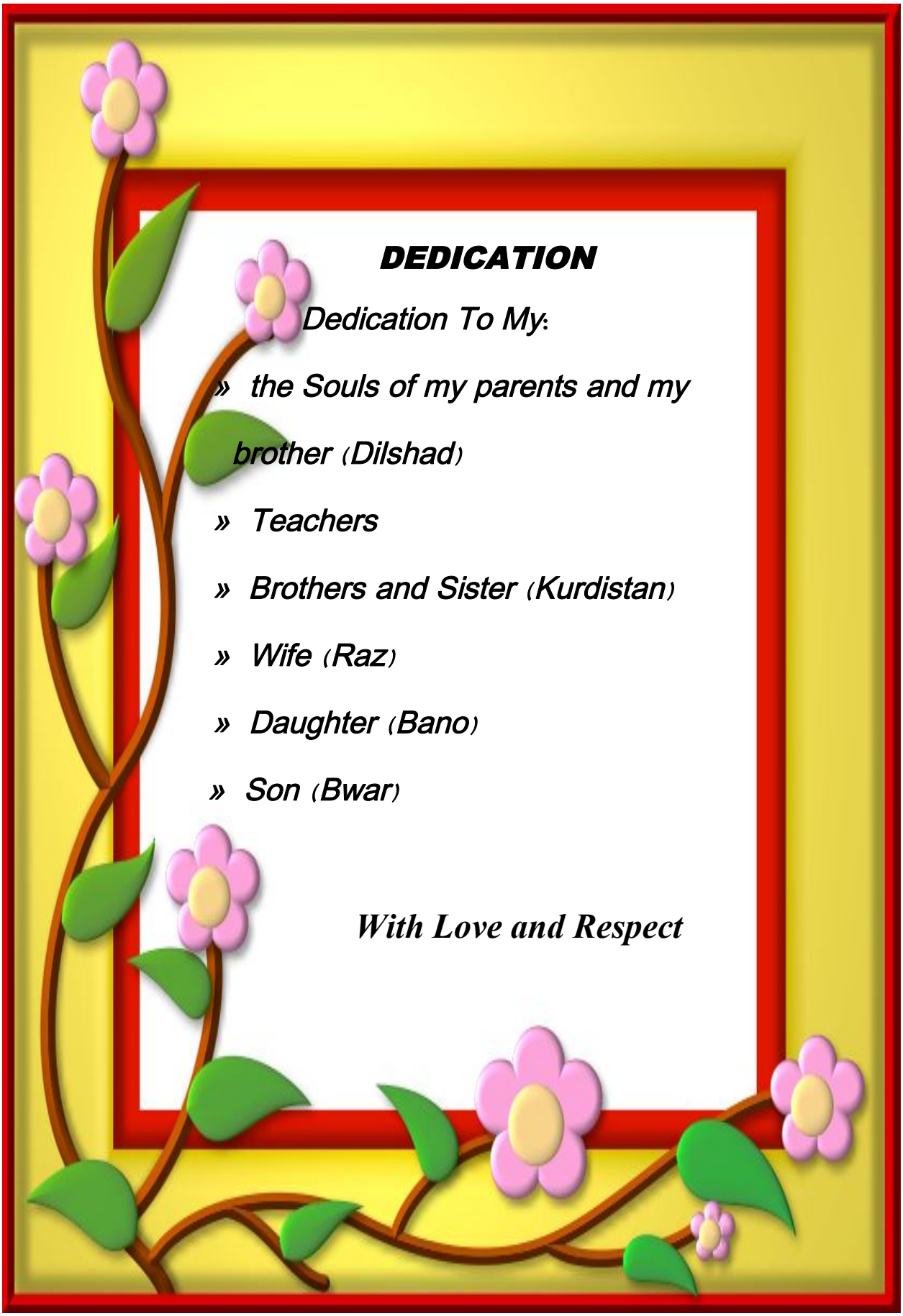

DEDICATION

Dedication To My:

- *the Souls of my parents and my brother (Dilshad)*
- *Teachers*
- *Brothers and Sister (Kurdistan)*
- *Wife (Raz)*
- *Daughter (Bano)*
- *Son (Bwar)*

With Love and Respect

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Your's Sincerely
Bahzad

SUMMARY

This study was carried out for the period from begining March 2009 to end of February 2010 in three zones I, II, and III (Mountainous, Semi-mountainous and foothills and plane) regions, respectively in Sulaimani governorate for distribution of ticks (Ixodidae) in infested sheep.

The prevalence rate of infested sheep in all zones was 298 **(**11.8%) in Sulaimani governorate, and the prevalence rate of infestation in zone-I was 85 (10.1%), in zone-II 94(11.1%), and in zone-III 119(14.3%). The rate of infestation was higher in March, April, May and July in all zones; no infestation was observed in zone-I and zone-II in November to February, but was observed in zone-III.

Hyalomma anatolicum anatolicum, H. marginatum, Rhipicephalus turanicus and *R. sanguineus* were found and identified, two species were more predominant among sheep *H. a. anatolicum* in zone-III (Garmian region) 353(70.0%) and *R. turanicus* in zone-I (Pishder region) 177(59.4%), and *H. a. anatolicum* was found through March 61(75.3%), April 89(69%), May 92(68.7%), and June 50(74.6%) in zone-III.

Hyalomma a. anatolicum and *R. turanicus* were found through March 22(53.6%), 12(29.3%): April 36(41.4%), 38(43.7%): May 46(46.9%), 33(33.7%), and June 41 (53.2%), 25(32.5%) in zone-II respectively, while *R. turanicus* was highly distributed in April 48(71.6%) and May 57(65.5%) in zone-I.

According to linear model of the percentage of infested sheep in any zone by number of ticks was recognized and it was high in zone-III [3.1 + 0.23 Number of ticks (X)]. The ratio of male to female tick infested sheep was 1: 2 during the study.

The site of attachment of ticks was observed; the highest number was noticed on the ears 492 (42.0%) and under tail 208 (17.7%).

The clinical signs were more severe in sheep infested by *Hyalomma spp*. than those infested by *Rhipicephalus spp.* this included; anemia, anorexia, emaciation and skin problems including skin irritation, loss of skin elasticity, alopecia and patches of hyperemia.

Hematological parameters were statistically different in values at $p \leq 0.01$ of total red blood cells (RBCs $10^6/\mu l$), packed cell volume (PCV %), and hemoglobin concentration (HgC g/dl) in *Hyalomma spp.* when compared to infestation with *Rhipicephalus spp*., mean corpuscular hemoglobin (MCH pg) in *Hyalomma spp*. infested sheep was significantly different at $p \leq 0.05$ when compared to *Rhipicephalus spp*. The total white blood cells (WBCs $10^3/\mu l$), mean corpuscular volume (MCV fl), and mean corpuscular hemoglobin concentration (MCHC g/dl) did not varies significantly in values infested *Hyalomma spp*. with *Rhipicephalus spp.*

A further study was done on rearing of engorged female of *Hyalomma a. anatolicum* under laboratory condition to determine times of oviposition and larval development; it was (27 ± 1.32) days and (22.4 ± 2.26) days respectively. And obtained hatched larvae were used for preparation of whole crude larvae extract antigen for immunization of rabbits by three subcutaneous injections with WCL.

Cell-mediated immune reactions was evidenced by statistically ($p \leq 0.01$) increased in the phagocytic index to *Staphylococcus aureus* in the immunized rabbits (7.2 ± 0.61) in compared to control groups.

Severe cutaneous delayed-type hypersensitivity reaction was investigated in immunized rabbits with inoculation of whole crude larvae I/d and it was

calibrated after 2, 4, 6, 12, 24, 36, 48, 72, 96 hours and 7days, and significantly ($p \leq 0.01$) increased in the skin thickness and it was found being 3.80± 0.34, 4.80± 0.47, 7.90± 0.52, 10.20 ± 0.38, 11.10 ± 0.26, 8.70 ± 0.64, 6.70 ± 0.49, 4.90 ± 0.24, 3.20± 0.30 and 2.60 ± 0.19 mm, respectively.

Quantitative humoral-mediated immune (immunoglobulin concentration) response to WCL was evaluated by biochemical reactions (glutaraldehyde coagulation test) and it was more than 600 mg/dl and (Sodium sulfite concentration test) it was more than 1500mg/dl) in immunized rabbits serum.

The Qualitative immunoglobulins concentration was evaluated by Radial immuno-diffusion plates kits and it was highly significant ($p \leq 0.01$) response of immunoglobulin-G in pre-immunized (control) sera rabbits (1.068 ± 0.050)mg/dl unconcealed when compared to immunized rabbits (2.758 ± 0.220)mg/dl.

There was obvious refusing of immunized rabbit to employed attachment of the growing larvae attachment on the ears of rabbits in compared to the control rabbits.

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nCHAPTER ONE

INTRODUCTION

There are two well established families of tick, the Ixodidae (hard tick), and Argasidae (soft tick), both are important vectors of disease-causing agents to human and animals throughout the world **(Vredevoe, 2007).** Tick is obligatory blood sucking arachnid arthropods; infesting mammals, birds, reptiles and amphibian. They act as vectors of diseases, causing anemia, dermatitis, paralysis, otoacariasis as well as loss of production **(Schmidt and Roberts, 1989).** These parasites generate direct effect in cattle on terms of milk production and gain weight reduction (**Peter *et al.,* 2005).** In Ixodidae, the hard ticks, there are several genera and species. Those of veterinary importance are in the genera Boophilus*,* Rhipicephalus, Amblyomma, Haemaphysalis, Hyalomma, Dermacentor and Ixodes **(de la Fuente and Kocan, 2003).**

Some species of hard ticks are important vector of *Nairovirus* (Crimean Congo Hemorrhagic Fever), theileriosis, babesiosis and anplasmosis **(Minjauw and McLeod, 2003).** Usually associated with tick of genera *Hyalomma spp*. and widely prevalent within those handling field tick, also infestation with ticks has a variety of direct and indirect effects on their host through blood loss, skin inflammation (Pruritus), hair and wool loss, toxic and allergic reaction **(Richard and David, 1997)**.

Identification of ticks should include description of the species life cycle stage, gender of adult, level of engorgement and status of mouthparts **(Winn *et al*. 2006).**

Control of ticks depending on acaricides and broad ranging resistance to these chemicals represents an extremely significant threat to animal health and production, resulting in economic losses to producer and major reduction in food supplies. The acaricides involved in the control of ticks belongs to the group of organophosphates amidines and synthetic pyrethroids. These acaricides were used at different intensities, depending on the zone and grazing system **(Rubaire-Akiiki *et al.,* 2004)**.

Problems of these chemicals with acaricides are toxic and costly, resistance in single and multiple host ticks and resistance exist for all major chemical groups used for hard tick control, chemical residues in food and the environment **(Rajput *et al.,* 2006).**

Effective control measures against tick borne disease are best achieved through combination of tick control, prevention of disease through vaccination and treatment **(Cunningham, 1981** and **Minjauw and de-Castro, 1999).**

Aim of study:

The aim of this study are to show the distribution and identification of hard ticks among sheep stock in different localities in Sulaimani governorate and effects of tick on the general health status including blood pictures and a trial of cellular and a humoral immunity producing against tick's infestation using rabbits as, the experimental model for this study.

CHAPTER TWO

REVIEW OF LITERATURE

1. TICKS:

Entomology is that branch of zoology, which deals with arthropods. The word Entomology is derived from the words “Entomon” which means insect and “Logos” means science **(Jain and Jain, 2006).** Ticks are related to animals such as spiders and insects, without a spine (invertebrates) belonging to a group called the phylum Arthropoda. All members of this group have an external skeleton (exoskeleton), a hard outer covering to which the muscles are attached internally and protect organs such as the gut and reproductive apparatus. Ticks are within a group called the order Acari, which consists mostly of mites and ticks belong to this order. Ticks are very similar to mites but are larger and all of them only feed as parasites **(Walker *et al*., 2003)**. Ticks are believed to have originated 120 million years ago, and that they speciated by ~ 92 into the main tick families as we know them today **(Mans, 2002)**.

Ticks are members of the phylum Arthropoda of the animal Kingdom as insects but are in a different class (Arachnida), the name of Arthropoda derived from the Greek word "Arthros" means a joint and 'Podos' means a foot. All other phylum both in number of individuals and in the diversity of their ecology distribution, arthropoda are approximately 80 percent of all known animals **(Jain and Jain, 2006).**

Tick species has preferred environmental conditions and biotopes that determine the geographic distribution of the ticks and consequently, the risk areas for tick borne diseases **(Parola and Raoult, 2001).**

2. TAXONOMY:

Ixodidea includes three families of ticks, namely Agrasidae, Ixodidae and Nuttallidae. There are approximately 860 species. in 22 genera and three families (**Keirans and Robbins, 1999),** of the three families the Ixodidae **(**hard ticks) is the largest, consisting of more than 650 species (**Hoogstraal, 1956**). *Hyalomma* is one of the most important genera of ticks, having a wide range of hosts and geographical distribution **(Kakar and Kakarsulemankhel**, **2008)**

2.1 Hard ticks classification (Krantz and Walter, 2009):

Domain**: Eukaryotia**

Kingdom: **Animalia**

Phylum**: Arthropoda (jointed limbs)**

Subphylum: **Chelicerata (anterior fangs/chelicerar)**

Class: **Arachnida (scorpions, spider, mites and ticks).**

Subclass: **Acari or Acarina**

Superorder: **Parasitiformes (Ticks and Mites)**

Order: **Ixodida (= Metastigmata: Ticks)**

Superfamily: **Ixodidea**

Family: **Ixodidae (Hard ticks).**

Genus	Species	Genus	Species
Hylomma	*H. anatolicum*	*Boophilus*	*B. annulatus*
	H. truncatum		*B. microplus*
	H. marginatum		*B. decoloratus*
	H. excavatum		*B. kohlsi*
	H. detritum	*Ixodes*	*I. ricinus*
	H. shulzei		*I. scapularis*
	H. aegyptium		*I. persulcatus*
	H. impeltatum		*I. hexagonus*
	H. asiaticum		*I. canisuga*
			I. simple
Rhipicephalus	*R. bursa*		
	R. sanguineus	*Dermacenteor*	*D. nitens*
	R. turanicus		*D. marginatus*
	R. appendiculatus		*D. anderso*
	R. evertsi		
		Haemophysalis	*H. punctat*
Amblyomma	*A. variegatum*		*H. bispinosa*
	A. americanum		*H. leach*
	A. hebraeum		*H. adleri*
	A. lepidum		*H. otophila*
	A. gemma		*H. sulcata*
			H. erinacei

The renaissance in tick research in the area was largely due to Harry Hoogstraal and his co-workers, including Carleton Clifford, Jim Keirans, Glen Kohls, Harold Trapido, and Hilda Wassef who were responsible for the description of a large number of new species, particularly in the genus *Haemaphysalis* is the second largest tick genus in the family Ixodidae, consisting of about 170 species and subspecies **(Horak *et al.,* 2002)**.

The Ixodidae is the largest important family, characterized by the presence of a tough, sclerotized plate (scutum) on the dorsal body surface **(Francis, 1993).** The family of *Ixodidae* is divided into two groups: 1- Prostriata: Anal groove is anterior to the anus, e.g. Ixodes. 2- Metastraita: Anal groove is posterior to the anus**.** This further divided into brevirostrate (short capitulum) e.g. gernera *Rhipicephalus* and Longirostata (long capitulum) e.g. genera Hyalomma **(Jain and Jain, 2006).**

3. TICK STRUCTURE:

Ticks have certain characteristics that distinguish them from other arachnids such as spiders. It has a rounded body, without a clear boundary between the anterior and posterior parts. The body is divided into a capitulum (gnathosoma) and the rest of the body (idiosoma). It has six pairs of appendages including the chelicerae, pedipalps, and four pairs of locomotors appendages **(Morel, 1989)**.

Ticks are ventrodorsally compressed; usually have no definite division between the head, thorax at abdomen**.** All ticks are blood sucking parasites, there is no distinct head region. Mouthpart comprises two palps (sensory function), two chelicerae (cutting) and hypostome (anchorage and feeding tube). The hypostome is armed with backward projecting teeth. The chelicerae are armed with movable denticles and the lateral stigmata are without sinuous peritremes, wingless and without antenna **(Jain and Jain, 2006)**.

3.1 Genus: *Hyalomma*.

In *Hyalomma anatolicum*; the male body is small and reddish brown in color Scutum, dark reddish brown, inornate, elongated oval in shape, eyes rounded, small and deeply socketed, spiracular plates are comma shaped, legs: Long, strong and pale yellowish brown bands. Capitulum: longer than wide, posterior margin of basis

capituli is concave and angular. Papli longer than wide. Hypostome: round tip, long and 3/3 dentition, teeth moderate. Female body: small, elongated, triangular and reddish brown in color. Scutum: Reddish brown, in-ornate. Spiracular plates ovate with short thick dorsal projections. Legs: As in male. Capitulum: Longer than wide, palps strong (**Kakar and Kakarsulemankhel, 2008).**

3.2 Genus: *Rhipicephalus*.

Genus Rhipicephalus include from (Rhipi = fan; cephalon = head) and contain 70 known species. It is commonly called "dog tick." It also occurs on a wide variety of mammals, they are reddish or blackish brown, inornate ticks. Eyes and festoons are present. Palpi and hypostome are short. Basic capitulum is hexagonal dorsally. Spiracles are comma shaped in both sexes. Some important species: *R. Sanguineus* (brown tick or kennel tick), *R. turanicus* **(Jain and Jain, 2006**). *R. Sanguineus* commonly infested a variety of domestic and wild mammals besides dogs **(Furman and Loomis, 1984).**

In other species, *Heamaphysalis spp*. has palpi with flared second segment, like *Ixodes,* these ticks lack eyes, but they differ in having festoons and a posterior anal groove. *Dermacenter spp*., have a rectangular basis capituli and II festooned. The scutum is ornamented (brightly coloured) the coxae, especially of the males, progress in size from I to IV. *Ixodes spp* have an anal groove which curves around anterior to the anus; have no eyes, festoons, or scutal ornamention. Their palpi are thickest at the paplal segments II and III (**Sonenshine, 1991).**

4. LIFE CYCLE:

The life cycle of Ixodidae ticks commonly takes 2-4 years, depending on species and availability of hosts **(Service, 2004)**. Tick also is classified on the basis of life cycle as one, two, or three- host ticks. Tick species are generally one-or three-host ticks; most tick species feed on blood three times during their life cycle **(Campbell and Thomas, 2006).** The 119 days required to complete one generation under laboratory condition is probably faster than occurs naturally where environmental conditions and availability of hosts might not be appropriate to render the life cycle rapidly **(Ochi, 2004)**.

Mating sometimes occurs on the ground. More often, it occurs on the host at the time of the blood meal. A virgin female cannot complete engorgement **(Kebede, 2004).** Oviposition proceeds more rapidly in constant darkness than in continuous light **(Snow and Arthur, 1966)**, and depending on temperature and relative humidity and usually begins few days later and continues for several days. The female dies soon whilst the males remain attached to the host for longer periods and may mate with other females **(Minjauw** and **McLeod, 2003)**. The adult female lays several thousands of eggs in one batch, depending upon species of ticks **(Sonenshine, 1991)**. **Jain and Jain (2006)** showed the female of genus *Hyalomma* laid about 10000 to 15000 thousand eggs, while the engorged female of the genus *Rhipicephalus*, lay up to 7000 thousand eggs in the cracks and crevices of the kennels.

All eggs hatching together, so present at one time. Ticks typically feed only once during each developmental stage. Duration of time larva, nymph, and adult spend feeding varies among species and developmental stage but typically takes several days **(Sonenshine *et al.*, 2002)**.

Rees (2004) considered that the moulting processes were occurred by hormones named ecdysteroids (moulting hormones)**. Minjauw** and **McLeod (2003)** suggested that the life-cycle of each tick species involves a characteristic number of host individuals. Life-cycle of ticks is divided into three groups according to the host **(Figure 1).**

4.1 One-host ticks.

In one-host ticks, the tick remains continuously on the host from the moment of access as an unfed larva until its departure as an engorged female. This type of life-cycle is characteristic of *Boophilus spp*. **(Adam *et al*., 1971** and **Minjauw and Mc Leod, 2003).**

4.2 Two-host ticks.

In two-host ticks, the tick remains on the host until the nymph has engorged; it returns to second host to feed as an adult**.** *Hyalomma spp*. and some *Rhipicephalus spp* belong to this group **(Adam *et al*., 1971** and **Golezardy, 2006)**

4.3 Three-host ticks.

Most hard ticks exhibited three life cycles **(Sonenshine *et al*., 2002),** this means the tick will feed on three separate hosts **(Sonenshine, 1991).** *Hyalomma anatolicum* as a three host tick it may feed as larva or nymphs on gerbils, then on cattle or sheep as adults. It can feed on hares as a two-host tick for entire life cycle **(Walker *et al.,* 2003).** *Amblyomma spp*., and majority of *Rhipicephalus spp*., belong this group **(Golezardy, 2006)**. *Rhipicephalus sanguineus* commonly known as the brown dog tick is a three host tick **(Torres, 2008).**

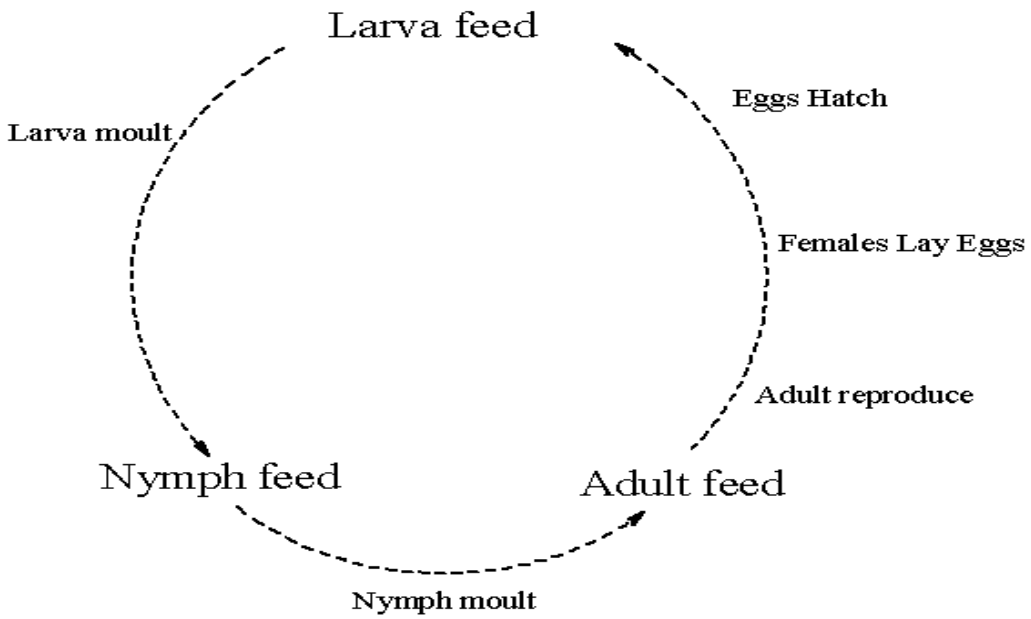


Figure (1): The life cycle of Ixodidae ticks

5. IMPORTANCE OF TICKS:

There are several hundred genera and species of hard ticks; that are of considerable economic importance, particularly in the transmission of the disease of livestock, *Ixodides, Rhipicephalus, Amblyomma, Hyalomma, Boophilus* and *Dermacenter* **(Francis, 1993).** Ticks are due to their ability to transmit protozoan,

rickettsial and viral diseases of livestock, which are of great economic importance world-wide. Tick-borne protozoan diseases (e.g. Theileriosis and Babesiosis) and rickettsial diseases (e.g. Anaplasmosis) and cowdriosis. The economically important Ixodid ticks of livestock in tropical regions belong to the genera of *Hyalomma*, *Boophilus, Rhipicephalus* and *Amblyomma* **(Frans, 2000).**

Jongejan and Uilenberg (2004) revealed that the ticks are reservoirs of certain infectious agents e.g. *Pasteurella multocida, Brucella abortus* and *Salmonella typhimurium* in human and animals. **de Castro *et al.* (1997)** revealed that when ticks attach to the host causes irritation of the skin, with subsequent ulceration and sometimes secondary bacterial infections. In addition, tick wounds may become infested by screw-worms or other agents of myiasis. **Brossard and Wikel (2004)** mentioned that ticks are responsible for a varity of losses, caused by direct effect of attachment (tick worry), and by the injection of toxins, or through the morbidity and mortality associated with the diseases that they transmit. **Chang *et al*. (2006**) described mechanical injury, caused by insertion of their capitulums into the skin, and tick paralysis caused by their poisonous secretion causes important harms of tick infestations in human and animals.

Ticks of the genus Hyalomma are well-known vectors of viruses and avid parasites of human. The considerable length of *Hyalomma* mouthparts provokes a painful bite. One of the most important diseases transmitted by *Hyalomma* ticks is Crimean-Congo Hemorrhagic Fever (CCHF), of which *Hyalomma marginatum* is one of the main vector ticks (**Estrada-Pena and Jongejan, 1999**).

Crimean-Congo Hemorrhagic Fever (CCHF) virus may be also transmitted by *Hyalomma spp*. and records that the *Hyalomma asiaticum* from foothill semidesert regions of Russia, China, Afghanistan, Pakistan, Iran and Iraq may parasitize human **(Kuo-Fan, 1991).** *Hyalomma marginatum* is well known as a vector of the dangerous viral zoonosis Crimean-Congo Hemorrhagic Fever (CCHF) in Turkey **(Ergonul, 2006** and **Abadi *et al*., 2010).**

6. PHYSIOLOGY OF TICKS.

Waladde (1987) observed that the stimuli emitted by the host can be sensed by tick through mechanoreceptors, thermo-receptors and olfactory receptors, which was found on the tarsi of the first pair of legs; but the role of vision is still not clear. Some ticks live in open environments and crawl onto vegetation to wait for their hosts to pass by. This is a type of ambush and the behaviour of waiting on vegetation is called questing. Thus in genera such as *Rhipicephalus, Haemaphysalis* and *Ixodes* the larvae, nymphs and adults will quest on vegetation. The ticks grab onto the hosts using their front legs and then crawl over the skin to find a suitable place to attach and feed. Adult ticks of the genera *Amblyomma* and *Hyalomma* are active hunters, they run across the ground after nearby hosts **(Walker *et al.,* 2003).**

Mencke (2006) showed that the "Haller's organ" located on the front legs detects a potential host by a variety of chemical and physical-chemical stimuli. Certain biochemical substances, such as carbon dioxide as well as heat, light shadow and movement serve as stimuli for questing behaviour subsequently.

Snow and Arthur (1966) observed that no correlation has been shown between female size and egg size and the number of eggs produced by a tick, the number of eggs laid is directly dependent on the size of the unfed female. Larvae and nymphs have high humidity requirements, while the adults can protect themselves better against evaporation because of their larger size and thicker tegument (**Kebede, 2004)**.

6.1 Tick salivary gland:

Buczek and Bartosik (2006) showed that tick saliva contains pharmacologically active molecules directed against host's immune system and effectors pathways. Ticks feeding induce hosts inflammatory and immunological reactions of host comprise both innate and acquired immunity.

Salivary glands include, cement to help anchor the mouthparts to the host, various enzymes and inhibitors, histamine agonists and antagonists, prostaglandins, antihemostatic factors, and immuno-modulating factors (**Sauer *et al.,* 1995)**. The important functions of salivary glands include the absorption of water vapor from unsaturated air by ticks, excretion of excess fluid for blood meal concentration (**Sauer**

***et al.,* 2000).** When the tick attaches to host, prostaglandin in the tick saliva are passed in to the skin, these prostaglandins may decrease the production of interleukin-1 (IL-1) and tumor necrosis factor- alpha (TNF-alpha) by macrophages and the secretion of interleukin-2 (IL-2) and interferon gamma by T-Lymphocytes, their actions have on inhibitory action on the host's local immune response. Apyrase, an enzyme in tick saliva, may maintain blood flow in to the bite by stimulating local vasodilatation and preventing platelet aggregation, there are also inhibitions of the coagulation cascade in tick saliva that enhance blood flow to the lesion, these factors combine to enhance the blood meal of the tick and facilitate transmission of infectious agents to the hosts **(Andy and Erika, 2001).**

Kocakova *et al.* (2003) revealed that the interleukin-8 plays a critical role in inflammatory processes, while the anti-IL-8 activity was studied in saliva to be important for successful feeding and for survival of the ticks of ixodid tick species.

The saliva inhibited interleukin-2 production by T cell hybridomas, an activity consistent with that of PGE2. Akininase was demonstrated, and this may counteract the algesia-and edema-promoting properties of PGE2, salivary gland components produce antihemostatic, anti-inflammatory, immunosuppressive and salivary apyrase may promote other effects important to a tick's successful feeding, as well as transmission of tick-borne pathogens **(Ribeiro *et al*., 1985)**, Salivary apyrase may prevent those inflammatory processes stimulated by ATP **(Ichikawa *et al*., 1972),** inducing mast cell deregulation (**Bloom *et al*., 1970)**. Apyrase converts adenosine triphosphate (ATP) to adenosine monophosphate (AMP), which is pharmacologically inactive or even inhibitory to purinergic P2 receptors **(Burnstock *et al*., 1983**). Histamine and serotonin are mediators of the itch response. Histamine increases vascular permeability and it is a mediator of inflammation, rich basophils concentrated in the area around the attachment site and observed very high level of resistance after a single infestation **(Wikel, 1982).**

Azzolini *et al*. (2003) showed that serine proteinase inhibitors from *Rhipicephalus sanguineus* are similar to inhibitors from *Boophilus microplus* and other hard tick species, suggesting a similar role of these inhibitors in hard against different ectoparasites with a unique antigen**.**

6.2 Ticks biting and feeding:

The duration of the tick's blood meal takes 2 to 14 days depending on weather in all stages **(Krober and Guerin, 2007)**. The feeding period of *R. sanguineus* depending on tick developmental stage, can vary from two days (e.g. larvae) to several weeks (e.g. females) and the feeding period of nymphs is longer than that of larvae and adult (e.g. engorgement of females may take longer on rabbits than on dogs) **(Troughton and Levin, 2007).**

Hess and Vlimant (1986) showed that the metastriate tick possesses 19 olfactory sensilla on each of the first leg tarsi that responses were employed as biological detectors in gas chromatographic analysis of host volatiles collected from breath, stables, animal rooms, and steer skin washes, apart from host volatiles, such as H_2S from the rumen and oral cavity **(Steullet and Guerin, 1992b)**.

Olfactory receptors in chemosensilla on appendages such as the antennae of insects and the first leg-pair of ticks permit these ectoparasites to find hosts from a distance. These include receptors for common respiratory products such as carbon dioxide and other breath components **(Guerin *et al.,* 2000).** Chemosensilla are strategically placed on the tips of legs and palps of arthropods and on the antennae of insects. Such sensilla are brought regularly into contact with the substrate as the organism walks **(Krober and Guerin, 1999),** and both palps and tarsi are used by male ticks to actively sample the female cuticle during mating **(de Bruyne and Guerin, 1998).** Ticks also attracts strongly to breath CO_2 **(Steullet and Guerin, 1992a)**. **Diehl *et al.* (1991)** found that the amount of aggregation-attachment pheromone composed of O-nitrophenol, methyl salicylate and nonanoic acid in a ratio of 2: 1: 8 µg emitted by a male of both species *Amblyomma variegatum* and *A. haebraeum* is considerable in the host. The combination of host odours and aggregation-attachment pheromone lead some of these species to suitable host-location mechanism, blood feeding and mating. O-nitrophenol, methyl salicylate and nonanoic acid are produced in the dermal glands type 2 associated with the ventrolateral cuticle, and found the methylsalicylate, an aggregation-attachment pheromone component in bovine and rabbit odour. When attached the hypostome of Ixodidea the site of skin host, produced a hyaline sheath of a concentric laminar structure surrounded the tissue

damage. The length of the hyaline sheath corresponds of the hypostome **(Uilenberg, 1990).**

The process to gain access to body fluids of the host through the skin can be divided in general into four phases. The first phase is the 'probing-phase' which identifies the most favorable site for skin penetration and feeding. This first probing phase is followed by the actual penetration of the skin (second phase). The tick will embed the hypostome into the skin and secrete a cement plug (short mouthpart), a glue-like substance called attachment cement, the cement will harden and helps to further anchor the tick firmly in place **(Anderson and Harrington, 2005).** When feeding, the tick is able to return about 70% of the fluid and ion content of the blood-meal into the host by salivation into the feeding site **(Bowman and Sauer, 2004)**.

The third phase is the 'feeding-phase', during blood-feeding the tick is well fixed to the host skin and introduces saliva containing anticoagulants to maintain a constant blood flow. In the final fourth phase, the withdrawal-phase**,** the tick pulls off from the host skin and leave the host **(Mencke, 2006).**

6.3 Diapauses:

Diapauses syndrome is defined as a neurohormonal mediated dynamic state of low metabolic activity. Associated with this is reduced morphogenesis, increased resistance to environmental extremes and altered or reduced behavioral activity **(Berkvens *et al*., 1994)**. Diapauses**;** state of inactivity and arrested development accompanied by greatly reduced metabolism as in many eggs. It is a mechanism for surviving adverse weather condition **(Stafford, 2004).**

Perry *et al*. (1991) showed that the diapause is an essential phenomenon in ticks' population changes. Lack of diapauses indicates that ticks are able to pass more than one generation each year especially when there is more than one rainy season, and high temperature increases the metabolic rate of poikilothermic organisms **(Chapman, 1976)**, which in turn increases the rate of physiological processes involved in egg production resulting in decrease in preoviposition and incubation period length.

Belozerov (1982) noticed that there are two types at diapauses: Behavioral diapauses are characterized by the suppression of host seeking activity by unfed ticks,

and morphogenetic diapauses' results from the blocking of some essential steps in development, such as embryogenesis, oogensis or metamorphosis of larvae and nymphae. Diapauses maintenance: In nature, one or more mechanism can maintain diapauses. Sensitivity to day length and seasonally changing environmental factors **(Berkvens *et al.*, 1994)**. Diapauses' termination: The termination of diapauses is dependent upon stimuli received from outside the tick **(Belozerov, 1982).**

7. ENVIRONMENTAL EFFECT OF TICKS.

Many species of ticks are adapted to seasonal variations in climate within their geographical range. In the tropics, there is usually to overcome the adverse effects of prolonged dry seasons. Dry environmental conditions are dangerous for ticks, particularly to the questing larvae which are very susceptible to drying out fatally **(Walker *et al.*, 2003).** Although ticks can tolerate considerable variations in temperature and humidity, most species are absent from both very dry and very wet areas, but certain *Hyalomma* species occur in arid areas **(Service, 2004).** Each species of tick is adapted to certain ranges of temperature and moisture, some occurring only in warm regions with a fair degree at humidity, while others are winter ticks most active in a dry climate **(Foster and smith, 2008).**

Yakhchali and Hosseine (2006) revealed that ticks were present on the small ruminant population throughout the year (*R. bursa, R. Sanguineus* and *B. annulatus*); their numbers seemed to increase particularly after the rainy seasons including spring and fall**.** The life cycle of *Hyalomma marginatum* is faster in southern parts of its distribution range (Northern Africa) with larvae active as early as February, but clearly slower in northern colder regions **(Hoogstraal *et al.*, 1961).** In laboratory, the optimal survival for *Hyalomma anatolicum* occurred at 85% relative humidity with 18 - 28 C° **(Al-Tamemi and Waiker, 1981)**. Humidity and temperature are important factors that influence tick activity and survival**.** Ticks need areas with a good cover of vegetation and a mat of decaying vegetation where a relative humidity of at least 80% remains throughout the driest times of the year. When it is too dry or too cold, ticks will withdraw to the litter area in order to prevent desiccation or freezing **(Gray, 2002).**

Alahmed and Kheir (2003) revealed that temperature is an important factor affecting the duration of preoviposition.

Yano *et al.* (1986) revealed that the temperature also affected the egg productivity and hatch-ratio. The number of deposited egg/per mg of body weight decreased markedly at 15C°, and the hatch ratio was lowered with dropped temperatures.

Short and Norval (1981) observed that peak adult activity occurs in the rainy season, whereas peak larval and nymphal activity occurs in the dry season. Adult activity is regulated by the combined influences of temperature, humidity, and day length**. Sonenshine (1991)** suggested that adverse environmental condition or a decline in the day length may cause ticks to enter diapauses where they may delay host seeking.

8. EPIDEMIOLOGY OF TICK-BORNE DISEASE:

Ticks transmit the widest varity of pathogens of any blood-sucking arthropods, including bacteria, rickettsia, protozoa, and viruses. Some human diseases of current interest in the United States caused by tick-borne disease include Lyme diseases, ehrichiosis, rocky mountain spotted fever, tularemia and tick-borne relapsing fever **(Vredevoe, 2007).** Four groups of tick-borne disease, are of important to the livestock industry; anaplasmosis, babesiosis, cowdriosis and theileriasis **(Minjauw and McLeod, 2003).**

8.1 Disease transmitted by ticks in domastic animals:

Ticks are both biological and mechanical vectors for viruses, bacteria, rickettsias; spirochaetas, protozoons and helminths, commonly found all around the world are obliged to suck blood from vertebrates such as mammals and birds during their periods of development **(Gazyagci and Aydenızoz, 2010)**.

8.1.1 Babesiosis (Red water fever, Texas fever and malignant jaundice):

Babesiosis is virulent, inoculable, non-contagious infectious disease that affects most domestic and wild mammals. Its causative agent is a sporozoa, the genus *Babesia* that obligatory transmitted after cyclic development in ticks. The pathology is

characterized by primary parasite hemolytic anemia giving rise to haemoglobinuria icterus, and severity of haemolysis **(Willadsen, 1999).**

Mustafa (2006) Showed that 5.6% of sheep were infected with *Babesia motasi* in Sulaimanyiah province, North Iraq. Both *Babesia motasi* and *B. ovis* have been reported from sheep and goats in Iran and Turkey **(Lewis *et al.,* 1981b)** and a large *Babesia motasi* isolated from naturally infected sheep from North Iraq **(Alani *et al.,* 1987)**. Sheep babesiosis is the biggest problem in the Zagros mountainous area, where *Rhipicephalus bursa* is the dominant tick species, but *R. turanicus* in the other part of Iran and *R. sanguioneus* can play as a vector of sheep babesiosis **(Shayan *et al*., 2007).**

Bitten by an infected tick, symptoms appear after two to three weeks, depended on the agent involved and the magnitude of the tick infestation **(de-Silva and Nadine, 2008).**

8.1.2 Thieleriosis (East cost fever):

Theileriosis is an infectious, virulent, inoculable, noncontagious disease that affects domestic and wild ruminants. It is caused by sporozoa of the *Theileria*, transmitted after cyclic development in ticks. *Theileria spp*. are difficult to differentiate morphologically. The pathology is mainly characterized by generalized febrile adenitis and edema except East Cost Fever by *Thieleria parva* demonstrates by high mortality **(Chartier *et al*., 1989** and **Uilenberg, 1990).** *Theileria lestoquardi* has been described for the central and southern part of Iraq **Hooshmand-Rad** and **Hawa (1973),** and **Latif *et al.* (1977)** recorded a low incidence of *T. lestoquardi* infection in two other province of north Iraq, namely in Sulaimaniyah and Mousal, with rates of infection for the north, middle, and south parts of the country at 7.33, 30.37, and 41.33%, respectively.

Mustafa (2006) demonstrated that (6.2%) of the sheep infected with *Theileria herci (lestoquardi)* in Sulaimani province, North Iraq. **El-Azazy *et al*. (2001)** observed in Saudi Arabia that all *Theileria* infected sheep were infested with *Hyalomma impeltatum* except the one that carried *Hyalomma anatolicum anatolicum.* *H. anatolicum anatolicum*, a three host tick vector transmitting the causative agent of bovine tropical theileriosis, is widely distributed throughout India **(Ghosh *et al*., 2008).** *Theileria annulata* is transmitted by *Hyalomma. a. anatolicum*, *H. marginatum*

isaaci, H. dromedarii and *H. scupense* and equine piroplasmosis **(Jain and Jain, 2006)**. The ovine malignant theileriosis caused by *Theileria lestoquardi* (*T. hirci*) considered as an important cause of disease and mortality in sheep and goats in the Mediterranean Basin, West Asia **(Rao *et at.*, 1991).**

8.1.3 Anaplasmosis (Gall sickness).

Anaplasmosis are infectious diseases, virulent, inoculable, and noncontiguous, that affect domestic and wild ungulates. Their causative agent is a rickettsia of the genus Anaplasma. The pathological signs are febrile, sub acute or chronic anemia, reduction of bile production, the different *Anaplasma spp* cannot be distinguished by their morphology. The criterion used is the marginal or central location of the parasite in the erythrocyte. *Anaplasma marginale* is pathogenic, while *A. centrale* is only slightly pathogenic and can be used for premunation **(Kebede, 2004).**

Anaplasmosis is also transmitted in a variety of mechanical ways for example through vaccination, tattooing or castration tools. Geography and climate are the two important factors determining what tick species are responsible for local cases of Anaplasmosis **(de-Silva and Nadine, 2008).**

9. TICKS DISTRIBUTION:

Several factors could undermine the passive-encounter mode of tick distributions among hosts, first; tick could actively orient towards some hosts, and weakly with others. Second; ticks could more readily reject and drop off some host species than others. Third, species-specific grooming behavior by host may result in larval ticks having a poorer success rate in feeding from some host species than others. Fourth, tick could orient towards particular microhabitat feasting introducing a bias in their probability of encountering particular hosts **(James and Oliver, 1990).**

Walker *et al.* (2003) revealed that the *Hyalomma marginatum marginatum* occurs in areas with the humid Mediterranean climate and cannot survive under desert conditions. *H. m. marginatum* is widely distributed in North Africa and is commonly reported from Morocco, Tunisia, Algeria, and more sparsely in Egypt. *Hyalomma anatolicum anatolicum* is adapted to areas of Mediterranean and desert climates.

Milutinovic and Radulovic (2002) observed that the greatest number of specimens of the species *Rhipicephalus sanguineus* was taken from hunting and stray dogs.

9.1 Iraq:

Leiper (1957) Showed that seven different species of ticks are found in Iraq, of domestic and wild animals include *Hyalomm anatolicum excevatum* and *Rhipicephalus sanguineus* were the commonest throughout the animal population, the other species *H. detritum*, *R. bursa*, *Haemaphysalis* spp., *Boophalus annulata* and *Dermacenter marginatus* are less common Greatest numbers were found in spring and throughout the summer months. **Eichler (1966)** revealed that the greatest numbers of ticks were found in spring and in summer on sheep were *H. detritum, R. bursa, Boophalus annulata, Amblyomma punctata, Dermacenter marginatus* occure in north of Iraq, and are common only in winter in animals that are housed at night. **Robson *et al*. (1968b)** reported that the *Rhipicephalus spp*. existed only in the northern parts of the country among sheep and goat in Iraq. These results support the finding of **Friedhoff (1997),** recorded that *Haemaphysalis, Rhipicephalus,* and *Hyalomma punctata* are the most common species of ticks in Iran and Iraq. **Mustafa (2006)** observed different species ticks, *Hyalomma anatolicum*, *Rhipicephalus bursa*, *R. sanguineus* and *Boophilus annulatus* in all area in Sulaimani governorate, North Iraq. While **Omer *et al*. (2007)** collected many species of ticks from sheep and goats include *Rhipicephalus bursa*, *R. turanicus* and *Hyalomma spp*. in Dohok province.

9.2 Neighboring countries.

9.2.1 Jordan:

Hoogstraal and Kaiser (1960) described *Boophilus kohlsi* from domestic animals in Jordan. **Main *et al.* (1990)** revealed that the 18 Ixodid species include: (*H. aegyptium, H. marginatum, H. rhipicephaloides, H. anatolicum, H. dromedarii, H. impeltatum, H. schulzei, H. detritum; Rhipicephalus sanguineus, R. turanicus, R. camicasi, R. bursa, Haemaphysalis erinacei, H. sulcata, H. parva, B. annulatus, B. kohlsi, Ixodes spp*. in East of Jordan.

9.2.2 Iran:

The distribution of tick species that are able to infest animals in Iran is briefly reviewed basic of published records, the tick studies were started by **Delpy (1936)** in Iran, Later, **Mazlum (1971)** described a list of adult ticks collected from domestic animals in different regions and emphasized the *R. bursa* is the dominant tick in the north areas of Iran and *Rhipicephalus sanguineus* in the south west of Iran..

Hashemi-Fesharki (1997) described that the major tick genera in Iran which infested sheep and goat are *Hyalomma*, *Rhipicephalus*, and *Haemophysalis***. Rahbari *et al. (*2006)** showed that the *Rhipicephalus spp*. is most important sheep tick, *R. bursa* occurs as a dominant sheep tick in the Zagros mountainous area and could not be found in the semidesert area and in the Arabian Gulf area, whereas *R. sanguineus* was the major species in the northern part (Caspian Sea area) and in the semidesert area. **Nabian and Rahbari (2008)** demonstrated that the fifteen Ixodidae tick species were identified over the period in Iran from cattle, sheep and domestic and wild goats and found that *Hyalomma anatolicum excavatum* is adapted to a variety of climatic conditions and were often less commonly found on livestock than *Hyalomma anatolicum anatolicum*.

9.2.3 Saudi Arabia:

Pegram *et al.* (1986) noticed that the *Rhipicephalus camicasi* parasitizes in both large and small domestic ruminants and is restricted to the arid and semi-arid lowlands of northeastern Africa and the Arabian Peninsula**.**

Al-Khalifa *et al.* (1986) showed that the *Hyalomma (Hyalommina) arabica* is the most abundant tick parasitizing goats and sheep in Makkah province, Saudi Arabia. Elsewhere in Saudi Arabia goats and sheep are parasitized chiefly by *Rhipicephalus turanicus, Hyalomma impeltatum* and *Hyalomma anatolicum anatolicum.*

Al-Khalifa *et al.* (1987) found that *R. turanicus* occurs in Al-Madina, Makkah and Asir province, while Jazan province is the main area of distribution of *H. a anatolicum* in the Kingdom, where cattle and sheep are the main hosts for adult ticks. *H. a excavatum* is mainly distributed on sheep and camels in eastern, central and northern Saudi Arabia.

9.2.4 Turkey:

Yukari and Umur (2002) identified that various tick species such as *Dermacenter marginatus, D. niveus, Haemaphysalis parva*, *Rhipicephalus turanicus, R. bursa, Hyalomma anatolicum excavatum* and *Ixodes ricinus* in infested sheep in the Border region, Turkey**.** *Hyalomma anatolicum anatolicum* was dominant species Ixodidae ticks collected from the cattle with 32%, *H. a. excavatum*, *Boophilus annulatus*, *Rhipicephalus bursa* represent 25% and *R. sanguineus* was the minor species with 8% in eastern Turkey **(Aktas *et al.,* 2006)**. Tick fauna is composed of about 32 species in two families and ten genera in mammals. The tick of veterinary significance in the family Ixodidae comprises seven genera with 28 different species. *Ixodes spp.* is mostly seen in northern Turkey. Tick of the genera, *Haemophysalis*, *Hyalomma, Boophilus, Dermacentor* and *Rhipicephalus* are wide spread throughout Anatolia, Turkey (**Aydin and Bakirci, 2007).**

9.3 Arab countries:-

9.3.1 Egypt:

Hoogstraal and Kaiser (1959) observed that the *Hyalomma anatolicum anatolicum* is usually more numerous than *Hyalomma anatolicum excavatum* and is more uniformly distributed, while *Hyalomma anatolicum excavatum* is absent in certain ecological zones where *Hyalomma anatolicum anatolicum* is present **(El-Baky 2001** and **Emre *et al*., 2001)**.

9.3.2 Tunisia

Bouattour *et al.* (1999) observed that *Hyalomma detritum detritum* was the most abundant and important vector of *Theileria annulata* species infesting cattle. *Hyalomma dromedarii* and *H. impeltatum* were collected on domestic ruminants in the arid and desertic zones. *H. m. marginatum* and *H. A. excavatum* were widespread and

9.3.3 Sudan

Osman (1997) recorded that 34 species of different genera were collected from sheep and goat, two belonging to the genus *Amblyomma*, seven to the genus *Hyalomma*, 22 to the genus *Rhipicephalus* and three genera of *Boophilus*.

Walker *et al.*, 2003 revealed that the *Rhipicephalus turanicus* species occurs in the eastern regions of the African continent from Sudan and observed that the adult *R. turanicus* have most frequently been collected in sub-Saharan Africa; often heavily infests sheep and sometimes found on horses. **Ahmed *et al*. (2005)** described five species of Ixodidae ticks in Riven Nile province, Sudan. They found that the *Hyalomma a anunatolicum* was the predominant species (73.6%) where as ticks belonging to the *Rhipicephalus sanguines* group (14.7%), *R. evertsi* (9.1%), *R. simus* (2%) and *Hyalomma dromidarii* (0.5%). **Mohammed and Hassan (2007)** identified four tick genera and eight species in sheep in Senner state, Sudan, they were *Amblyomma lepidum, H. a. anatolicum, H. truncatum, Rhipicephalus (Boophilus) decoloratus, R. camicasi, R. evertsi evertsi, R. guilhonix* and *R. muhsamai*. Seasonal pattern of activity was observed during rainy seasons**.**.

9.4 Europe:

9.4.1 Serbia:

Petrovic (1979) revealed that the *R. bursa* was found mostly on sheep, .which encountered at the end of April and May in southern region and in June in the western region of the country parts of Serbia. **Milutinovic and Radulovic (2002)** noticed that the hard ticks abundant in spring and less abundant in autumn. The considerable interchange between spring and autumn tick population can be attributed mainly to environmental conditions in Serbia.

9.4.2 Spain.

Merino *et al.* (2005) identified nine species of ticks, the most frequent being *Dermacentor marginatus* (55.7%), *Ixodes ricinus* (12.4%) and *Rhipicephalus bursa* (11.9%)

9.5 Other countries:-

9.5.1 India:-

The *Hyalomma* tick fauna of the Indian area is characterized by the presence of several distinct, endemic species as well as by the small size of individual ticks in local populations of species widely distributed in this area and elsewhere in Asia, Africa, and southern Europe **(Kaiser and Hoogstraal, 1964).**

Shahardar *et al.* (1998) reported various genera of ticks viz. *Boophilus, Haemophysalis, Hyalomma, Amblyomma, Rhipicephalus* from cattle and buffalo in India. In Pakistan very, limited data is available on *Hyalomma* ticks**. Ghosh *et al.* (2007)** identified seven genera of hard ticks and three genera of soft ticks in India, the most important genera involved were *Boophilus, Hyalomma, Haemophysalis*, *Rhipicephalus* and *Argas*.

9.5.2 Pakistan:

(Manan *et al.,* 2007) observed that the most commonly prevalent ticks were belonging to genus *Boophilus* (46.1%) followed by *Hyalomma* (31.25%), *Rhipicephalus* (17.93%) and *Amblyomma* (4.61%) among the domestic animals in Peshawar, Pakistan. **Ghosh *et al.* (2007)** reported that *H. anatolicum* was the most abundant followed by *R. sanguineus* in camels &sheep in Pakistan.

Sajid *et al.* (2008) showed that the *Hyalomma anatolicum*, was predominant tick species both in cattle, buffaloes and goats followed by *Rhipicephalus sanguineus* in Pakistan. The prevalence of *H. anatolicum* and *R. sanguineus* was 41 and 25. 5% in cattle and buffaloes, respectively. In caprines, the prevalence of *H. anatolicum* and *R. sanguineus* was 42.7 and 37.6%, respectively.

9.5.3 Ethiopia:

The major tick genera reordered during tick distribution survey 1989-1991 in south western Ethiopia were Amblyomma (40%), Rhipicephalus (37%), Boophilus (21%), and Hyalomma (1.5%). Amblyomma, Rhipicephalus and Boophilus ticks are mainly parasite of livestock **(Mekonnan *et al.*, 2001).**

9.5.4 Israel:

Mumcuoglu *et al.* (1993) observed that the adult *Rhipicephalus turanicus* were found on sheep throughout the year in both valleys with two peaks in January and September, adult of *R. turanicus* were found more often on goats. *R. sanguineus* and *R. turanicus* ticks are common in Israel and found on a large variety of domestic and wild animals. **Yeruham *et al.* (1996)** revealed that the most common tick species of sheep in Israel was *Rhipicephalus spp*. which was followed by the *Haemaphysalis* tick**. Yeruham *et al.* (1999)** showed that the *Hyalomma marginatum* ticks have a worldwide distribution and have been documented on mountain gazelles and Nubian ibexes in Israel.

10. IMMUNITY OF TICKS:

A successful host-parasite relationship is a balance between limiting the host defense and the ability of the parasite to modulate, evade or restrict the host response and could enhance the ability of the arthropoda to obtain a blood meal and facilitate pathogen transmission **(Wikel *et al*., 1994)**. The degree of host resistance to ixodide infestations can be measured by the following indicators, decrease of body weight after engorgement with consequent reduction of oviposition, increase of feeding period, and reduction of larval hatchability rate and molting rates **(Wikel *et al*., 1996). Wikel (1999)** demonstrated that immunological interactions at the tick- host interface involve innate and specific acquired host immune defenses and immunomodulatory countermeasures by the tick.

10.1 Innate (natural) or nonspecific immunity:

Brossard and Wikel (2004) revealed that both innate and specific acquired immune defenses are involved in the responses of vertebrate hosts to infestation, ticks have evolved countermeasure to circumvent host immune defenses.

Morrisan (1989) showed in primary infestation reactions (figure 2), mild inflammatory response leading to erythema and edema, there are few obvious clinical features. The specific immune response involves the activation of T-and B-lymphocytes.

A: Primary immune response:

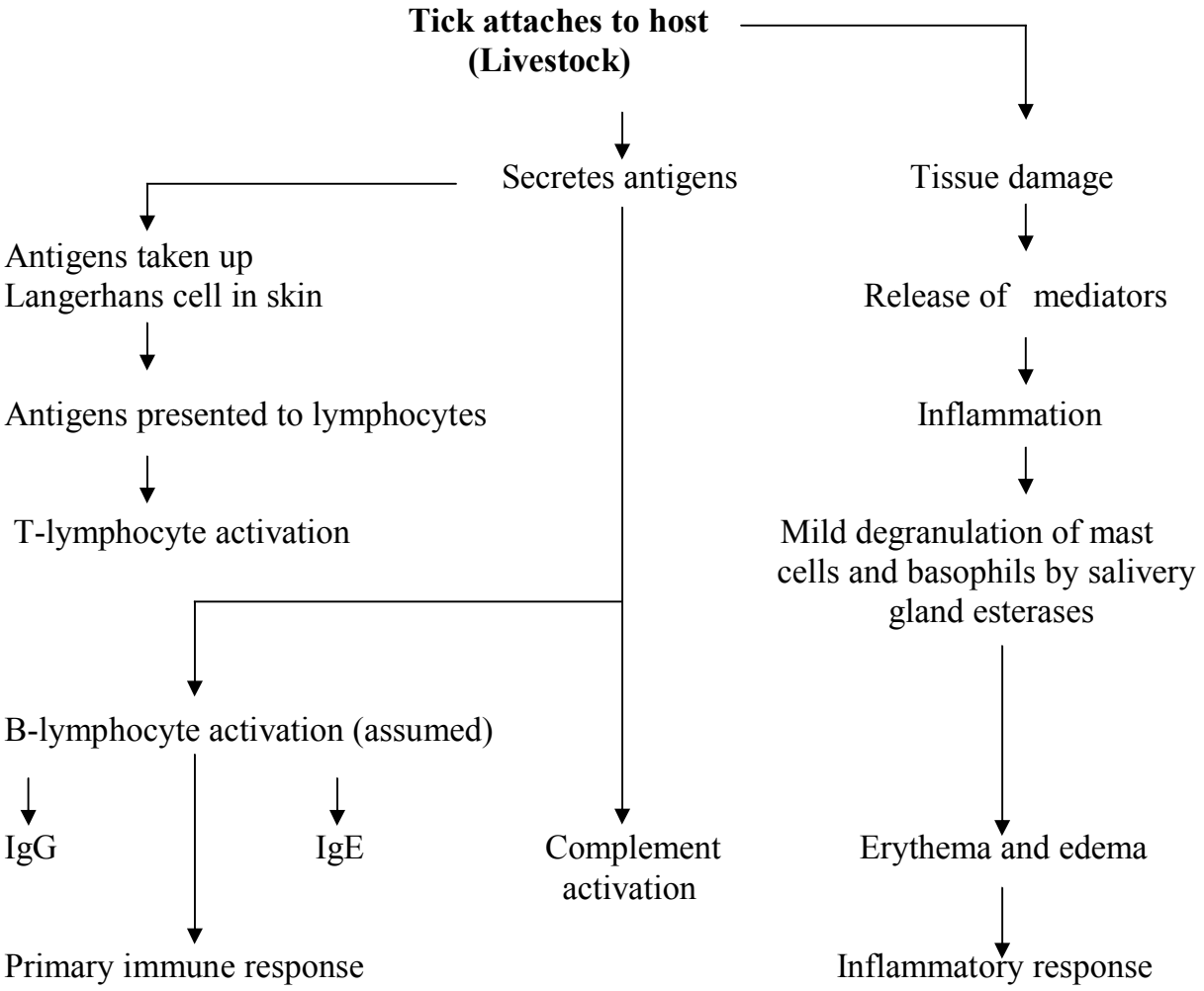


Figure (2): Host reaction to tick challenge by primary infestation (Morrisan, 1989).

Complement components C3, C4, C5 bind to cells and promote acute inflammation, neutrophil chemotaxis, mast cell activation. **(Brossard and Wikl, 2004**). Innate immunity mediates resistance of ticks infestation in some animal species. These non-adaptive immune factors are capability of some breeds of animals that can consciously move their skin, self grooming activity, swelling, skin color and thickness, area of skin available for tick infestation or length of fur **(Kashino, 2005)**.

Tick responds to the host immune attack by secreting saliva containing pharmacologically active molecules and attachment site facilitates both tick feeding and enhances the success of transmission of pathogens from tick into the host **(Fourie *et al.*, 1996b)**. **Utech *et al.* (1978)** noticed that several factors affecting resistance to ticks have been shown to influence the resistance of various breeds of beef cattle and

dairy cattle when infested with larval ticks that failed to survive to maturity following infestations; *Bos indicus* Brahman beef cattle were the most resistant, followed by *Bos indicus* × *Bos taurus* and *Bos taurus* British cattle. In the dairy breeds, *Bos taurus* Jersey cattle were more resistant than Guernsey cattle to *Boophilus microplus*, pregnancy and lactation reduced resistance, young cows were more resistant than older cows and males were less resistant than females.

10.2 Acquired or specific immunity:

Acquired or specific immunity is immunity induced by exposure to an antigen, naturally or using vaccination (**Kuby, 1994),** can be further divided into two subcategories: humoral and cell mediated immunity. The humoral branch of the immune system involves the interaction of B cells with extracellular antigen and their subsequent proliferation and differentiation into antibody-secreting cells that are specific for a certain antigen.

Antibodies secreted by B cells function as the effector of the immune humoral response by binding to an extracellular antigen and neutralizing and/or facilitating its elimination. Cell-mediated immunity involves the interaction of T cells and their associated cytokines to eliminate intracellular pathogens **(Galyean *et al.*, 1999)**.

During the secondary infestation **(figure 3),** mast cells and basophil degranulation and the release of mediators are much greater than during the primary infestation, and basophils rapidly invade the feeding lesion, degranulate and liberate vasoactive mediators leading to causes edema and might contribute to the formation of blister-like epidermal vesicle underneath the attached and leading to rejection of ticks **(Morrisan, 1989).**

B: Secondary infestation:

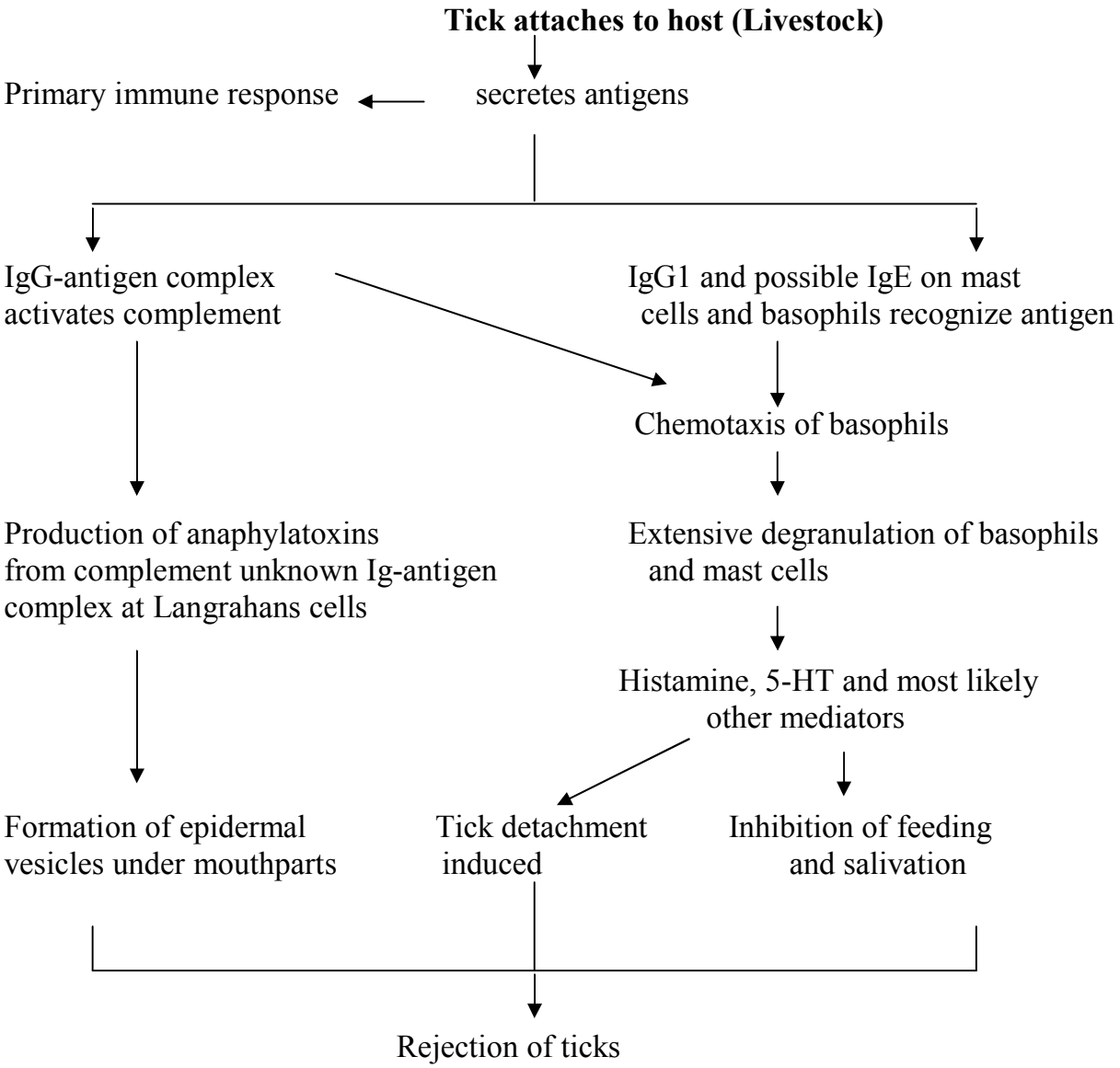


Figure (3): Host reaction to tick challenge by secondary infestation (Morrisan, 1989)

Host resistance to tick infestation has an immunological basis involving complement-dependent cellular and antibody-mediated effector mechanisms (**Wikel and Allen 1982**). In Anaphylatoxin, the role of complement in immune defense bind the components C3a, C4a, C5a to cells and promote acute inflammation, neutrophill chemotaxis, mast cell activation **(Brossard and Wikel, 2008**).

Wikel (1996) cleared that tick countermeasures to host defenses reduce T-lymphocyte proliferation, elaboration of the TH1 cytokines interleukin-1 and tumor, and antibody responses**. Wikel (1999)** revealed that tick feeding stimulates host

immune response pathway involving antigen-presenting cells, cytokines, B-cells, T-cells, circulating and homocytotropic antibodies, granulocytes, and an array of biologically active molecules and noticed when the tick response to host immune defense, tick-mediated host immunosuppressive countermeasure inhibit: host antibody response; complement activation; T-cell proliferation; and cytokines elaboration by macrophage and Th1-lymphocyte. **Allen and Humphreys (1979)** demonstrated the acquired host immunity against tick infestation following immunization with tick antigens as an alternative method of tick control. **Rechav *et al.* (1991)** suggests that humoral immunity may have an important role in the acquired resistance to ticks.

Brossard and Wikel (2008) reported that the tick-induced suppression of host immune defense is characterized by reduced ability of lymphocytes from infested animals to proliferation and diminished primary antibody responses to T-cell dependent antigen, and decreased elaboration of macrophage (IL-1 and TNF-α) and Th1 lymphocyte cytokines. **Wikel *et al.* (1994)** showed that the tick salivary gland derived materials that can modulate host cytokines; antibody and cell mediated immune responses, and observed the host immune response involved cytokines, antibodies, complement and T lymphocyte regulatory and effector pathways.

Xu *et al.* (2005) demonstrated in tick salivary glands that numerous genes are induced during the feeding process; many of these newly expressed proteins are secreted in tick saliva and may play a role in modulating host immune response and pathogen transmission, performed by suppression subtraction hybridization use in the control of ticks and to prevent transmission of tick-borne diseases.

Ghosh *et al.* (2005) noticed that when immunoprophylactic measures against multi-tick infestation, two glycoproteins of 34 and 29 KDa were isolated from the larvae of *Hyalomma anatolicum anatolicum* and *Boophilus microplus* and observed a direct correlation between anti-glycoproteins antibody response and protection against infestation. **Abdul-Amir and Gray (1987)** described that the sheep acquired resistance after the first infestation and the ticks showed suppressed feeding and oviposition success and the histology of tick-bite lesions revealed a cellular infiltrate consisting predominantly of neutrophils and this was followed by the infiltration and then degranulation of basophils.

11. TICK CONTROL:

The prevention of tick bites in animals reduces tick-transmitted diseases such as anaplasmosis, babesiosis, theileriosis and heart water disease **(Krober and Guerin, 2007)**. Control should be based on an understanding and management of ecologic factors responsible for tick infestations and selection of appropriate acaricides **(Dryden and Payne, 2004).** The current methods for controlling of ticks are primarily based on the use of acaricides. Their use however, has limited efficacy in the reduction of tick infestation and is often accompanied by serious drawbacks including selection of acaricide-resistant ticks and environmental contamination **(de la Fuente and Kocan, 2003)**. Irritation at the site of feeding stimulates grooming response by the hosts, which limits successful engorgement of ticks. This reaction has to do with the ability of hosts to control their tick burden by virtue of their natural resistance **(Hassan and Osman, 2003).** Control of ticks by vaccination would avoid environmental contamination and the selection of drug resistant ticks due to repeated acaricides application **(de la Fuente *et al*., 1998).** Some hosts become resistant after multiple infestations and often display immune response to substances found in tick saliva **(Wikel and Whelen, 1986).**

11.1 Types of tick control:

Control methods, such as predators and parasite pasture spelling i.e. leaving pasture unstocked to break the tick life-cycle, sterile male release, tick –resistant cattle, and vaccination with tick antigens **(Minjauw and de Castro, 1999).** Controlling ticks is difficult and generally requires a combination of cultural, preventive, and pesticide central methods specific for the tick question, body or ear, the body ticks require a complete skin drench best achieved by dips **(Campbell and Thomas, 2006).**

11.1.1 Housing in tick proof buildings:

There should be no cracks and crevices in the buildings as the ticks hide and breed there. Caulking of the walls of the animal's sheds is an inexpensive measure that significantly reduces the tick burden **(Muhammad *et al.,* 2008)**. Pasture spelling and

rotational grazing have been shown to be capable of greatly reducing the population of one-host Ixodid tick *Boophilus microplus* on dairy farms in Australia. Pasture spelling is rarely used for tick control and not very effective for the control of multi-host ixodid ticks (e.g., *Hyalomma anatolicum anatolicum*) because of the long survival periods of the unfed nymphs and adults. Duration of the spelling period varies from 2 to 3 months in summer and 3 to 4 months in winter, but these intervals need to be determined for each area **(David, 2005). Sutherst *et al.* (1979)** indicated that a single annual spelling period in summer of between 8 and 12 weeks would substantially reduce tick populations.

11.1.2 Manual removal of ticks:

Farmers remove the ticks manually generally at the time of milking. For manual removal of ticks, using the forefingers, first grasp the tick close to the animal's body and then twist it counter-clock wise, should be given to the possible hazard to humans from pathogens present in these ticks. Even after the tick is separated from the skin, careful attention is needed, because the capitulum remained in the skin could cause the tick-bite pyrexia **(Chae *et al.,* 2000). Masika *et al.* (1997)** noticed that ten percent of livestock owners in Eastern Cape Province of South Africa either cut ticks off with blades or scissors. The burning of grasses, weeds, stubs of crops, periodical ploughing of grazing grounds, cultivation of land help in reducing member of ticks **(Jain and Jain, 2006).** Pasture burning and use of certain grasses and legumes are also practiced for inhibition or killing of ticks (**Chiera *et al.,* 1984**). **Powell (1977)** reported that planned dipping and pasture spelling had improved the efficacy of tick control.

11.1.3 Tick pheromones:

Tick pheromones that regulate assembly, attraction, aggregation, attachment and mating behavior have been described. Most of the compounds regulating these behaviors are purines, substituted phenols, or cholesteryl esters. Other phenomenal compounds include organic acids, hematin, or ecdysteroids, combined these pheromones with an acaricide and applied to tick-infested vegetation or directly to the body surfaces of livestock or companion animals, these derives are effective for tick

control **(Sonenshine, 2006)**. Using pheromones-acaricide-impregnated plastic tail-tag decoys demonstrated excellent efficacy for control of the bont tick (*Amblyomma hebraeum*) on cattle in Zimbabwe **(Norval *et al.*, 1996**).

Stachurski (2000) demonstrated that attraction- aggregation- attachment pheromones had influenced on initial tick activation and host finding. Using efficacy of a 2, 6-dichlorophenol (2, 6-DCP) of extra lures on the horse's tail may help to control populations on the hindquarters **(Borges *et al.*, 2007).**

11.1.4 Chemical control:

The conventional control methods include the use of chemical acaricides with partially successful results but this treatment has certain implicit drawbacks, such as the presence of residues in the milk and meat and the development of chemical resistant tick strains **(Nolan, 1990).** The use of acaricides has disadvantages, such as the selection of resistant tick populations and harmful effects on the animals, human beings and the environment **(Garcia-Garcia *et al*., 2000).**

Crampton and Gichanga (1979) showed that the three major species to be resistant to or tolerant of toxaphene; *Boophilus decoloratus* 99.3%, *Rhipicephalus eversrtsi evertsi* 77.3% and *R. appendiculatus* 62.5% and only one sample of *Hyalomma* (*H. marginatum rufipes*) was tolerant of toxophene. Organophosphours resistance was noted in one species only, *R. sanguineus*, collected from suburbs of Nairobi. Arsenic was the first effective method for controlling ticks and tick-borne disease, and was used in many parts of the world before resistances to the chemical become a problem **(George, 2000).** Farmers also used alternative methods such as used engine oil (12%), Jeyes fluid (household disinfectant 24%) **(Mbati *et al.*, 2002)**.

Abdel-Shafy and Zayed (2002) examined the acaricidal effect of plant extract of Neem seed oil (*Azadirachta indica*) on egg, immature and adult stages of *Hyalomma anatolicum excavatum* and concluded that Neem can be used for tick control at economic concentrations of 1.6% to 3.2%.

Acaricides are needed to control tick infestations and tick born diseases. However, the uses of acaricides are constrained by their high costs, tick resistance, concerns about residues in food and in the environment **(Mekonnen, 1996).** The

acaricides are more toxic and harmful to arthropods than to warm-blooded vertebrates **(Okello *et al*., 2003).**

11.1.5 Ecological control:

Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks. Vegetation is periodically removed by burning, but spontaneous or induced fires have little direct effect on ticks since they occur in the season when adults are not active. The influence of burns on tick abundance varies markedly with the time of year, intensity of burn, and the tick species present **(Baars, 1999).** Various stages of some ticks (e.g., *Boophilus* spp.) attach themselves to the blades of grass and other vegetation and stealthily attach to the cattle passing nearby. Though clearance of vegetation will annihilate their places of shelter, this type of action, however, may encourage soil erosion and may be detrimental to the ecosystem **(Muhammad *et al.,* 2008).**

11.1.6 Biological control:

Jain and Jain (2006) studied that biological control is a nonchemical parasitic control, use such biological agencies, animals, plants, organisms and worms…. etc, which destroy or reduce the population of ticks which are vectors of protozoal disease such as *Babesia bovis* can reduce the egg production (60%), egg hatching, duration of oviposition, larval survival time and death (98%) of infected female tick. Fungi such as, *Aspergillus*., *Nematodes* such as *Steinernematidae*., bacteria such as *Enterobacterium*., Predators, including, tick canabolism, including birds, rodents, shrews, ants and spiders play some role in tick control **(Muhammad *et al.* 2008).**

The activities of the hyperparasites *chalcid* flies *Hunterellus* are probably important in nature, but they are difficult to evaluate. It is still more difficult to manipulate or reproduce them for practical use. Predators are most effective, especially ants and birds (*Buphagus spp.* or *oxpeckers*, *Crotophagus*, various magpies, village fowl) depending on the conditions **(Samish and Alekseev, 2001).** Using of entomopathogenic fungi to control ticks may reduce the

frequency of chemical acaricide use and the need for treatment for tick-borne diseases **(Kaaya and Hassan, 2000)**.

11.1.7 Sterilization of males:

Jain and Jain (2006) noticed that using sterile males of *Hyalomma anatolicum excavatum* and *Rhipicephalus appendiculatus* can be obtained by irradiating at 2k rad or more used for eradication of a low of level of population**.** **Osburn and Knipling (1983)** showed that *Boophilus annulatus* x *Boophilus microplus* mating produce fertile females and sterile males, backcrossing of the fertile female progeny also produces sterile males and fertile females, through three to six generations.

Davey and Hilburn (1991) showed that hybrid males are less reproductively competitive than pure *Boophilus microplus* males, mating less than half as frequently as them

11.1.8 Vaccination:-

The immunological control of ticks is gaining importance and encouraging results have been achieved in the past by immunizing various animals (cattle, guinea-pigs, rabbits, mice and dogs) with the respectable tick antigens against *Boophilus microplus*, *Rhipecephalus appendiculatus*, *Amblyomma americanum, Dermacentor variabilis*, *H. dromedarii*, *Ixodes ricinus* and *Rhipecephalus sanguineus* infestation **(Ogden *et al.,* 2002).** Vaccines could produce much more sustainable control strategies and revolutionize the economics of controlling ticks and Tick-borne disease **(Minjauw and McLeod, 2003)**. Host immunization with antigens from tick saliva can induce anti-tick resistance and is seen to be able to induce immunity against pathogens transmitted by ticks. Such the *Bos indicus* breeds are more resistant to ticks than others due to an innate resistance in cattle **(Fourie *et al.,* 1996a).**

Garcia-Garcia *et al*. (2000) observed that the Bm95 antigen from strain A of *Boophilus microplus* was able to protect against infestation with Bm86-sensitve and Bm86-resistant tick strains, suggesting that Bm95 could be a more universal antigen to protect cattle against infestations by *B. microplus* strains from different geographical areas. **Almazán *et al.* (2003)** revealed that the identification of protective antigens for

the control of *Ixodes scapularis* infestation using cDNA expression library immunization. **de Vos *et al.* (2001)** concluded that a good responses to vaccination with Bm86 were seen in *Hyalomma dromedarii,* while good results were obtained in *Hyalomma anatolicum anatolicum*, and *Boophilus decoloratus*.

Andreotti *et al.* (2002a) studied that *Boophilus microplus* trypsin inhibitors (BmTIs) present in larvae were preliminarily characterized as active proteins, approximately 10-18 kDa, showed 72.8% efficacy to interfere in tick development with 69.7% and 71.3% reduction of both tick number and egg weight and suggested that *B. microplus* serine proteinase inhibitors may play a role in the tick larvae fixation and feeding processes. **Ghosh *et al.* (1998)** showed significant reduction in engorged percentage and weight of engorged female and egg masses were observed in female fed on immunized rabbits, compared to the female ticks fed on control rabbits.

Kimaro and Opdebeeck (1994) revealed that the membranes of eggs (EM) antigens did not protect cattle against challenge with ticks, despite high levels of anti-egg antibodies in the sera of the vaccinated cattle, while vaccinated with membranes guts (GM), had high levels of antibodies against GM and were protected significantly against challenge with *Boophilus microplus*. **You and Fujisaki (2009)** revealed that the rCHT1 (recombinant Chitinase protein from hard tick)) stimulated a specific protective anti-tick immune response in the mice as evidenced by the significant longer feeding periods in the larval ticks and significant difference in the egg weights, lower molting rate (76.7%) compared to 96.7% for the control, The result suggesting that the rCHT1 might be a useful vaccine for biological control of the tick. **Sahibi *et al.* (1997)** demonstrated that immunized groups of three tick-naive calves with *Hyalomma marginatum marginatum*, a salivary extract (SE) and intestinal extract (IE) of the ticks, they noticed that attachment percentage was inhibited by the infestation, salivary extract, and not affected by the intestinal extract generated immunity. The percentage of engorgement was reduced by the infestation, SE, and the IE. The length of feeding was prolonged by the infestation, shortened by the SE, and not affected by the IE. **de la Fuente and Kocan (2006)** revealed that the development of vaccines against multiple tick species may be possible using highly conserved tick-protective antigens or by antigens showing immune cross-reaction to different tick species.

CHAPTER THREE
MATERIAL AND METHODS

1. SURVEY:

A survey study was carried out to show the distribution of ticks in Sulaimani governorate. Sulaimani governorate is located at north east of Iraq and southeast of the Iraqi Kurdistan region. The international border with Iran represents the eastern boundary of the governorate. It is bounded in north and north-west by Hawler governorate, west by Kirkuk governorate and Salahaddin governorate, south and southwest by Diala. Sulaimani governorate was divided in to three different zones, depended on topography and climate factors for study on the distribution of ixodidae. The three zones were; Zone I, Mountainous areas; Zone II, Semi-mountainous areas and Zone III, foothills and plane areas **(Figure 4).**

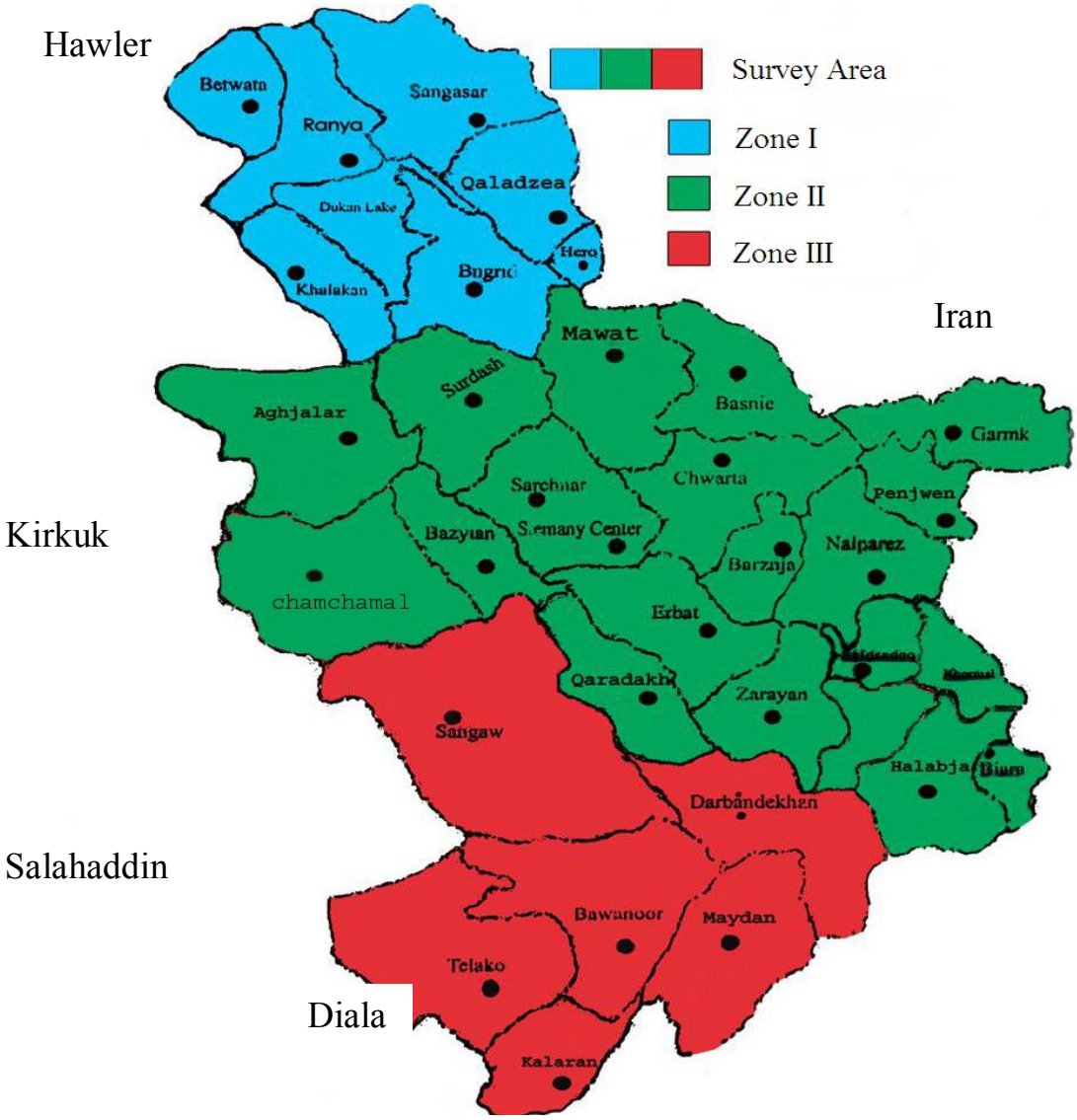


Figure (4): Geographical distribution (zones) surveyed areas in Sulaimani governorate.

1.1 Tick sampling:

Ticks were collected and isolated from 2525 local breed sheep from various flock, in three different zones (Zone-I: 840, Zone-II: 850, and Zone-III: 835) through periods of beginning of March 2009 to end of February 2010 **(Table 1).**

Table (1): Number of the sheep examined in different zones during March, 2009 to February, 2010.

Zone	Location	NO. of sheep examined
Zone-I	**Peshder region (Qaladaza- Sangasar-Rania-Betwata-Bingird)**	**840**
Zone-II	**Sulaimani region (Qaradakh-Mawat-Penjwen-Halabja-Chamchamal, Bazyan)**	**850**
Zone-III	**Garmian region (Kalar-Kifri- Maydan-Darbandekhan, Sangaw)**	**835**
Total		**2525**

1.2 Tick collection and identification:

Recently dropped off the ticks and fully engorged female ixodid ticks were collected by tissue forceps and cotton, soaked in ethanol. They were placed individually into clean universal glass vials of dimensions (2.5×8.0) cm and covered with plain muslin cloth to provide adequate ventilation. The covers were bound with rubber bands to avoid escaping of ticks. The tick samples were brought to the laboratory, cleaned and kept in 70% ethanol in room temperature.

Ticks were counted and identified, based on morphological features according to **(Hoogstraal, 1956)** which represented in **(Table 2)** using a dissecting microscope (Dissecting microscope, Motic-Education, China.), magnifying-hand lens and the binocular microscope (Altay, Biovision-103B, China), and identification of collected ticks samples referenced by Iraqi museum of entomology.

Table (2): Keys for classification of the genera of hard ticks (Ixodidae) (Hoogstraal, 1956).

Structures	The genera of ticks						
	Ixodes	*Hyalomma*	*Amblyomma*	*Dermacentor*	*Haemaphysalis*	*Rhipicephalus*	*Boophilus*
Gnathosoma	Long			short			
Basis capituli	rectangular dorsally					hexagonal dorsally	
Eyes	absent	Present			absent	present	
Anal grooves	Anterior	Posterior					
Coxae I	not forked	bifid	with two spurs	bifid	not forked	with two spurs	bifid
Festoons	absent	present/ absent	Present				absent
Scutum color	inornate or ornate		ornate		Inornate		
Males ventral Shields	cover all the surface	three pairs of shield	absent			two pairs of shields	

A total number of 1171 ticks were collected form three different zones (Zone-I: 298, Zone-II: 369, and Zone-III: 504) from 2009-2010 in Sulaimani governorate as shown in **(Table 3).**

Table (3): Number of the tick collected from sheep in different zones.

Zone	Location	NO. of tick collected
Zone-I	**Peshder region (Qaladaza- Sangasar- Rania-Betwata-Bingird)**	**298**
Zone-II	**Sulaimani region (Qaradakh-Mawat-Penjwen-Halabja-Chamchamal, Bazyan)**	**369**
Zone-III	**Garmian region (Kalar-Kifri- Maydan-Darbandekhan, Sangaw)**	**504**
Total		**1171**

1.3 Rearing and breeding of *Ixodidea* under laboratory condition:

In vitro engorged female of *Hyalomma anatolicum anatolicum* (**figure 5)** were collected in sterile glass with bijou bottle and covered by muslin cloth and incubated at 27±1.6°C, kept in desiccator jars with relative humidity (RH%) 85±1.4% (using saturated solution of potassium chloride for humidity control) both temperature and humidity measured by (Humidity-Temperature meter, France). Observation was made twice daily at the morning and evening hours until eggs were laid, in oviposition times of (27 ± 1.32) days after incubation **(figure 6)**, with hatching times (22.4± 2.26) days. The eggs were hatched to larvae, with duration of hatching 6.16±1.02 days. Larvae were left in the incubator (WTC-binder, Germany) as same condition of temperature and humidity required for hatching within two weeks, the larvae (pre-feeding period) became sclerotized and Hardening **(figure 7)**. The larvae were placed into a deep freezer (arçelk, 2031D, Turkey) at –20°C for preparation of antigens for immunization, and some of these larvae were also applied for challenge infestation procedures. All methods were carried out in Department of Animal Production, College of Agriculture –Bakrajo farm.

Figure (5): Engorged female of *Hyalomma anatolicum anatolicum* in sterile glass (Bijou tube).

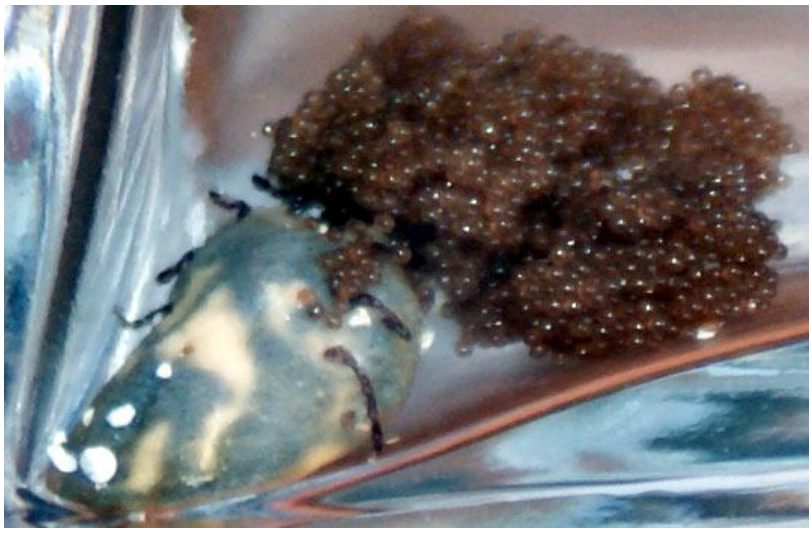


Figure (6): Oviposition (eggs laid).

Figure (7): Larva of *Hyalomma anatolicum anatolicum* (three pairs legs).

1.4 Sampling of sheep:

Fifty of highly infested sheep were selected from the total infested sheep, which were examined through the study to identify and influence of ticks on sheep. The methods of identification and keeping were discussed above. Three milliliters of blood were drawn from jugular vein; the area was prepared aseptically after clipping and soaking with alcohol and deposited into disposable clean plastic tube with anticoagulant (DMD-Dispo, S.A.R.) used for estimation of hematological parameters. The animals investigated for presence of piroplasmosis (*Babesia spp*. and *Theileria spp*.) by appropriate Giemsa stained blood smear and positive one was excluded in this study.

1.4.1 Hematological examination in sheep:

All the hematological parameters were under taken as described by **(Coles, 1986).** These parameters include**;** total erythrocyte count (RBC $\times 10^6/\mu l$), total leukocyte count (WBC $\times 10^3/\mu l$), packed cell volume (PCV %), hemoglobin concentration (HbC gm/dl), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH pg) and mean corpuscular hemoglobin concentration (MCHC g/dl).

1.4.1.1. Total erythrocyte cell (RBC) Count:

The total erythrocyte count was estimated using hemocytometer (Hemocytometer, Germany) method. Blood was carefully drawn to the 0.5 mark of the red blood cell diluting pipette and isotonic solution Hayem's solution was drawn to the 101 marks to dilute the blood (1:200). After well mixing, the diluted blood put on the hemocytometer counting chamber and allowed to settle for several minutes. The total number was determined and multiplied by 10000. This value represents the total number of erythrocytes / micro liter.

1.4.1.2 Total white blood cells (WBCs) Count:

The hemocytometer (Hemocytometer, Germany) was used for estimation of total white blood cells count, blood was drawn to diluting pipette slightly to the 0.5 mark. The proper diluting fluid (Turke's solution) was drawn to the 11 marks, and the blood and diluting fluid were mixed by shaking the pipette vigorously for 2-3 minutes. The

dilution factor is 1-10. Cover slip fixed on Neubauer chamber and small drop of mixture attached to the edge of cover slip to draw small portion of mixture between chamber and slip and let to stand for few minutes then examined under x40 power of microscope, four squares counted numerate and divided by four and multiplied by 200 to obtain number of white blood cells per micro liter.

1.4.1.3 Estimation of Packed Cell Volume (PCV %):

The hemocrit method (Microhematocrit method) was applied for determination of packed cell volume (PCV%), using a capillary hematocrit tube approximately 7 mm in lengths and having a bore about 1 mm. The tubes were filled by capillary action, the outside was carefully dried with a piece of gauze, and the opposite end of the tube was sealed with special clay. Sealed tubes were placed in a special speed centrifuge (Micro-Hematocrit centrifuge, Taiwan), the lid closed and spinned at 12000 rpm for 5 minutes. After centrifugation, the tubes were placed carefully on a special reader for determining the percentage of packed red cells.

1.4.1.4 Estimation of hemoglobin concentration (Hb g/dl):

The acid hematin method (Sahli method) was used. This method depends upon the conversion of hemoglobin into acid hematin, 0.1 N hydrochloric acid was added to whole blood and the mixture is allowed to stand until acid hematin has developed. The color of the blood and the acid mixture was compared with standard, and the reading was made either in percentage or grams per deciliter of blood (**g/dl**).

1.4.1.5 Mean corpuscular volume (MCV):

The mean corpuscular volume was determined by dividing the volume of packed red cell per 1000 ml of blood by the total red cell count in millions per micro liter. The result of this calculation is expressed in femtoliter (**fL**).

$$\text{Equation (fL)} = \frac{\text{Pcv \%}}{\text{RBCs } 10^6/\mu\text{l}} \times 10$$

1.4.1.6 Mean corpuscular hemoglobin (MCH):

Mean corpuscular hemoglobin is determined by dividing the hemoglobin percentage in grams per 1000ml of blood by the erythrocyte count in millions per micro liter. The result of this calculation is expressed in picogram (**pg**).

$$\text{Equation (pg)} = \frac{\text{Hbc } \mathbf{g/dl}}{\text{RBCs } 10^6/\mu l} \times 10$$

1.4.1.7 Mean corpuscular hemoglobin concentration (MCHC):

Mean corpuscular hemoglobin concentration is determined by dividing the hemoglobin in gram per 10000ml of blood by the volume of packed cell volume per 100ml of blood. Results are expressed in grams of hemoglobin per deciliter (**g/dl**)

$$\text{Equation } \mathbf{(g/dl)} = \frac{\text{Hbc } \mathbf{g/dl}}{\text{Pcv \%}} \times 100$$

1.4.2 Hematological examination in rabbits:

1.4.2.1 Total white blood cells (WBCs) Count:

Total white blood cells counts (WBC $X10^3/\mu l$) in rabbits were determined as described by **(Coles, 1986)**.

1.4.2.2 Differential leukocytes count (DLC):

Differential leukocyte counts were estimated by microscopical examination of Giemsa stained blood smears. One hundred leukocytes were counted from each smear of different types, including Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils and are reported in percentage (%).

2. IMMUNIZATION:-

2.1 Preparation of the whole tissue antigens extracts:

The protocol of the antigen preparation was performed as described by **(Ghosh *et al*., 1998)** with some modifications. That unfed larvae weighed and placed onto pre-sterilized Petri dishes containing an ice-cold 0.15 M Phosphate buffered saline (PBS), pH 7.2. They were thoroughly washed thrice in PBS, air- dried and then wrapped with aluminium foil papers. Approximately 3.0 gm of unfed larvae were homogenized in 10 ml of 0.15 M PBS, pH 7.2 containing 1.0 µm/l disodium ethylene diamine tetra acetic acid (Disod. EDTA), employing sterile pestle and mortar placed on an ice-bath. The homogenate suspensions were filtered free of cuticle and debris with a double layered muslin cloth into a sterile beaker. Washing by PBS and centrifuged (Speed centrifuge-80-1, Italy) at 5000 rpm for 10min three times and added 5ml of PBS to precipitate protein and kept in sterile glass vial at -20°C until further use **(Van den Broek *et al*., 2003).** Total protein concentration of antigen was estimated by modified Lowry's method **(Schacterle and Pollack, 1973).**

2.2 Determination of total protein concentration of the antigen:

Protein concentration of larval antigen was determined by modified Lowry's method, which depends on reduction of phosphotangestate or phosphomolybdate salts by amino acids, tyrosine and tryptophan **(Schacterle and Pollack, 1973).** Bovine serum albumin (BSA) 1.0mg/ml of distilled water was used as a standard solution with an extinction coefficient of 0.670 at a wavelength of 280nm **(Holme and Peck, 1988).** Employing UV/Visible spectrophotometer (Eppendorf, bioPhotometer, Germany).

Standard protein curve was constructed, using different concentrations of bovine serum albumin (BSA) standard solution as form (62.5, 125, 250, 500, 750 µg/ml) versus the absorbance of (0.08, 0.2, 0.33, 0.6, 0.88) at 650 nm. Then (5 µl of antigen + 995 µl of distilled water) were measured. Absorbance was measured against the blank solution at 280 nm. The absorbance of both antigens were plotted into the standard curve to determine protein concentration of each antigen **(Figure 8).**

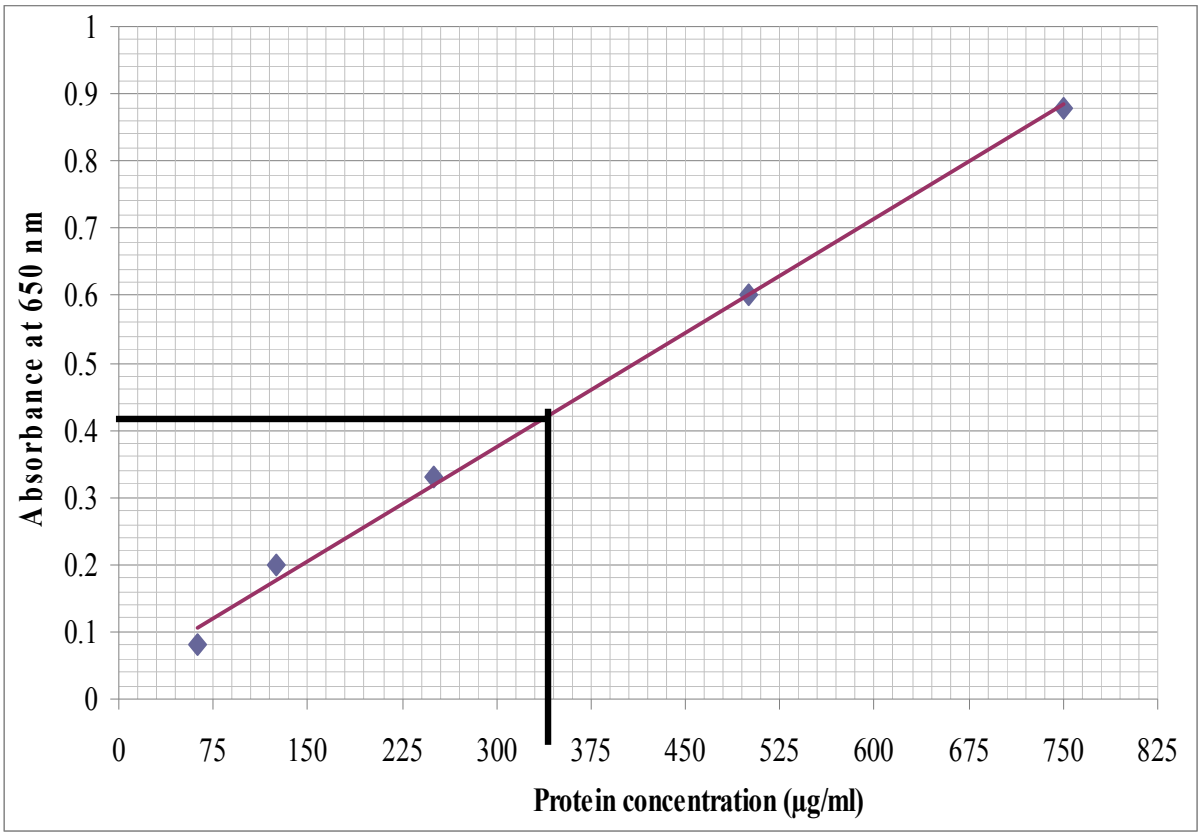


Figure (8): Standard protein curve: protein concentration (µg/ml) versus absorbance at 280 nm.

2.3 Immunization procedures:-

2.3.1 Experimental design:

Twenty local breed rabbits (rabbit more available and easy handling *in vitro* and suitable for rearing and molting of stages of the ticks), 3-6 month old rabbits were used (10 rabbit for control and 10 rabbits for immunization) each rabbit was shaved the back of neck region and wiped with 70% ethanol followed by tincture iodine, each rabbit was vaccinated subcutaneously (s/c) on back of the neck region, using a sterile syringe and needle of 23 gauge and inoculated with 0.1 ml (6.8 mg/ml) larval antigen mixed with vegetable oil given in three successive doses at weekly interval. A group of 10 rabbits was inoculated with phosphate buffer saline as a control. At fourth weeks post inoculation 5 ml of blood was drawn directly from heart by cardiac puncture of each rabbit. One ml deposited in disposable clean plastic tube with anticoagulant (DMD-Dispo, S.A.R.) used for estimation of hematological parameters

and the remainder four milliliters of blood deposited without anticoagulant in free plastic tube, to obtain serum by ordinary centrifuge 5000 rpm (Speed centrifuge-80-1, Italy) spanned for five minutes and separated serum kept at - 20C° for estimation of serological analyses of both control and immunized rabbits **(Van den Broek *et al*., 2003)** including:-

2.4 Serological analyses:

These analyses include the activity of total serum protein, total serum albumin and total serum globulin execute on the experimental rabbits (pre-immunization and post-immunization rabbits), using spectrophotometer for examination of specific wavelength (Spectrophotometer, Apel- AD-303, Japan).

2.4.1 Total serum protein:

Biuret method was used for estimation of total protein. In which Cu^{+2} reacts in alkaline solution with the peptide linkages of proteins to form a violet-colored complex. The intensity of the color produced is proportional to the protein concentration. Manual Biuret method was applied by mixing 0.2 ml serum sample with 2.8ml distilled water and then added 5ml of Biuret reagent. The mixture, mixed well and let to stand in water bath at $37C^{o}$ for 10 minutes and read at 540nm **(Schalm *et al*., 1975).**

2.4.2 Total serum albumin:-

Albumin concentration in serum was measured by using **Biolabo kits** and as follows:-

Reagent Composition.

Vial R1: Bromocresol Green.

- Succinic acid 83 mmol/L
- Bromocresol green 167 µmol/L
- Sodium hydroxide 50 mmol/L
- Polyoxyethylene monolauryl ether 1.00g/L
- Preservative

Vial R2: Standard

Bovine albumin serum 5.0 g/dl.

Procedure:

Pipette into well identified test tube	Blank	Standard	Assay
Reagent	**2ml**	**2ml**	**2ml**
Dematerialized water	**10 µl**		
Specimen			**10 µl**
Standard		**10 µl**	

The absorbance of the Assay (A sample) and the standard, (A sample) were measured against the reagent blank at 630 nm.

Calculation:-

The calculations of result were as followed:

$$\text{Albumin concentration} = \frac{\text{Assay}}{\text{Standard}} \times \text{Standard concentration 5g/dl}$$

2.4.3 Globulin determination:

The determination of globulin concentration in blood serum was done through a simplified mathematical method by subtraction of the albumin concentration that previously estimated from total protein concentration.

Golobulin concentration = Totale protein concentration – Albumin concentration.

2.5 Quantitative evaluation of immunoglobulin concentration in rabbits:

2.5.1 Glutaraldehyde coagulation tests (Mary *et al.*, 2004):

1- A solution of 10% solution of glutaraldehyde was prepared (usually prepared via dilution of a 25% solution to a 10% solution).
2- Placed 0.5% ml of serum into a tube.
3- Fifty µl (0.05 ml) of the 10% glutaraldyhade reagent was added to the tube.
4- Mixed immediately, and then incubated at room temperature.
5- The tubes were examined at intervals for as long as 1 hour, looking for evidence of coagulation.
6- The results were interpreted as follows:

a- Complete coagulation is indicative of more than 600 mg/dl of immunoglobin.

b- Semisolid clot is indicative of 400 to 600 mg/dl of immunoglobin.

c- No coagulation is indicative of less than 400 mg/dl of immunoglobin.

2.5.2 Sodium sulfite precipitation tests (Mary *et al.*, 2004):

1- Three solutions of sodium sulfite were prepared (14%, 16% and 18%) from anhydrous sulfite and distilled water.

2- Placed 1.9 ml of sodium sulfite solution into each of three test tubes.

3- Added 0.1 ml of serum into each of the three tubes.

4- Mixed immediately, and then incubated at room temperature for 1 hour.

5- After 1 hour, the tubes were examined for evidence of precipitation.

6- The results were interpreted as described in **(Table 4).**

Table (4): Interpretation of sodium sulfite precipitation test.

Immunoglobulin concentration	Sodium sulfite concentration		
	14%	16%	18%
< 500 mg/ dl	-	-	+
500- 1500 mg/dl	-	+	+
> 1500 mg/dl	+	+	+

Precipitation after 1 hour (cloudiness without visible flakes is a negative test). Visible flakes when noted were considered positive.

2.6 Evaluation of immune response:

2.6.1 Evaluation of cell-mediated immunity:

2.6.1.1 Evaluation of delayed-type hypersensitivity cutaneous reaction:

The test was carried out after the method of **(Kaura and Sharma, 1982)**. The flank skin of each immunized (post-immunization) rabbits was carefully freed of hair, using hair clipper. A circular area was marked and wiped with 70% ethanol 0.05 ml, 3.4 mg of whole crude larvae extract were inoculated I/D, using 1.0 ml tuberculin syringes into the right side of flank region of each rabbit in the center of the marked circular area, while 0.05 ml of normal saline was done into the left side in all rabbits. Observation was made within 0 hr, 2hr, 4hr, 6hr, 12hr, 23hr, 36hr, 48hr, 72rh, 96hr and 7days. Post-inoculation to exclude an immediate-type cutaneous hypersensitivity reaction and monitor degree of swelling and redness of skin. The mean diameter and skinfold thickness were measured by a caliper (Vernier/ Electronic digital caliper/ 0-150mm, Lezaco, China **(Figure 9).**

Figure (9): Vernier - Electronic digital caliper for measuring skin fold thickness in experimental rabbits.

2.6.1.2 Evaluation of phagocytosis:

The ingestion of *Staphylococcus aureus* test: This test was performed as stipulated by **Burrell (1979).** *S. aureus* colonies (Children General Hospital, Sulimani) were taken out with sterile platinum loop being on Bunsen flame, into diluted (1:10) peptone broth (1gm of peptone diluted to 9 ml distilled water) in two test tubes and incubated at 37°C for 18 hrs, 0.05ml of the culture medium was mixed with 1.0 ml of freshly prepared heparinized blood obtained from the immunized and control rabbits. The mixture was incubated at 37°C for 1/2 1hr. Smears were prepared on clean glass slides, air-dried and stained with 10% Giemsa stain for 30 min. Examined under oil immersion microscope (× 100) for estimation of cells engulfing bacteria (*S. aureus*) as well as the number of phagocytic cells.

$$\text{Phagocytic Index (PI)} = \frac{\text{Number of phagocytosed cells}}{\text{Total number of phagocytic cells}} \times 100$$

2.6.2 Evaluation of humoral-mediated immunity

2.6.2.1 Determination of the lgG protein:

Determination of the lgG protein (Shaheed Hadi Consultation Clinic), using radial immunodiffusion plate (Radial immunodiffusion plates, IgG RID, Italy) which contained specific antiserum in agarose gel, 0.1M phosphate buffer pH 7.0, 0.1% sodium azide as a bacteriostatic agent, 1 mcg/ml amphotericin B as an antifungal agent. The plates contained 0.002M ethylene-diaminetetraacetic acid. The kit included plates (Radial immunodiffusion plates, IgG RID, Italy), the plates contained agraose gel and a specific antiserum IgG. Radial immunodiffusion was based on the diffusion of antibody from a circular well radial into a homogeneous gel containing specific antiserum IgG. A circle of precipitated antigen and antibody forms, and continues to grow until equilibrium is reached. After 48 hr of incubation, the zone diameter of control and samples was measured and conversion with **(Appendix 1).**

Samples:

Serum or plasma.

Reagents:

Plate: Agarose gel containing the goat antiserum lgG.

2.6.2.1.1 Performance of test:

The plates removed from the refrigerator to room temperature (approximately 30 minutes) before filling the wells. The excess of moisture was removed from the plate in the bag by removing the cover until evaporation has dried the surface and the wells. For the best results, three (3) wells were filled with control sera for each plate. The location of each was noted. The specimen (5 µl) delivered to the well by placing the pipette tip at the bottom of the well. The well allowed to fill to the top of the agarose surface and the bubbles was removed to ensure proper volume and diffusion of the sample. The plate replaced in the bag. The plates incubated in an upright position on a flat surface at room temperature (20-24°C) for 16-20 hours for overnight readings and over 48 hours for end point readings. After 48h of incubation, the precipitating ring diameter of control and samples were measured.

3. CHALLENGE LARVAL INFESTATIONS

After two weeks of pre-feeding, the sclerotized uncalculated numbers of larvae *in vitro* were obtained for attachment and feeding process as indicated by accumulation of flat larvae onto muslin covers and their attempt to suck blood from nearby finger. The content of each bijou bottle (unfed larvae of *Hyalomma a. anatolicum*) was placed on and clipped and shaved ear-pinnae of immunized (post-immunized) and control (pre-immunized) rabbits put inside tubular ear-bags (muslin cloth) (5 X 10) cm and fixed at the base of each ear by thread (cotton) which present in the edge of ear-bags using for prevent ticks escaping from ears. Ear movement was limited by tightening tips of the ear-bags with a nylon thread around the neck figure (10), after 3 days changes in each ear of rabbits detected for attachment and feeding.

Figure (10): Experimental rabbit for tick infestation in immunized rabbit.

4. STATISTICAL ANALYSIS

Statistical analysis was conducted using SPSS for windows (version 18). The variance analysis (ANOVA), Chi square, Linear regression, and t-test were done to find the significant differences between the zones, seasons, species and sex of ticks at level 5% and 1% using statistical tool **(JMP, 2007).**

CHAPTER FOUR

RESULTS

1. TICK SPEICES AND DISTRIBUTION:

In this study two genera of hard ticks, *Hyalomma* and *Rhipicephalus* were observed and for each genus, two species (*Hyalomma anatolicum anatolicum, H. marginatum*) and (*R. turanicus, R. sanguineus*) were identified according to morphological features, depending on the keys mentioned by **(Hoogstraal, 1956)** and **Walker *et al*., 2003)**.

Table (5) shows four species of ticks which were isolated from the genera Hyalomma (*H. a anatolicum*, *H. marginatum*), the highest number and percentage species were *H. a anatolicum* 608(51.9%), *H. margimatum* 122 (10.4%), and genera Rhipicephalus (*R. sanguineus* 90 (7.7%) and *R. turanicus* 351(30%) during the year.

Table (5): Number and percentage of different tick species identified in 3 zones in Suliamani governorate from March, 2009 to February 2010.

Zones	Genus: Rhipicephalus		Genus: Hyalomma		Total No. Ticks
	No. (%) *R. sanguineus*	No. (%) *R. turanicus'*	No. (%) *H. a anatolicum*	No. (%) *H. marginatum*	
Zone- 1	23 7.7	177 59.4	64 21.5	34 11.4	298
Zone- II	20 5.4	124 33.6	191 51.8	34 9.2	369
Zone-III	47 9.3	50 10.0	353 70.0	54 10.7	504
Total	90 7.7	351 30.0	608 51.9	122 10.4	1171

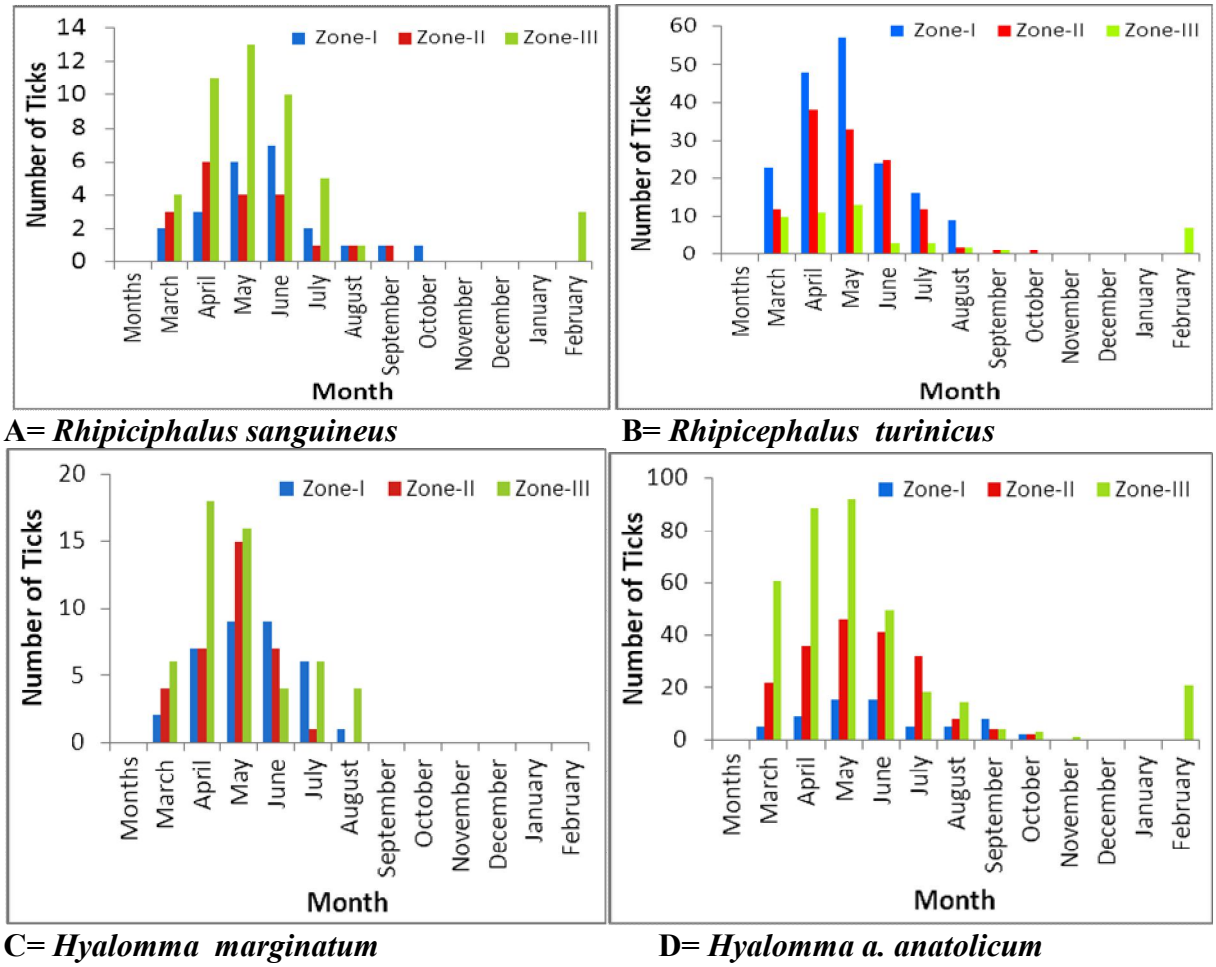
According to **table (6)** and **figure (11)** the distribution of tick species during different months of the study was referred to the distribution of different species of ticks, which were zones dependent according to Chi square test showed fluctuation in the occurrences. The number of *Hyalomma a. anatolicum* was highly distributed in March, April, May, and June 61(75.3%), 89 (69%), 92 (68.7%), and 50 (74.6%),

respectivily in zone-III and *Rhipicephalus sanguineus* was highly distributed in April, May, and June 11 (8.5%), 13 (9.7%), and 10 (14.9%) respectivily in zone-III in comparison with other zones, while the number of *Hyalomma marginatum* was highly distributed in April and May 18 (14%) and 16 (11.9%) in zone-III comparing to other zones and the number of *Rhipicephalus turanicus* was highly distributed in zone-I in March, April and May 23 (71.8%), 48 (71.6%), and 57 (65.5%), respectively.

Table (6): Distribution of tick species according to months in different zones.

Months	Zone-I				Zone-II				Zone-III			
	R. spp (No. %)		*H. spp* (No. %)		*R. spp* (No. %)		*H. spp* (No. %)		*R. .spp* (No. %)		*H. spp* (No. %)	
	*R. s**	*R. t**	*H a.a**	*H m**	*R. s*	*R. t*	*H a.a*	*H. m*	*R. s*	*R. t*	*H. a*	*H. m*
March	2 6.3	23 71.8	5 15.6	2 6.3	3 7.3	12 29.3	22 53.6	4 9.8	4 4.9	10 12.4	61 75.3	6 7.4
April	3 4.5	48 71.6	9 13.4	7 10.5	6 6.9	38 43.7	36 41.4	7 8	11 8.5	11 8.5	89 69	18 14
May	6 6.9	57 65.5	15 17.2	9 10.4	4 4.1	33 33.7	46 46.9	15 15.3	13 9.7	13 9.7	92 68.7	16 11.9
Spring//Total	11	128	29	18	13	83	104	26	28	34	242	40
June	7 12.7	24 43.6	15 27.3	9 16.4	4 5.2	25 32.5	41 53.2	7 9.1	10 14.9	3 4.5	50 74.6	4 6
July	2 6.9	16 55.2	5 17.2	6 20.7	1 2.2	12 26	32 69.6	1 2.2	5 15.6	3 9.4	18 56.3	6 18.7
August	1 6.2	9 56.3	5 31.3	1 6.2	1 9.1	2 18.2	8 72.8	0 0.0	1 4.8	2 9.5	14 66.7	4 19
Summer/Total	10	49	25	16	6	39	81	8	16	8	82	14
September	1 11.1	0	8 88.9	0	1 16.7	1 16.7	4 66.6	0	0	1 20	4 80	0
October	1 33.3	0	2 66.7	0	0	1 33.3	2 66.7	0	0	0	3 100	0
November	0	0	0	0	0	0	0	0	0	0	1 100	0
Autumn/Total	2	0	10	0	1	2	6	0	0	1	8	0
December	0	0	0	0	0	0	0	0	0	0	0	0
January	0	0	0	0	0	0	0	0	0	0	0	0
February	0	0	0	0	0	0	0	0	3 9.7	7 22.6	21 67.7	0
Winter/Total	0	0	0	0	0	0	0	0	3	7	21	0
Total Number	23	177	64	34	20	124	191	34	47	50	353	54
Total ticks	298				369				504			
Total Number	1171											
χ^2cal P < 0.01	281.6 **											

R. s. = Rhipicephalus sanguineus; R. t. = Rhipicephalus turanicus; H. a. a. = Hyalomma anatolicum anatolicum; H. m. = Hyalomma marginatum.



A= *Rhipiciphalus sanguineus* **B= *Rhipicephalus turinicus***

C= *Hyalomma marginatum* **D= *Hyalomma a. anatolicum***

Figure (11; A, B, C and D): Distribution of tick species according to months in different zones.

Table (6) and figure (12) show the influence of the seasons on the distribution of different species of ticks. The distribution of ticks was highest in spring, followed by summer, then the number of ticks was decreased in both fall and winter seasons.

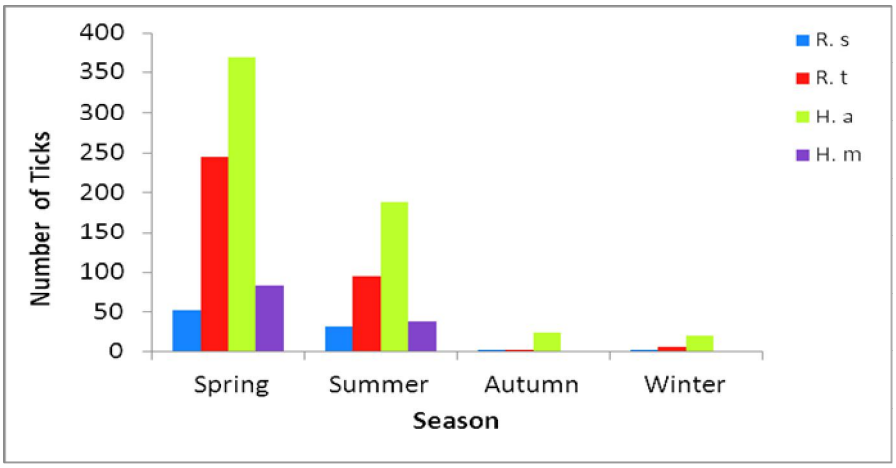
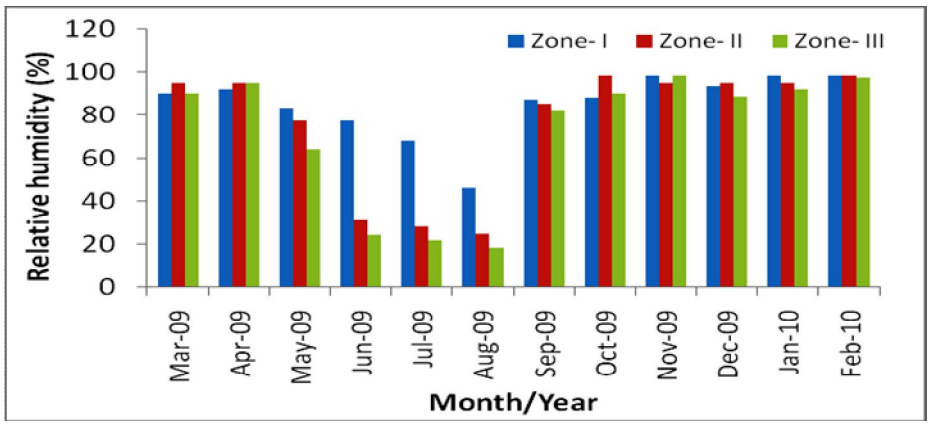
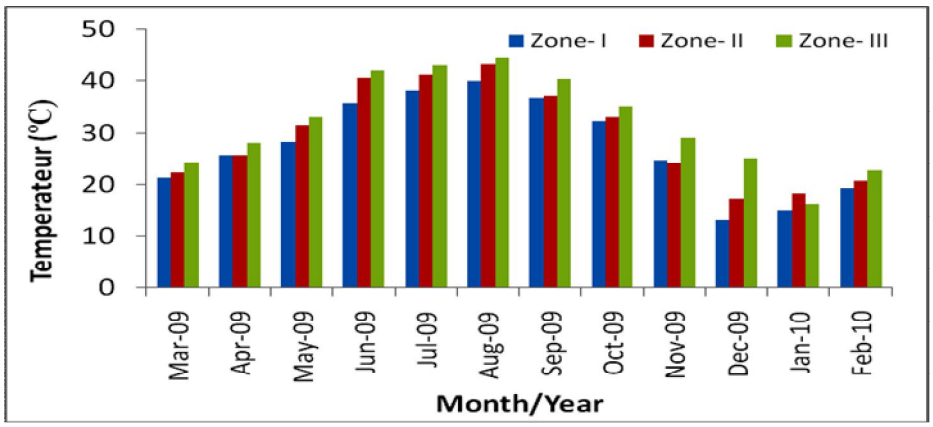


Figure (12): Distribution of tick species according to season in different zones.

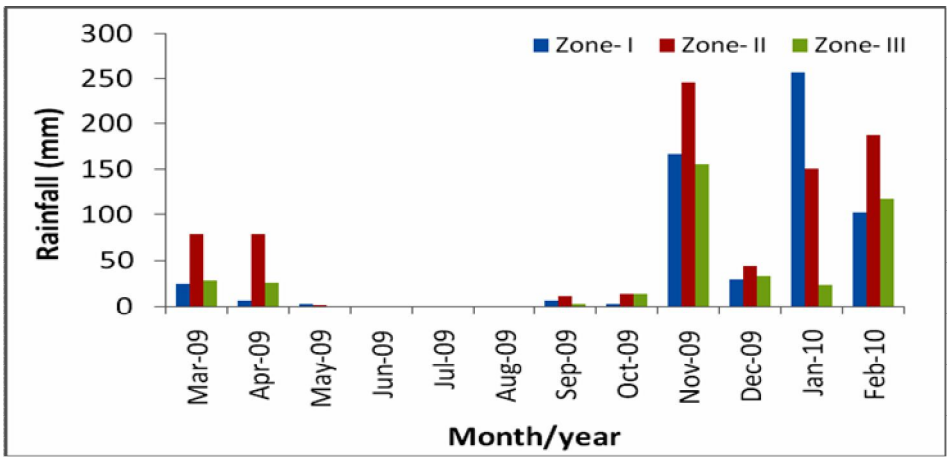
It was found that the main role of this difference in prevalence was due to the humidity, temperature and rainfall which were shown in the meterological data (**Figure 13** and **Appendix 2).**



A: Monthly relative humidity (%) for Sulaimani governorate during March, 2009 to February, 2010.



B: Monthly temperature C° for Sulaimani governorate during Mar, 2009 to Feb, 2010.



C: Monthly rainfall (mm) for Sulaimani governorate during Mar, 2009 to Feb, 2010.

Figure (13; A, B, C): The effect of monthly environmental factors on distribution of ticks in different zones.

Table (7) shows the mean value (Mean ± SE) of distribution of different species of tick: *Hyalomma a. anatolicum* was highest in zone-III (35.3 ± 18.4) followed by zone-II 23.9 ± 9.1 and zone-I 8 ± 2.5 respectively, while in *Haylomma marginatum* the difference did not vary significantly among the zones, *Rhipicephalus sanguineus* was highest in zone-III 6.7 ± 2.8 in comparism to zone-I, 2.9 ± 1.2 and 2.9 ± 1.1 in zone-II, while the species *Rhipicephalus turanicus* distribution was highest 29.5 ± 2.9 in zone-I followed by zone-II 15.5 ± 7.6 and zone-III 6.3 ± 3.5 .

Table (7): Distribution of tick species (Mean ± SE) according to different zones in Sulaimani governorate

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Zone	Tick species			
	H. a anatolicum	*H. marginatum*	*R. sanguinus*	*R. turanicus*
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
I	**8.0 b ± 2.5**	**5.6 a ± 2.1**	**2.9 b ± 1.2**	**29.5 a ± 2.9**
II	**23.9 ab ± 9.1**	**6.8 a ± 3.2**	**2.9 b ± 1.1**	**15.5 ab ± 7.6**
III	**35.3 a ± 18.4**	**9.0 a ± 4.3**	**6.7 a ± 2.8**	**6.3 b ± 3.5**

Numbers with the same letter within a column are not significantly different from each other according to Duncan's multiple range tests ($P \leq 0.05$)

Table (8) and Appendix (3) show the distribution of tick sexes. It was found the number of infested female tick was greater than the male and the ratio of male tofemale was 1:2 in all species of tick(.

Table (8): Distribution and ratio of ticks according to sex.

Specimens of adult ticks	Male No. %	Female No.%	Total No. %	♂/♀
Rhipicephalus sanguineus	**27 (30.0)**	**63 (70.0)**	**90 (7.7)**	**1:2**
Rhipicephalus turanicus	**110 (31.3)**	**241 (68.7)**	**351 (29.9)**	**1:2**
Hyalomma anatolicum anatolicum	**177 (29.1)**	**431 (70.9)**	**608 (51.9)**	**1:2**
Hyalomma marginatum	**40 (32.8)**	**82 (67.2)**	**122 (10.5)**	**1:2**
Total	**354 29.4**	**817 70.6**	**1171**	**1:2**

Figure (14) and Appendix(3) confirmed that the highest distribution of female *Hyalomma anatolicum anatolicum* in zone-III 183 and female species of *Rhipicephalus turanicus* in zone- I 90 and both species in the spring season collected.

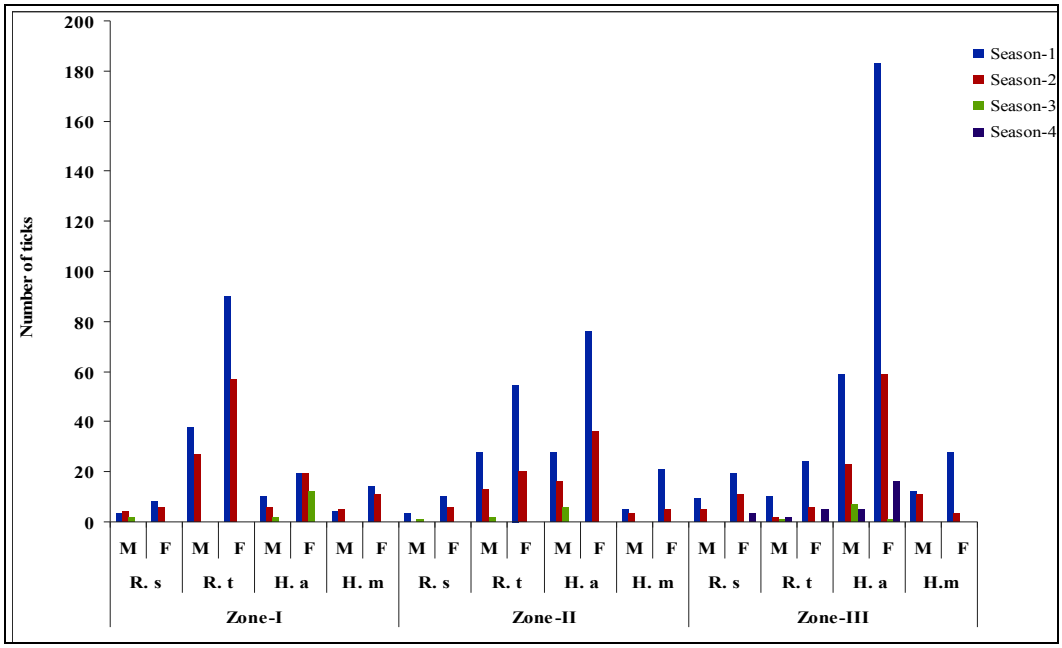


Figure (14): Monthly distribution of tick according to species and sex in different zones (seasone1= spring, seasone2= summer, seasone3=autumn and seasone4=winter).

Table (9) and **Figure (15; A and B)** show that the number of adult tick was mostly engorged female of both genera 554(47.3%) and non-engorged females 263 (22.5%) and male of both genus 354(30.2%). *Hyalomma a. anatolicum* gave the highest number of engorged females 319(57.6%) and male 177(50%), followed by *Rhipicephalus turanicus* females 147(26.5%) and males 110(31.1%), *H. marginatum* females 49 (8.9%) and males 40(11.3%) and *R. sanguineus* females 39 (7.0%) and males 27(7.6%), respectively. No nymph and larvae were found.

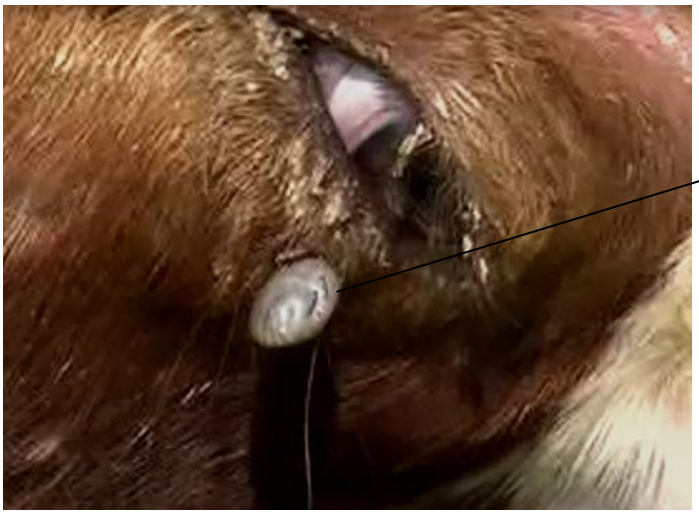
Table (9): Number and percentage of tick stages on the infested sheep in different flocks.

Tick stage	Tick species				Total Ticks No. %
	Rhipicephalus spp. No. %		*Hyalomma spp* No. %		
	R. s	*R. t*	*H a. a*	*H. m*	
-Adult female - Engorged female	39 (7.0)	147 (26.5)	319 (57.6)	49 (8.9)	554 (47.3)
- Non-engorged female	24	94	112	33	263 (22.5)
Total	63	241	431	82	817
Adult male	27 (7.6)	110 (31.1)	177 (50)	40 (11.3)	354 (30.2)
Nymph	0		0		0
Larvae	0		0		0
Total	441		730		1171

Table (10) and **Figures (15; A, B and 16)** show the sites of attacks of the tick on the body of infested sheep. The highest number 492(42.0%) of ticks was on ear, followed by under tail 208(17.7%), udder 139(11.9%), between thighs 112(9.6%), under axilla 105(9.0%) and testes 95(8.1%).. The lowest number was on eyelid 20(1.7%)

Table (10): Number and percentage of hard tick species on different sites of the body.

Site of body	*Rhipicephalus.* spp		*Hyalomma.* spp		Total ticks No. and %
	R. s No .and %	*R. t* No .and %	*H. a.a* No .and %	*H. m* No .and %	
Ear	52 (10.6)	159 (32.3)	248 (50.4)	33 (6.7)	492 (42.0)
Eyelid	1 (5.0)	8 (40.0)	10 (50.5)	1 (5.0)	20 (1.7)
Under axilla	8 (7.6)	30 (28.6)	47 (44.8)	20 (19.0)	105 (9.0)
Between Thigh	6 (5.4)	35 (31.2)	55 (49.1)	16 (14.3)	112 (9.6)
Udder	9 (6.5)	37 (26.6)	82 (59.0)	11 (7.9)	139 (11.9)
Testes	7 (7.4)	29 (30.5)	56 (58.9)	3 (3.2)	95 (8.1)
Under tail	7 (3.4)	53 (25.5)	110 (52.9)	38 (18.2)	208 (17.7)
Total	90 (7.7)	351 (30.0)	608 (51.9)	122 (10.4)	1171



A; Various stages of engorgment of *Hyalomma spp*. on the eye.

B; Several times of feeding of *Hyalomma spp*. on the ear.

Figure (15; A and B): The sites of infestation by hard tick (A; eye. B; ear).

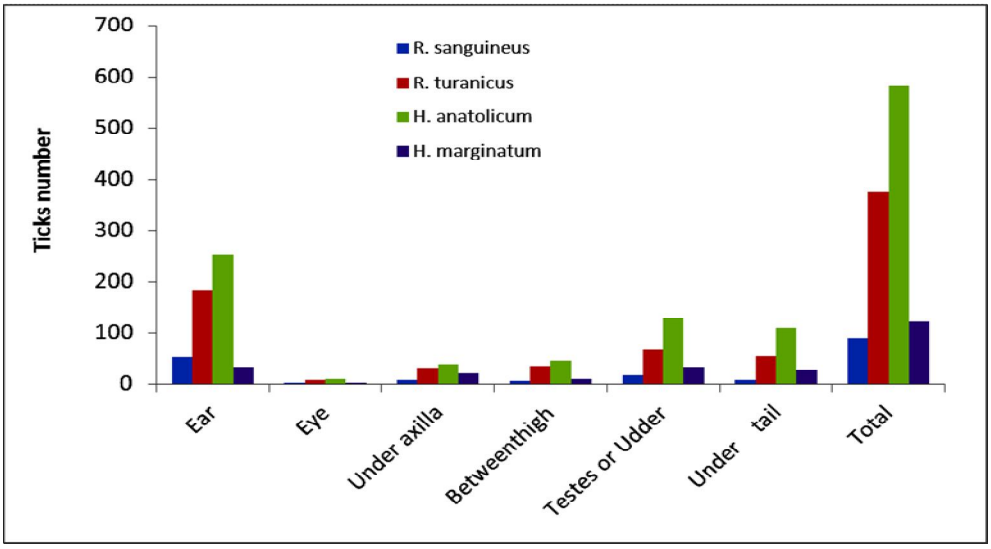


Figure (16): Distribution of hard tick on the different sites of the body.

2. PREVALENCE:

The results in **table (11)** and **figure (17)** show that out of the 2525 sheep, 298 (11.8%) were infested with tick in all three zones, and the prevalence rate of infested sheep in zone-I, zone-II, and zone-III were (10.1%, 11.1%, and 14.3%), respectively. Although the number and rate of tick's infestation of sheep was higher in zone-III than that of the zone-I and II, but statistically not significant.

The rate of infestation was higher in May (22.0, 23.3 and 36.0) in three zones respectively than other months of the study, and no infested sheep was observed in zone-I and zone-II from November to February, while in zone-III the rate of infestation was (2.0, 0%, 0.0 and 15.0), respectively.

Table (11): Number and percentage of infested sheep with hard ticks (Ixodidae) related to monthly occurrence in different zones in Suliamani governorate.

Months	Zone-I			Zone-II			Zone-III		
	No. sheep examined	No. sheep infested	Sheep infested %	No. sheep examined	No. sheep infested	Sheep infested %	No. sheep examined	No. sheep infested	Sheep infested %
March	85	9	10.6	90	11	12.2	85	21	24.7
April	110	18	16.4	95	19	20.0	100	27	27.0
May	95	21	22.0	90	21	23.3	75	27	36.0
June	90	16	17.8	90	18	20.0	60	15	25.0
July	65	9	13.8	90	13	14.4	80	8	10.0
August	70	6	8.6	60	6	10.0	75	6	8.0
September	70	4	5.7	80	4	5.0	60	3	5.0
October	50	2	4.0	40	2	5.0	50	2	4.0
November	50	0	0	55	0	0	50	1	2.0
December	50	0	0	60	0	0	60	0	0
January	55	0	0	50	0	0	75	0	0
February	50	0	0	50	0	0	60	9	15.0
Total	840	85	10.1	850	94	11.1	830	119	14.3
M ± SE	12.36a ± 2.2			13.73a ± 2.46			15.67a ± 3.7		
Total Number sheep				2525					
Total number of infested sheep				298					

Numbers with the same letter within a column are not significantly different from each other according to Duncan's multiple range tests ($P \leq 0.05$).

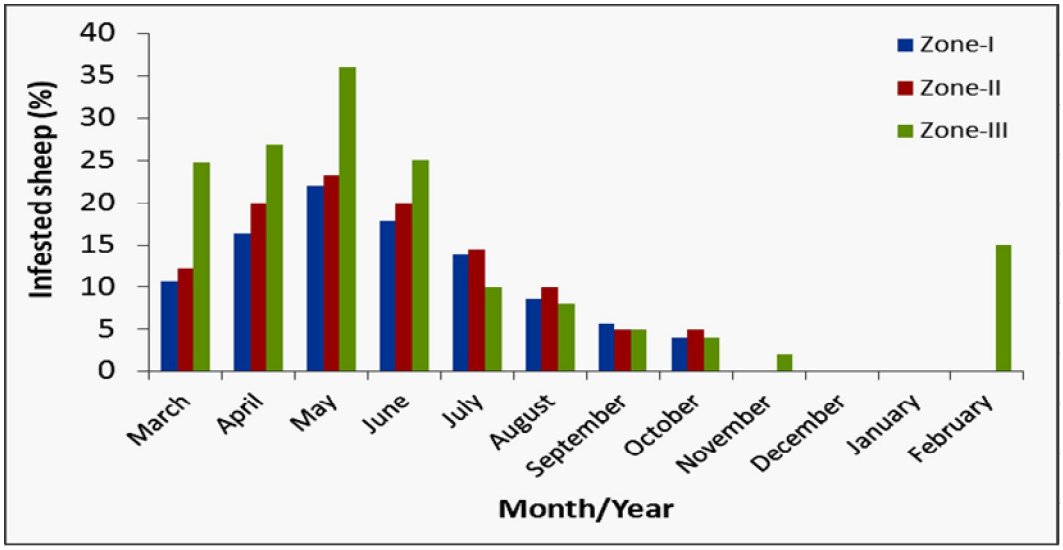


Figure (17): Number and (%) of infested sheep with hard ticks in different zones in Suliamani governorate.

Table (12) and Figure (18) show the total numbers of tick 1171 which was collected from three zones, the distribution of tick's species were not different significantly among the zone-I, zone-II, and zone-III (298, 396, and 504), respectively. Although the number of ticks in zone-III was higher when compared with the zone-I and zone-II. The higher number of ticks collected from sheep in April, May and June 32, 67, and 87 in zone-I, 41, 87, and 98 in zone-II and 81, 129, and 134 in zone-III respectively, but the number was low in July till October 29, 16, 9, and 3 in zone-I, 46, 11, 6, and 3 in zone-II and 32, 21, 5, and 3 in zone-III respectively, and no tick was collected in November till February in zone-I and zone-II, except in zone III only 31 ticks were collected in February.

Table (12): Number of collected tick from infested sheep from different zones according to months.

Months	Zone-I	Zone-II	Zone-III
	No. of tick collected	No. of tickcollected	No. of tick collected
March	32	41	81
April	67	87	129
May	87	98	134
June	55	77	67
July	29	46	32
August	16	11	21
September	9	6	5
October	3	3	3
November	0	0	1
December	0	0	0
January	0	0	0
February	0	0	31
Total	298	369	504
M ± SE	37.25a±10.51	46.12a±13.39	50.4a±15.88
Overall ticks	1171		

Numbers with the same letter within a column are not significantly different from each other according to Duncan's multiple range tests ($P \leq 0.05$).

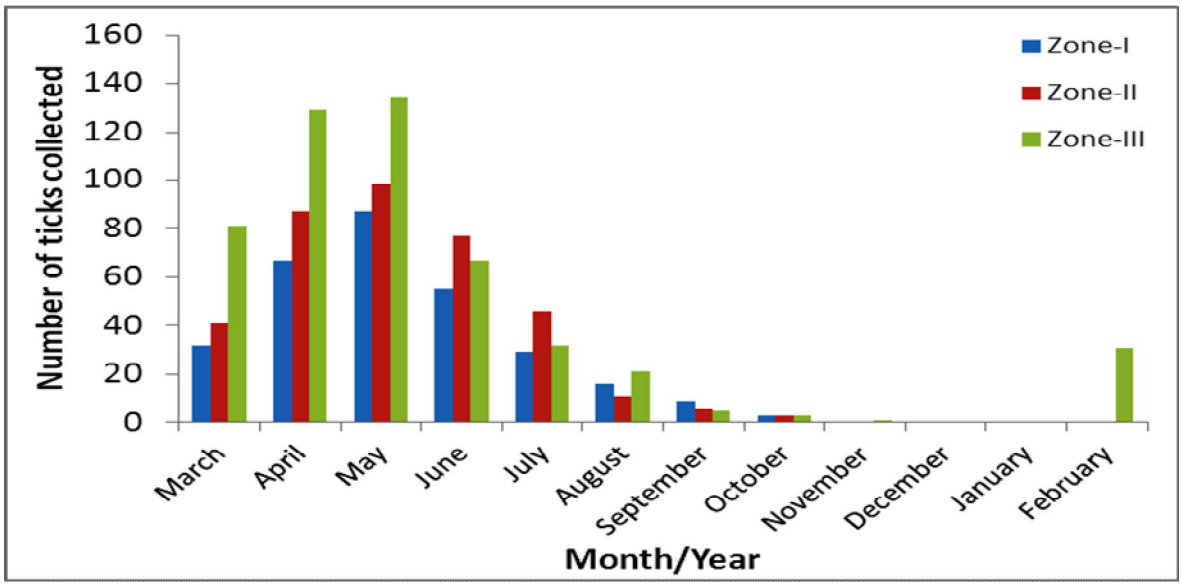
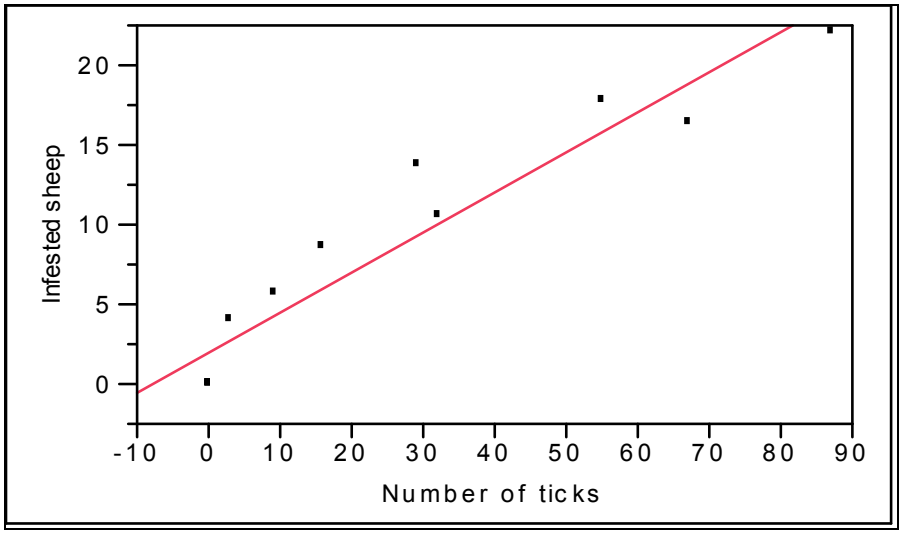
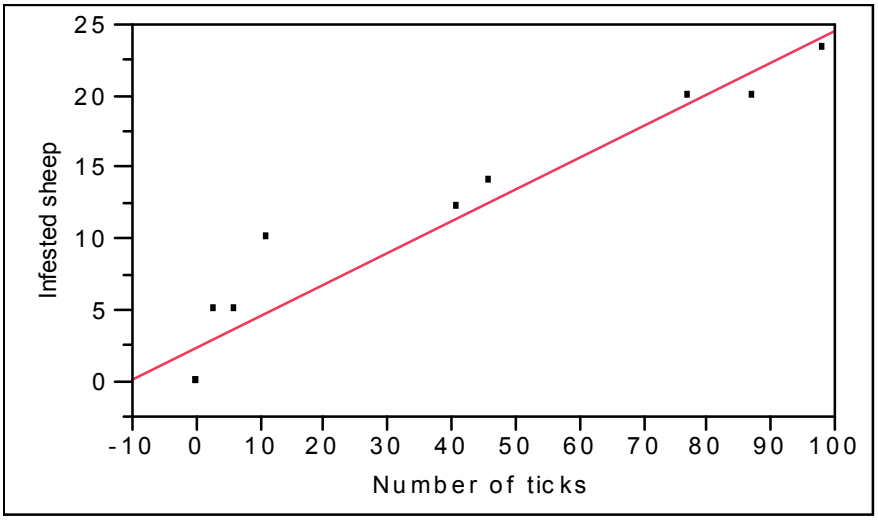


Figure (18): Distribution of hard ticks on the sheep in different zones in Sulaimani governorate.

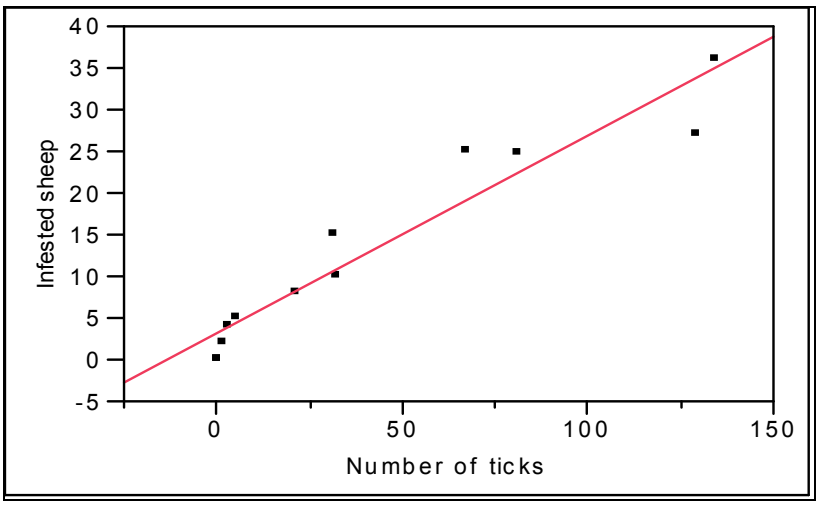
The percentage of infested sheep in any zone by number of ticks was recognized from the linear model in **(Figure 19; A, B and C)**. There were difference infestation rates in different zones, the highest infestation was recorded in zone III which was (3.1) followed by zone II which was (2.3) and the lowest rate (2.0) recorded in zone I. The linear model in zone-I , II and III showed that the **r²** were highly significant, and the number of ticks was an indicator for estimation of the infested percentage. Hence each tick infestation was increased in rate 0.25 in zone-I, and in the zone-III, each tick showed that the increasing of infested rate by 0.23, but the influence in zone-II showed that the tick infestation increased in rate 0.22.



A-zone-1: Infested sheep (Y) =2.0 + 0.25 (X) (X=Number of ticks). $R^2 = 0.91^{**}$



B-zone-II: Infested sheep (Y) = 2.3 + 0.22 X (X=Number of ticks). $R^2 = 0.92^{**}$



C-zone-III: Infested sheep = 3.1+ 0.23 X (X=Number of ticks). R^2= 0.92**

Figure (19; A, B and C): Linear equation of estimation of infested (%) with number of ticks in 3 different zones.

Table (13) shows the total number of tick which infested per sheep, it was 3.5, 3.9, and 4.2 in zone-I,II, and III, respectively during the study and the highest number of ticks infested per sheep was in May 4.1, 4.7 and 5.0 in all zones respectively, and the lowest number was detected in October and absent in November till February in all zones, except in zone-III, the number of tick per infested sheep was one in November and 3.4 in February.

Table (13): Ratio of hard ticks per sheep in different zones in Suliamani governorate.

Months	Zone-I	Zone-II	Zone-III
	No. Tick / infested sheep	No. Tick / infested sheep	No. Tick / infested sheep
March	3.6	3.7	3.9
April	3.7	4.6	4.8
May	4.1	4.7	5.0
June	3.4	4.2	4.5
July	3.2	3.5	4
August	2.7	1.8	3.5
September	2.3	1.5	1.7
October	1.5	1.5	1.5
November	0	0	1
December	0	0	0
January	0	0	0
February	0	0	3.4
Total	3.5	3.9	4.2

3. CLINICAL SIGNS:

The clinical signs observed in 50 sheep which highly infested with ticks 26 by *Hyalomma spp*., 18 by *Rhipicephalus spp*. and 6 by mixed infestation. These include; anemia, anorexia, emaciation and skin problems including skin irritation, loss of skin elasticity, loss of wool (alopecia) and patches of hyperemia. However the clinical signs were more severe in sheep infested by *Hyalomma spp*., than those infested by *Rhipicephalus spp*. **(Table 14** and **Figure 20)**.

Table (14): Clinical signs of infested sheep with ticks.

Cases	Sheep infested with *Hyalomma spp.*	Sheep infested with *Rhipicephalus spp.*
I - Anemia (Paleness of mucous membrane)	++	+
II - Anorexia	++	+
- Emaciation	++	+
III- Skin problem		
- Skin irritation	++	++
- Loss skin elasticity	++	+
- Loss of wool (alopecia)	++	+
- Patches of hyperemia	++	+

Figure (20): Sheep infested with tick; Emaciation, Loss of wool (alopecia) and Patches of hyperemia at the site of tick attachment.

HEMATOLOGICAL FINDINGS:

Table (15) shows the hematological parameters in sheep infested with *Hyalomma spp* and *Rhipicephalus spp*. There were statistically significant differences in p≤ 0.01 in values of total red blood cells (RBCs $\times 10^6/\mu l$), packed cell volume (PCV%), and hemoglobin concentration (HgC g/dl) with mean values of 5.9 $\times 10^6/\mu l \pm 0.18$, 20.5% ± 0.54, and 7.1 g/dl ± 0.17 respectively in sheep with *Hyalomma spp* in comparison to *Rhipicephalus spp* with mean values were $8.2 \times 10^6/\mu l \pm 0.25$, 25.7% ± 0.85, and 8.6g/dl ± 0.18 respectively, and the mean corpuscular hemoglobin (MCH pg) was 12.2 pg ± 0.54 in sheep infested with *Hyalomma spp* and was significantly different at p≤ 0.05 than those infested with *Rhipicephalus spp*. 10.6 pg ± 0.42. The mean corpuscular volume (MCV fl) 35.7 fl ± 1.93 and mean corpuscular hemoglobin concentration (34.9 g/dl ± 1.07). In *Hyalomma spp* infested sheep was not vary significantly from those infested with *Rhipicephalus spp*. with mean corpuscular volume of 32.0 fl ± 1.36 and mean corpuscular hemoglobin concentration 33.6 g/dl ± 1.15. Also there was no significant difference in values of total white blood cell (WBC) count in both *Hyalomma spp*. 11.8 $\times 10^3/\mu l \pm 0.25$ and *Rhipicephalus spp* $11.2 \times 10^3/\mu l \pm 0.31$. The type of anemia in both tick infestations was inductive by hematological examination and classified as normocytic normochromic. Observed data was also compared with normal values of the blood parameters, are shown in **(Appendix 4).**

Table (15): Hematological parameters of sheep infested with hard tick (*Hyalomma spp*. and *Rhipicephalus spp*.).

Blood parameter	Sheep infested with *Hyalomma spp.*	Sheep infested with *Rhipicephalus spp.*
Total RBCs$\times 10^6/\mu l$	5.9** ± 0.18	8.2 ± 0.25
TotalWBCs$\times 10^3/\mu l$	11.8 $^{n.s}$ ± 0.25	11.2 ± 0.31
- PCV%	20.5** ± 0.54	25.7 ± 0.85
- HgC g/dl	7.1** ± 0.17	8.6 ± 0.18
- MCV fl	35.7 $^{n.s}$ ± 1.93	32.0 ± 1.36
- MCH pg	12.2* ± 0.54	10.6 ± 0.42
- MCHC g/dl	34.9 $^{n.s}$ ± 1.07	33.6 ± 1.15
t (42) = 2.02 t (42) = 2.69 0.05 0.01	N.s.= not significant	

5. IMMUNIZATION

5.1 Evaluation of cell-mediated immunity response.

5.1.1 Leukocyte and Differential count

Evaluation of responses of immunized rabbits with whole larval crude extracts by estimation of cellular defense activity, mainly by estimation of white blood cells (Total Leukocyte number) and differential leukocyte count (LDC). The results indicated a significant increasment in the values of total Leukocyte $13.5\times10^3/\mu l \pm 0.58$ in immunized rabbits in comparison with control group $7.4 \times10^3/\mu l \pm 0.22$. There was statistically increase in numbers of monocyte 18.1 ± 1.1 and neutrophils (50.1 ± 1.17) in immunized rabbit in comparison to control rabbit 14.5 ± 1.1 and 42.1 ± 1.63 respectively **(Table 16).** .

Table (16): Total and differential leukocyte count in immunized and control rabbits.

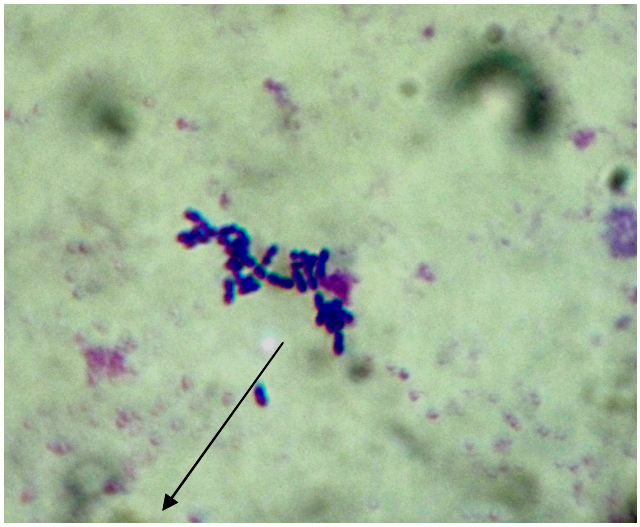
Experimental .rabbits	WBCs $\times 10^3/\mu l$	Deferential Leukocyte count				
	Mean ± SE	Monocyte	Lymphocyte	Neutrophil	Esonophil	Basophil
Control	7.4 ± 0.22	14.5 ± 1.1	38 ± 1.46	42.1 ± 1.63	6.1 ± 0.5	2.6 ± 0.3
Immunized rabbit	13.5 **±0.58	18.1* ± 1.1	30.9** ± 0.93	50.1** ± 1.17	3.1**0.34	0.0** ± 0.0
t (18) = 2.10 0.05	t (18) = 2.87 0.01					

5.1.2 Phagocytic ingestion activity:

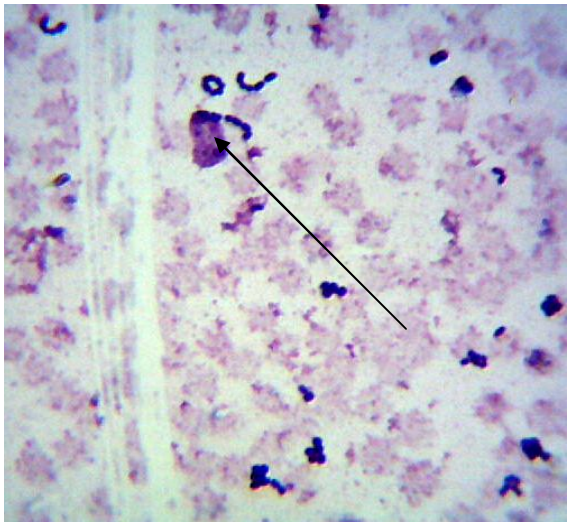
The phagocytic ingestion activity was observed in immunized rabbits with larval crude antigen extract by estimation of phagocytic Index. It was revealed that the significantly increasing in number of phagocytes ingested *Staphylococcus aureus* 7.2 ± 0.61 in immunized rabbits was higher than control group of pre-immunized rabbits 1.8±0.24 **(Table 17** and **Figure 21).**

Table (17): Phagocytic index in control (pre-immunized) and immunized in rabbits with whole crude larval extract.

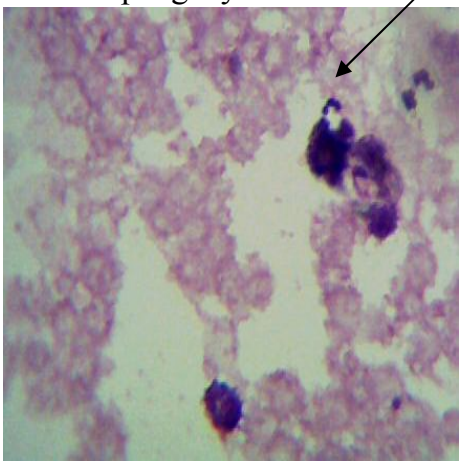
Experimental rabbits	Phagocytic cells
Control (pre-immunized)	1.8 ± 0.24
Immunized rabbit	7.2** ± 0.61
t (18) = 2.87 0.01	



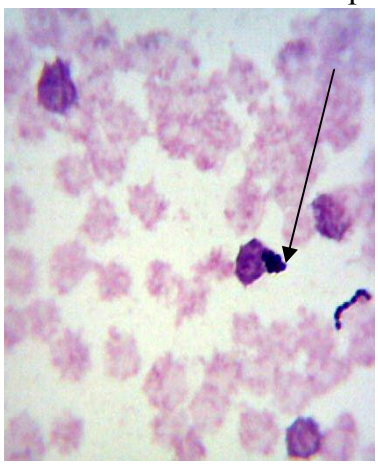
A): Aggregation of *Staphylococcus aureus* around phagocytic cell.



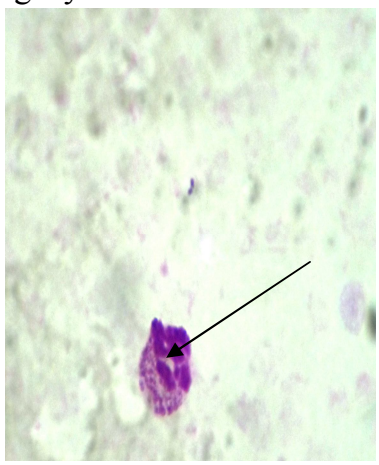
B): *S. aureus* attachment with the whole phagocyte.



C): Starting of phagocytosis Development.



D): Engulfing developmen



E): Complete phagocytosis.

Figure (21; A, B, C, D, and E) (Gram stain × 1000): Stage of phagocytosis by phagocytic cells of *Staphylococcus aureus* in stained blood of immunized rabbits by whole crude larval extract.

5.1.3 Delayed-type hypersensitivity by cutaneous reaction (Intradermal skin test):

Skin intradermal test reaction was used with Ag (larval crude extract antigen) for typical type of hypersensitivity when Ag introduced intradermally, an inflammatory and thickness of the skin response occurred after many hours and reached maximum after 12 to 24 hr (10.20 ± 0.38 mm, 11.10 ± 0.26 mm) respectively., and reaction was subsided and reached to thickness as zero time (2.60 ± 0.19 mm) at 7 days post-inoculation (immunized) rabbits **(Table 18** and **Figure 22 and 23).**

Table (18): Skin fold thickness (millimeter) of rabbit's inoculated (l/d) with whole crude larval extract *(Hyalomma anatolicum anatolicum)*.

Experimintal Rabbits	Skin fold thickness (mm) at 0 time	Post- inoculation(l/d) of whole crude larvae/ skin fold thickness(mm)									
		2h	4h	6h	12h	24h	36h	48h	72h	96h	7 days
Post-immunized	2.10± 0.15	3.80± 0.34	4.80± 0.47	7.90± 0.52	10.20± 0.38	11.10± 0.26	8.70± 0.64	6.70± 0.49	4.90± 0.24	3.20± 0.30	2.60± 0.19

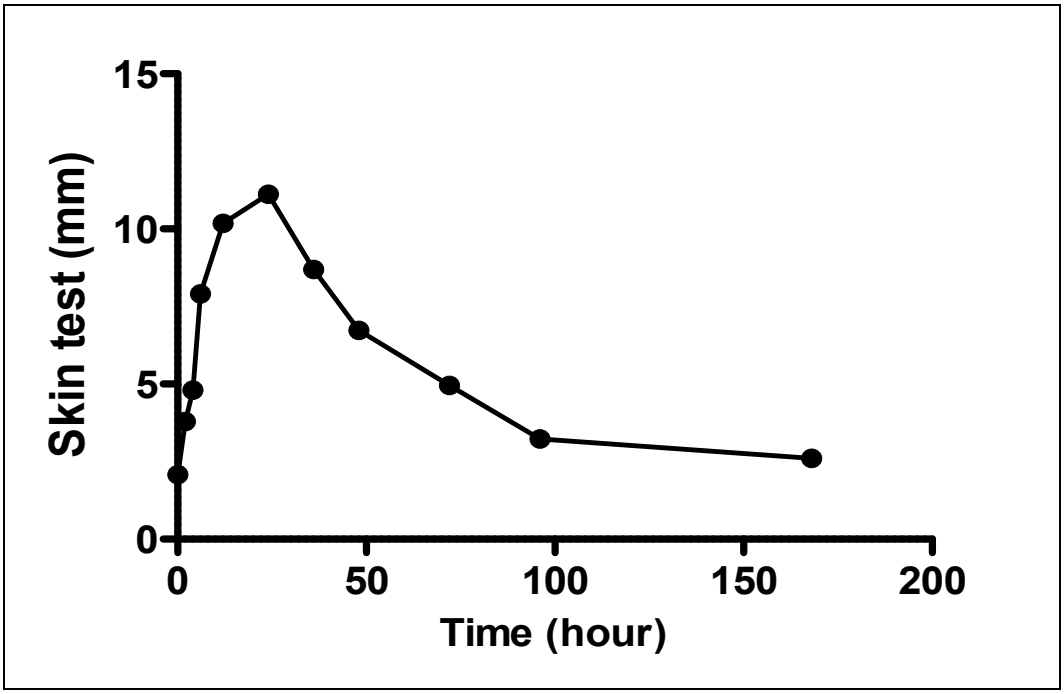


Figure (22): Skin test curve after 3 inoculations of whole crude larval extract *Hyalomma anatolicum anatolicum*.

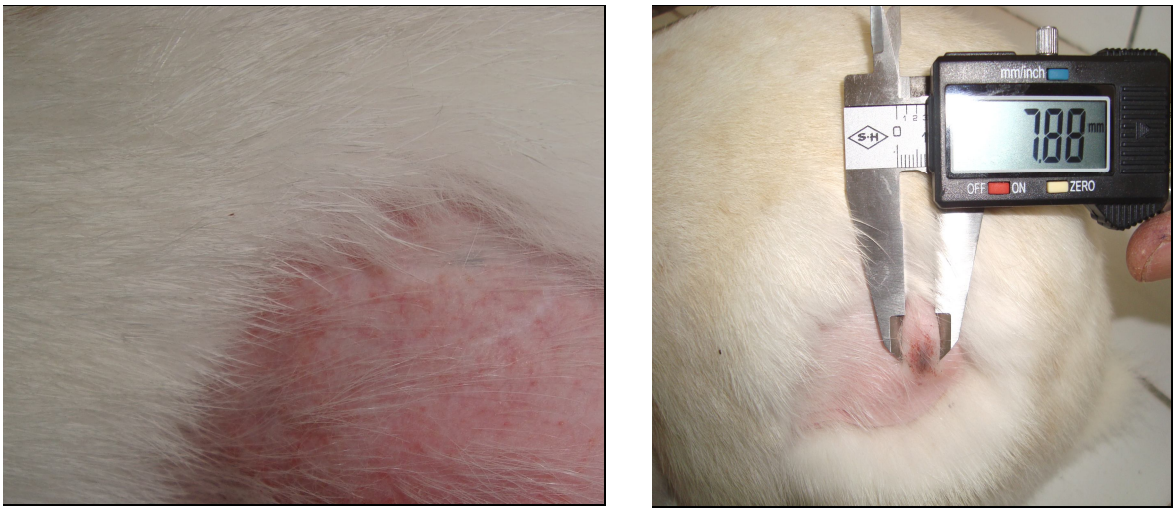


Figure (23): Intradermal inoculation in rabbit with larvae crude extract antigen; left, skin fold thickness (mm) at 0 time), right, increase in the thickness of skin celebrated by caliper (increase in skin thickness fold reach to peak at 24 hrs after inoculation.

5.2 Evaluation of humoral-mediated immunity.

5.2.1 Total protein (Albumin/ Globulin).

The content of serum total protein, albumin and glubulin were determined in immunized rabbit with whole crude larval extract. These values were highly significant p≤ 0.01 elevated (9.677 ± 0.16), (4.349 ± 0.08) and (5.328 ± 0.13) respectively in immunized rabbits compared to control rabbit (6.466 ± 0.21), (3.858 ± 0.10) and (2.608 ± 0.14) respectively **(Table 19).**

Table (19): Level of total protein, albumin, and globulin in immunized and control rabbits.

Experimental rabbits	Total serum protein-gm/dl	Total serum albumin- gm/dl	Total serum globulin-gm/dl
	Mean± SE	Mean± SE	Mean± SE
Control	6.466 ± 0.21	3.858 ± 0.10	2.608 ± 0.14
Immunized	9.677**± 0.16	4.349**± 0.08	5.328** ± 0.13
t (18) = 2.1 0.01			

5.2.2 Quantitative values of immunoglobulin:

5.2.2.1 Biochemical reaction to determination of immunoglobulin (Ig) concentration.

The biochemical analysis was used for determination of the quanlitative values of concentration of nonspecific immunoglobulin (lg) in serum of immunized rabbits with whole crude larval extract. The result of glutaraldehyde coagulation test used to estimate the concentration of lg in serum of immunized rabbits. It was higher than 600 mg/dl (0.6gm/dl) **(Figure 24** and **Appendix 5A).**

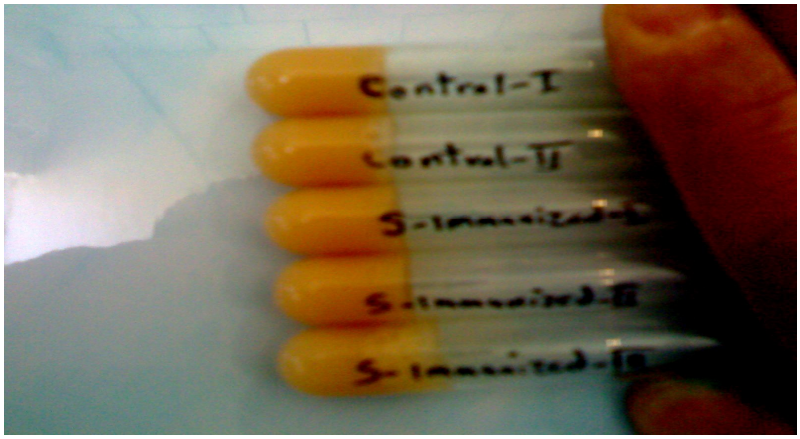


Figure (24): Glutaraldehyde coagulation test for determination of immunoglobulin Concentration.

Figure (25) and Appendix (5B) show the results of sodium sulfite precipitation reaction, the concentration of lg was more than 1500 mg/dl (1.5 gm/dl) in comparison with control group which was between 500-1500 mg/dl (0.5-1.5 gm/dl) as described in materials and methods **(Table 3).**

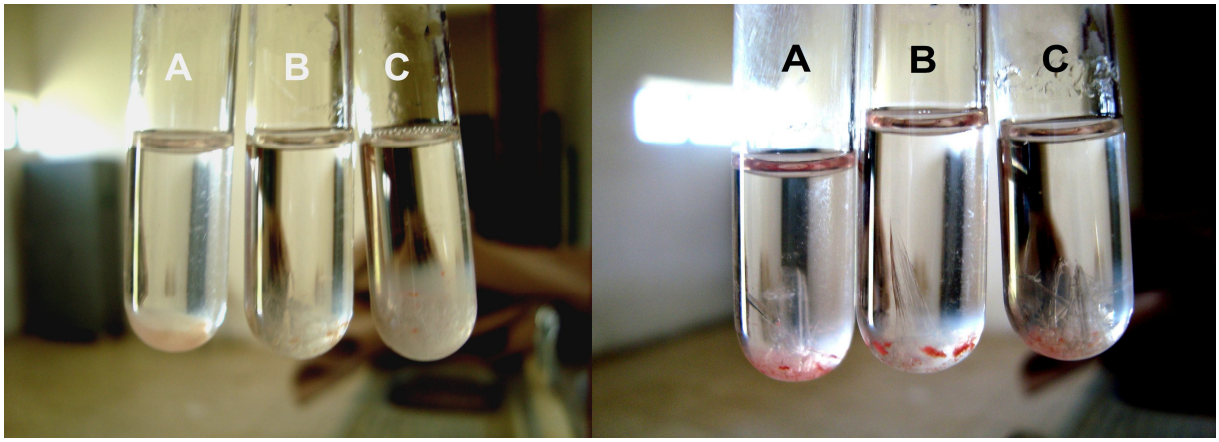


Figure (25): Sodium sulfite pricipitation test
-Left (control); A, neg (precipited and not flakes noted) B and C, positive reaction (flakes noted) the results 500-1500mg/dl.
-Right (immunized); A, B and C positive reaction (flakes noted) as results were >1500mg/dl.

5.2.3 Qualitative values of immunoglobulin:

A radial immunodiffusion plate was used for determination the concentration of specific immunoglobulin type-G (lgG). The result was indicated by measuring the diameter of diffusion ring in which represented the concentration of IgG in serum according to **(Appendix 1)**. It was 2.76± 0.22 gm/dl significantly high in immunized rabbit than control rabbit 1.068± 0.05 gm/dl at $p \leq 0.01$ **(Table 20** and **Figure 26).**

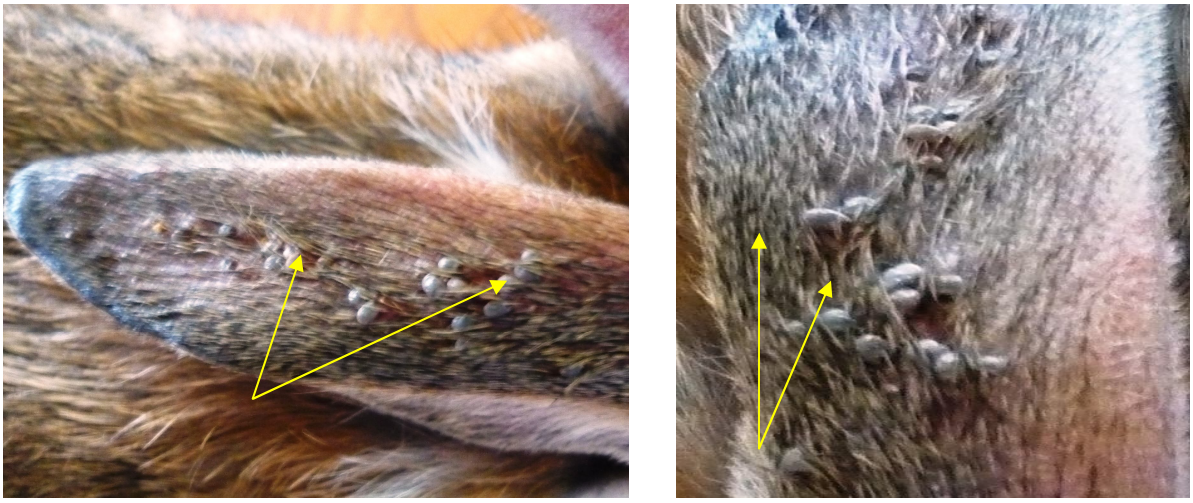
Table (20): The level of immunoglobulin type-G (IgG) in control and immunized rabbits (gm/dl).

Experimental rabbits	Immunoglobulin concentration (IgG) gm/dl
	Mean ± SE
Control	1.068± 0.05
Immunized rabbit	2.76 **± 0.22
t (18) = 2.87 0.01	

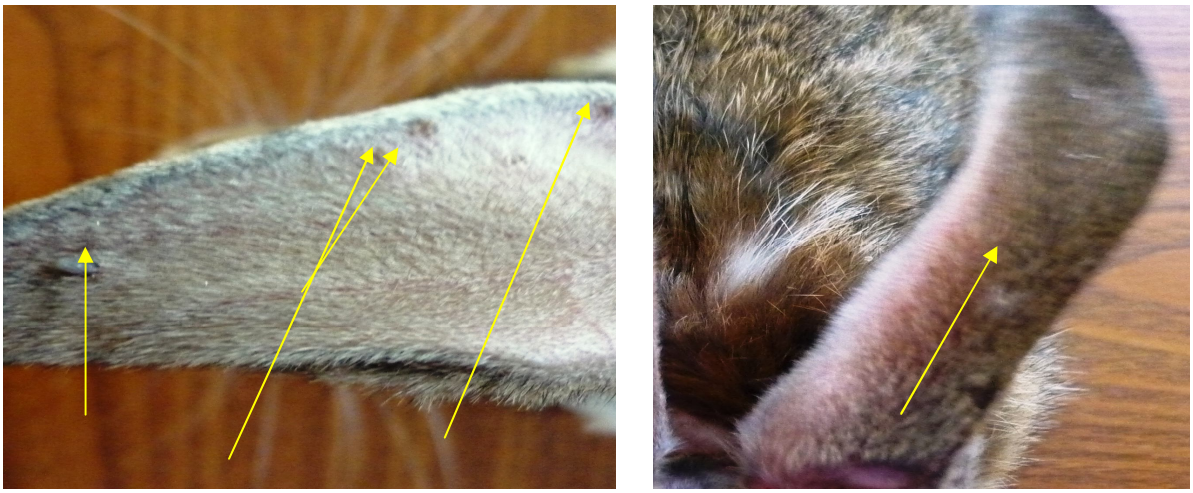
Figure (26): The Precipitated ring (IgG concentration); the above numbers 1, 2, 3, 4, and 5 shows the control and the number 6, 7, 8, 9, and 10 show the immunized rabbits.

6. CHALLENGE OF LARVAL INFESTATIONS:

During the challenge of larvae infestation, all rabbits terribly attempted to groom the infested ear-pinnae, particularly in the early stages of feeding and when the ear-bags removed after 3 days showed congestion, papules, edema and small abscesses have been developed on the infested region in post-immunized rabbits. In addition, totally fed-rejected and abnormality dropped larvae onto ear-bags, individual engorged larvae were noted and number of larvae were dead in post-immunized rabbits, while in non-immunized rabbits, high numbers of engorged larvae were still sucking blood, low number of fed-rejected and abnormality dropped larvae on to ear-bags were present **(Figure 27; A** and **B)**.



A



B

Figure (27; A, B): Tick challenge with *Hyalomma anatolicum anatolicum* larvae; A: In control rabbit, highest feeding larvae (engorged) in ear site. B: In immunized rabbit, the lowest of larvae attached, Papules and hemorrhage have been developed at the site of attachment.

CHAPTER FIVE
DISCUSSION

In this study the survey of ticks was carried out in Sulaimani governorate to show the prevalence of tick infestation in sheep, and it was observed the rate of 11.8% in all three geographic zones (zone-I, II, III), but the rate differed in the three zones 2.0, 2.2 and 3.1, respectively. Four species of ticks were observed; *Hyalomma a. anatolicum* 608(51.9%), *Hyalomma marginatum* 122(10.4%), *Rhipicephalus turanicus* 351(30%), and *Rhipicephalus sanguineus* 90(7.7%). *H. a. anatolicum* was common and dominant species collected among infested sheep in the foothills and plane areas zone-III followed by *Rhipicephalus turanicus* in the mountainous area zone-I.

Twelve tick's species were identified in three different zones in Iraq among reptiles, bird and mammals. *Rhipicephalus turanicus, Hvalomma anatolicum anatolicum* and *Hyalomma* nymphs are widespread. Thus, in mixed infestation, *Rhipicephalus turanicus* and *Hyalomma anatolicum anatolicum* were most dominant. Hyalomma nymphs infested mainly wild birds and hares **(Shamsuddin and Mohammad, 1988). Hawa *et al.* (2000)** observed that the *Hyalomma a. anatolicum* is the highly and widely distributed ixodidae tick, incriminating Iraqi livestock and causing substantial economic loss to livestock development worldwide. *H. a. anatolicum* is adapted to conditions in dry areas where a constant supply of hosts may not be available. Prevalence rate of ticks was 25.4% in sheep in Turkey **(Yukari and Umur, 2002). Razmi *et al.* (2003)** demonstrated five ixodid species from sheep and goats, the *Rhipicephalus sanguineus* and *Hyalomma marginatum*, were the most common species in sheep and goats. Other tick species encountered were *H. a. anatolicum*, *H. asiaticum* in sheep in Iran. **Esmail and Pour (2005)** from in Iran, showed that the major numbers of ticks were *Rhipicephalus* and *Hyalomma* among sheep and goat. **Radfar (2005)** showed that the major kinds of ticks are *Hyalomma* species in domestic ruminants in Iran**. Yakhchali and Hosseine (2006)** identified the highest number of adult tick collected, *Rhipicephalus bursa* (90.7% of sheep and 88.8% of goats) followed by *R. sanguineus* (6.9%), *Boophilus annulatus* (2.4%), Ixodid tick distributions per animal were 2.5 for sheep and 4.3 for goats in Urmia suburb, Iran. **Nabian *et al.* (2007)** observed the highest prevalence of *R. songuineus* in the sheep in Mazandaran province **Nabian *et al.* (2009)** indicated that the *Hyalomma*

a. anatolicum, H. asiaticum, H. marginatum and *H. detritum* were present in all zones when studied the distribution of *Hyalomma spp* on domestic animals in four zoogeographical zones in Iran, while **Abadi *et al*. (2010)** identified seven species of Ixodidae, and showed that the *Hyalomma spp* were higher population frequencies than other species and high number of *Rhipicephalus sanguineus* collected on sheep and goats 11.84% and no detected on other hosts (Cow and Camel) in Yazd province, Iran. **Nasiri *et al.* (2010)** identified two genera (*Hyalomma* and *Haemaphysalis*) and five species including *Hyalomma marginatum* (44.67%), *H. anatolicum* (43.17%), *H. asiaticum* (6.37%), *H. dromdareii* (5.55%), *Haemaphysalis sulcata* (0.24%) and the rate of tick frequency in mountainous region was 48.15% and it was 51.85% in plateau regions and the rate of tick infestation in sheep was 11.41% in Abdanan, Iran.

Sixteen species of ticks were recorded infesting sheep and goats in Pakistan**.** The most prevalent species of ticks include *Hyalomma a. anatolicum* and *Rhipicephalus turanicus* **(Hussain and Kumar, 1985).** In Pakistan **(Khan, 1993)** found that the sheep and goat were infested with *Rhipicephalus sanguineus*. **Sajid *et al*. (2008)** determined that the *Hyalomma anatolicum* was the most abundant followed by *Rhipicephalus sanguineus*. In caprines, the prevalence of *H. anatolicum* and *R. sanguineus* was 42.7% (309/723) and 37.6% (272/723), respectively. Mixed infestation of both tick species was found in 33.5% (264/789) of infested animals in Pakistan.

The number of ticks per infested sheep in zone I, II and III were (3.5, 3.9, and 4.2) respectively during the study according to the month or season, this difference occurred may be due to change of environmental condition, and showed that the higher number of ticks per infested sheep was in May (4.1, 4.7 and 5.0) in zone-I, II and III, respectively, and indicated that ticks were present on sheep during every month of the year but there was a reduction in the number of ticks per sheep during the dry season.

Robson and Robb (1967) described that in June and August the overall average of 29.8 ticks per infested animal is comparatively heavy compared to the 8.3 ticks per infested animal in 4 Liwas of Baghdad, Kut, Amara, and Basra in March and April in 1965, Iraq. **Robson *et al.* (1968a)** found that the average number of ticks per infested animal was 31.3 in June and August in 4 Liwas of Hilla, Karbala, Diwaniya and Nasiriya in Iraq. **Ahmed *et al.* (2005)** found the mean number of ticks on infested

sheep was 11.2 per sheep in River Nile province of northern Suden. The mean number of ticks on each animal ranged between 10-20 ticks per animal in Iran **(Rahbari *et al.*, 2007).** The distribution of tick species influenced by the seasons/month during the year, showed fluctuations in the occurrences and observed the number of ticks from March to July in 2009 – February, 2010 immediately following the beginning of rainy season, such as the number of *Hyalomma a. anatolicum* which was high in March, April, May, and June, *Rhipcephalus sanguineus* is highly distributed in April, May, and June and *Hyalomma marginatum* was highly distributed in April and May in zone-III (Garmian region). The number of *Rhipicephalus turanicus* was highly distributed in April and May in zone-I (Pishder region), and showed the highest number of *Hyalomma spp*. collected in spring and summer in zone-II and zone-III, while the highest number of *Rhipicephalus turanicus* collected in zone-I, this difference may be due to the environmental conditions in both seasons which are favorable to growth and development.

The main spring season was an optimum suitable environment for this distribution (suitable for activity of ticks, growth, development and reproduction in all zones in Sulaimani governorate), while the number of ticks decreased significantly in either fall and winter season, and it was found that the main role of this differences among the zones according to the humidity, temperature and rainfall, i.e. the relation of humidity in spring season reached up to 88%, 89%, and 83% in zone-I, II and III, respectively, when temperature was favorable (25°C, 26°C, and 28 °C) in zone-I, II, and III respectively with exist the rainfall in the season, may be suitable for the growth and development of tick distribution

In summer high temperatures (39°C) in zone-I, (42°C) in zone-II and (33°C) in zone-III were unfavorable for growth and combined with insufficient relative humidity which reached up to 64%, 28%, and 22% in zone-I, zone-II, and zone-III respectively with absent rainfall in all zones. While the relative humidity of fall reached to a favorable range of tick growth but combined with a high temperature of 30°C, 32°C, and 35°C in zone-I,II, and III respectively, which are showed the unfavorable conditions for tick development and growth, that will explain the low number of tick infestation among zones.

In winter the relative humidity with 96%, 96%, and 92% in zone-I, II, and III and lower temperature 16°C and 19°C in zone-I and II and absent of ticks, while few number of ticks appeared in zone-III, because the relative humidity (93%) and temperature (22°C) was favorable for growth of ticks. **Yakhchali and Hosseine (2006)** demonstrated that the Ixodid ticks were present on the animals throughout the year, being most abundant in summer and the least in fall, seasonal fluctuation of ticks was also determined in Kayseri region of Turkey. *Rhipicephalus* species were generally found in spring, while others like *Hyalomma* were found in late spring, summer, and early autumn in the Kayseri region of Turkey **(Ica *et al.,* 2007)**. Tick infestation was higher in late summer and lower in winter. The effect of age, status of body condition and post treatment effect of acaricides was found with no-significant difference **(Manan *et al.,* 2007)**. **Abadi *et al.* (2010)** revealed that the high prevalent of ticks occurrence during summer and spring, and the density and activity of ticks in winter was low. The highest seasonal activity was observed in spring and the lowest seasonal was in winter **(Nasiri *et al.,* 2010)**.

Khan (1967) found the seasonal activity of *Hyalomma* ticks was prevalent during June, July and August (summer months) and absent during December, January and February (winter months). **Walker *et al.* (2003)** demonstrated that all stages of *Hyalomma a. anatolicum* is active throughout the year; It is adapted to conditions in dry area and feeding activity is restricted mainly to the summer in areas with a distinct winter season but it may be active along the year. It has a great diversity of diapauses mechanisms which regulate its seasonal activity and developmental rhythms in cold climates, *Hyalomma marginatum* adults are present on animals between March and November with a peak of activity in Spring (April to May), while the adults of *Rhipicephalus turanicus* generally are most numerous during the late rainy to early dry seasons, all stages of *Rhipicephalus sanguineus* are found on dogs from October to May. **Rehman *et al.* (2004)** observed that the density of ticks were recorded in the months of August, September, and October, when the temperature was 27°C and relative humidity 84%.

Daniel *et al. (*2003)** emphasized that the temperature had relationship with the prevalence of tick infestation**.** The temperature during the summer months (May, June and July) was suitable for the activity of ticks, their growth, development and reproduction and the infestation rates were higher in summer months as compared to winter and spring seasons **(Jouda *et al.,* 2004**).

It was found that the rainfall was a main climatic factor which influences the seasonal variation in tick infestation as from June to November, the rainfall and humidity were low, and there was sharp reduction in number of ticks. This finding is in agreement with **(Pegram *et al.,* 1982).** Ixodid ticks are very sensitive to desiccation and cannot survive relative humidities of less than 80% for any length of time **(Kahl and Knulle, 1988). Jain and Jain (2006)** showed that the genus *Hyalomma* is tough and hard and can survive in low humidity and extreme climatic conditions.

Rainfall and the directly related relative humidity are the main climatic factors influencing tick distribution and activity **(Kebede, 2004). Latha *et al.* (2004)** found that the rainy season had high influence on ticks' infestation on sheep and goat in India. Changes in climate and in the length of the different seasons will directly affect survival, activity, and development of ticks, changes in development rates will make tick collection available to different diapauses windows (largely determined by day length), thus changing patterns of seasonal activity **(Gray *et al.,* 2009)**.

Climate change may extend or curtail host-seeking tick activity periods, potentially increasing or decreasing tick abundance and distribution, and effects on tick development rates can change seasonal activity patterns by altering the proportion of the tick population that are exposed to regulatory mechanisms such as diapauses **(Gray *et al.,* 2009).** Tick biology and ecology are under direct influence of climate factors, such as temperature and humidity **(Torres, 2010).**

Both the questing and developing stages are sensitive to desiccation and require a relative humidity of at least 80% throughout the year**,** abundance of host-seeking ticks is strongly influenced by the seasonal availability of suitable hosts, diapause in Ixodid ticks can occur at several different life cycle stages and may manifest in different ways and occurrence when environmental conditions are unsuitable for host seeking **(Belozerov, 1982)**. The diapauses mechanisms seem to enable the ticks to avoid entering host-seeking phases at unfavorable times of the year, such as high summer

and midwinter, areas which have hot summer and cold winter are most likely to show both forms of diapause in the majority of the population. They help to explain the extremely flexible life cycle of these ticks, which may take 2-6 years for completion and also the highly variable patterns of seasonal activity **(Gray, 1991).** Diapauses development should consider numerous interacting and constantly changing variables, such as reactions of organisms and seasonal changing environmental factors **(Berkvens *et al.*, 1994)**.

In this study, the ratio of male to female was 1:2 and the females were dominant in number, percentage of female 70.6% was higher than male 29.4% in all species of ticks and showed the highest number of females recorded, *Hyalomma a. anatolicum* and *Rhipicephalus turanicus*. **Gray *et al.* (2009)** observed that the adult females of *Hyalomma marginatum* were mainly introducted of feeding on wild and domestic ruminants via the Middle East and the Balkans**.**

Sex ratio calculated from field data can depend on the season and venue of collection, may shift the sex ratio of questing ticks in favor of females, particularly late in season of adult activity **(Yuval *et al.*, 1990).**

Fourie *et al.* (1996b) showed that the female was dominant in domestic stock and wild ungulates, except on adult on angora goats where the sex ratio was biased in favour of the males, the sex ratio is an important parameter which characterized the state and dynamics of natural populations of animals and the monthly variations in the sex ratio of the tick on hosts are believed to be related to the large fluctuations in sex ratios of questing ticks. **Filippova *et al.* (1976)** described that the larvae feed on small animals, such as the great gerbil, the nymphs also on small mammals and birds, and found the adults prefer cattle and sheep. The adult of ticks found on large hosts, wherease the immature stages infest hares and ground-frequenting birds **(Horak *et al.*, 1991)**. **Mekonnen *et al.* (2001)** described the number of male ticks which were equal to or exceeded females collected for each genus; *Amblyomm*a, *Hyalomma* and *Haemophysalis*, while in the genus of *Boophilus*, the females more than male number. **Ogorea *et al.* (1999)** found that the main attachment sites of fully engorged female of ixodid ticks were ears, head, body sides, perianal and scrotal/udder regions. Over the three sampling periods, 87% of the ticks counted were on the ears in sheep.

Regarding the oviposition, it was found that the times of engorged female with *Hyalomma a. anatolicum* was 27.11 ± 1.32 days and eggs hatching times (22.4 ± 2.26) days. **Snow and Arthur (1966)** concluded that largest eggs are laid from 0 to 48 hours (peak of egg production). **Snow (1969)** showed that elevation in ecological temperature had reduced oviposition period as the metabolic processes used to increase rapidly with an increment in temperature. **Ghosh *et al.* (1998)** found that the pre-oviposition of *Hyalomma a. anatolicum* was 12.09 ± 0.27 days, while, **Ochi, (2004)** observed that the pre-oviposition, oviposition and incubation period of eggs of *Hyalomm a. anatolicum* were 11.46 ± 0.72, 22.08 ± 0.24 and 27.51± 0.62 days respectively. This may be attributed to variations in temperature and relative humidity.

Also the site of attachment of ticks was observed; the maximum being in both genus (Hyalomma and Rhipicephalus) were collected during the year from ear of sheep 492 (42.02%), under tail 208(17.76%), followed by udder 139(11.9%), between thigh 112(9.56%), under axilla 105(8.97%), testes 95(8.1%) and the lowest was in eyelid 20(1.71%) and showed the *Hyalomma a. anatolicum* was highest infestation among species followed by *Rhipicephalus turanicus*, *R. sanguineus* and *H. marginatum* in each sites mentioned above. **Rehman *et al.* (2004)** showed the cow, goat and sheep were infested with maximum numbers of ticks in external ears.

Most ticks preferred the sites with the thinner skins and shorter hair, the infestation may vary with the tick species and with animal host e.g. that ear, neck and inside of thigh, the favored sites of attachment of *Hyalomma* in cattle. This tendency is suitable for ticks because it allows easy penetration of their mouth parts into the blood vessels for feeding **(Tatchell, 1987)**.

Fourie and Kok (1995) showed that the preferred site of attachment for *Hyalomma marginatum* on both Dorper and Merino sheep was the anogenital and inguinal areas (75-76%). On Dorper sheep, *Hyalomma truncatum* attached predominantly to the anogenital and inguinal areas (67.7%). On Merino sheep, most adult of this species attached to the feet (26%). Almost equal percentages also attached to the anogenital/inguinal and brisket areas (21.6% and 22.9%), respectively.

Hyalomma anatolicum ticks have long hypostome which was found in more numbers in the proximal portions of the ears, eyelids, lips, where as the adults were mostly found in the inguinal region **(Vathsala *et al*., 2007**). Rhipicephalus ticks have

short hypostome and attach more superficially (particularly on ears) in comparison with other ticks e.g., most species of *Amblyomma* and *Ixodes*, can attach firmly to the host skin **(Torres, 2010)**

In this study the clinical manifestation were more severe in sheep infested with *Hyalomma* spp. than *Rhipicephalus spp.,* these included anemia, anorexia, emaciation and skin problem. This may be due to the Hyalomma (male and female) possess long mouth parts (proboscis) deeper into the feeding sites, reaching more vascularized areas and consequently causing more damage to the host tissues, this agreed with **(Latif *et al.*, 1990). Sutherst *et al.* (1979)** indicated that the ticks can be harmful to livestock and causes direct effects include irritation and allergy, udder wounds, myiasis, sever toxicosis, and having long and massive hypostoma (*Amblyomma* and *Hyalomma*) may induce abscesses due to secondary bacterial infection and some short hypostome ticks (*Boophilus* and *Rhipicephalus*) when present in large amounts may also cause devaluation of hides due to tick bites **(Jongejan and Uilenberg, 2004**).

The hematological findings in sheep highly infested with *Hyalomma spp.* were significantly different in white blood cells(WBC), packed cell volume(PCV%), and hemoglobin concentration(HgC g/dl) than *Rhipicephalus spp.* and significantly only in mean corpuscular hemoglobin (MCV) and indicated that the normocytic normochromic type of anemia due to infestation of *Hyalomma spp*. and *Rhipicephalus spp*. but more severly occurred in *Hyalomma spp*. than *Rhipicephalus spp*., while the **Tyler and Cowell (1996)** and **Pfaffle *et al.* (2009)** classified the type of anemia as macrocytic normochromic depending on values of (MCV), (MCHC) to lesser extent on (MCH) values in tick infestation.

The immunity status of the body in rabbits against whole crude extract were studied, in the present study, by the total number of white blood cells and differential leukocyte percentage values in immunized rabbits were compared with control rabbits, some blood parameters were significantly higher in immunized rabbits than control rabbits, such as total white blood cells, monocyte and neutrophil, while the lymphocytes, esonophil and basophil were significantly lower than in control group. This may be due to migration from blood stream to biting site of tick lesion causes degranulation and produced histamine. It has been shown that the skin reaction at the

attachment site on resistance response appears as cutaneous basophil hypersensitivity reactions **(Allen, 1973)**, and decreased lymphocytes with an increase in neutrophils can be an indication of an inflammatory or an immune response due to pathogen infection **(Thomas, 2007).** More degranulated mast and eosenophil cells were observed after the third day of tick infested with *Ixodes ricinus* **(Brossaed and Fivaz, 1982),** and the intensity of the inflammatory response appeared to be more dependent on the quantity of antigenic substances introduced rather than to the size and insertion of mouth parts of the ixodides (**Latif *et al.,* 1990).**

Phagocytosis is a vital biological process in elimination of a foreign agent from the body, revealing a non-specific cell-mediated immune reaction; accordingly, the mean phagocytic index (PI) in immunized rabbit (7.2 ± 0.61) was significantly higher than control rabbits (1.8 ± 0.24). The phagocytic activity among the immunized animals may be attributed to increase of their nutrophils percentage

In this study the delayed-type hypersensitivity reactions on the skin was done and when inoculated (I/d) with 0.05 mg/ml of whole crude larvae antigen of *Hyalomma a. anatolicum* after three weeks increased thickness in immunized rabbits at 4 hrs and reached to peak at 24 hrs and decreased at 36 hrs to 96 hrs a, and recovery to normal in 7 days. While no significant changed areas were noted on the skin and skin fold thickness were compared to the immunized rabbits inoculated with normal saline. This may be due to the antibodies remained in immunized rabbits serum more than one month, this phenomena was shown by **Allen (1973)** who demonstrated that guinea-pigs infested with larval tick of *Dermacenter andersoni* had exhibited three month duration of antibodies in their sera. **David and David (1972)** observed that the ability of sensitized T-cells to produce migration inhibitory factor (MIF) could be used in assessing delayed-type hypersensitivity reactions. **Ellenberger *et al.* (1984)** found that the thickening and the reddening of skin in immunized rabbits are attributed to vasodilation that causes increase capillary permeability and local influx of mononuclear cells at the site of inoculation.

Cutaneous hypersensitivity reaction involved basophils accumulation at tick attachment sites in the skin of resistant animals and degranulated in response to tick salivary antigens, releasing histamine and other mediators. The mediators may directly cause ticks to cease salivating and feeding and then to detach, or they may induce

reflex grooming reactions by the host, leading to the removal of ticks from the itching skin **(Allen, 1989).** The immediate skin hypersensivity had been a potency reaction in immunized and in repeated experimental infection against crude salivary extract antigens of the ticks, this is due to heavy circulatory memory cells included immunoglobulin IgG type **(Tizard, 2004).**

Cutaneous hypersensitivity test was used to know host resistance to ticks and type of reaction (cellular immunity) to unfed larval extract. numerous physiologically active agents are injected by the parasite into the feeding lesion inducing strong inflammatory, vasodilatory and immunological responses by the host **(Krober and Guerin, 2007)**. Tick infestation induce to a complex variety of immune responses, involving antigen presenter cells, T lymphocytes, B lymphocytes, antibodies, cytokins, complement, basophils, mastocytes, eosinophils, and number of bioactive molecules **(Brossard and Wikel, 2004**). Anaphylatoxins produced by the complement activation and the histamine originated from leukocyte degranulation at the tick fixation site are responsible for the increased permeability of vascular endothelium. As a consequence, there will be local edema and serum exudation (**Andreotti *et al.,* 2002b**). Three high molecular weight portions isolated from saliva gave immediate hypersensitivity reactions in intradermal inoculation into rabbits which had previously been exposed to ticks **(Gill *et al.,* 2007). Brown (1984)** found that young guinea-pig expressed significantly greater level of acquired resistance to challenge by larval *Amblyomma americanum* ticks than older guinea-pigs.

Cutaneous reactions at tick attachment sites on cattle and laboratory animals expressing acquired immunity contain infiltrates of basophils and eosinophils **(Brossard and Fivaz, 1982)**, this type of reaction is termed cutaneous basophil hypersensitivity, which is a form of delayed type hypersensitivity mediated by Th1 cells.

Qualitative and semi-quantitive estimation of immunoglobulin concentration by several screening tests for estimation of immunoglobulin concentration are available. These tests can be performed in clinical practice and they can provide qualitative or semi-quantitative estimates of immunoglobulin concentration by using total protein, sodium sulfite precipitation test and glutaraldehyde coagulation test **(Mary *et al.,* 2004).**

The sodium sulfite precipitation test is based on the fact that immunoglobulins can be selectively precipitated from serum using concentration of a hydrous sodium sulfite ranging from 14%, 16% and 18%. A hydrous sodium sulfite concentration is required to cause precipitated in serum containing lower immunoglobulin concentration undergoing precipitation when mixed with a sodium sulfite with low concentration e.g. 14%, whereas sera with low Ig concentration do not undergo precipitation when mixed with same solution of sodium sulfite. The latter sera may undergo precipitation when mixed with sodium sulfite solution of higher concentration e.g. 16%-18% depending on the Ig concentration of the serum **(Mary *et al.*, 2004).**

The glutaraldehyde coagulation test is based on the fact that at low concentration, glutaraldehyde forms soluble complex with immunoglobulin, there by resulting in coagulation of the test mixture. The glutaraldehyde solution not form coagulation in sera with Ig concentrate of less than 400mg/dl and complete or partial coagulation in sera with Ig concentration of greater than 600mg/dl.

The values of total serum protein, albumin and globulin in post-immunized rabbits were significantly higher than pre-immunized ones, after third inoculated with whole crude larvae of *Hyalomma a. anatolicum*, **Rechav *et al.* (1989)** also concluded that the concentration of serum beta globulins increased only in Guinea pigs infested with immature ticks for the entire larval and nymphal feeding period.

Rechav *et al.* (1994) found the concentration of globulins, was highest in the Guinea-pigs exposed to medium and high numbers of ticks with long infestation intervals.

Banerjee *et al.* (1990) revealed that the immunized calve with *Hyalomma anatolicum* showed a significant increase in the level of serum gamma globulin. **Njau and Nindo (1987)** detected that the highest humoral antibody activity after the third tick infestation. **Kumar and Kumar (1995)** showed that the immunized rabbits had a significant reduction in tick yield weight and reduced feeding and reproductive performance of the ticks, when inoculated subcutaneously on days 0, 14 day 21 (three times) with a dose of 1 mg antigen (gut supernatant) per rabbit.

Determination of the lgG by using radial immunodiffusion plate executed in control and immunized rabbits in the study, showed that immunoglobulin concentration in immunized rabbits was significantly higher 2.758 ± 0.22, than control

rabbits 1.068 ± 0.05 when inoculated intradermally with 6.8 mg protein (whole crude larval extract). Immunoglobulins are major components of host serum, and they can cross the tick gut and react with tissues in the haemocoel (**Allen and Humphreys, 1979).**

Brown (1982) noticed that lgG1 antibodies are responsible for the ability of immune serum to transfer cutaneous basophile-associated immune resistance against tick feeding in Guinea pigs. Increase of IgG antibody titre following successive tick infestations (**Gill and Luckins, 1987**). **Njau and Nindo (1987)** showed that the maximum humoral antibody activity was detected after the third infestation in rabbits, slightly reduction in antibody activity occurred in the hosts during a tick period of 24 days after the third challenge. **Rechav *et al.* (1989)** concluded that the concentration of serum beta globulins increased only in Guinea pigs infested with immature ticks for the entire larval and nymphal feeding period.

Ghosh *et al.* (1999) observed that the unfed larvae *Hyalomma a. anatolicum* ticks provide a relatively easily available source of antigen for immunization of cattle against both larvae and nymphs. Larval antigen immunized rabbits showed significant antibody level from 28-126 days while with homogenous nymph antigen elevated antibody levels were recorded up to 112 days **(Ghosh *et al.,* 1998)**.

Kebede (2004) showed the highest reduction of the number of the ticks engorging and ability of the female ticks to lay eggs, when the female were feeding on the vaccinated cattle leads to uptake of antibodies and other components of the host's humoral immune system, resulting in damage of the gut. **Nikpay *et al.* (2008)** observed increased antibody level in calves at one week and reached in a peak at eight weeks post infection, then decreased in nine weeks, which occurred against larval body antigen. **Ali *et al.* (2009)** revealed that the antibody level in vaccinated buffalo as well as in vaccinated rabbits with whole *Hyalomma* tick reached to peak level on day 45 post inoculation and started declining thereafter, and revealed that the hard tick homogenate vaccine with more than 7.5 mg protein per dose is effective in inducing antibodies in the dairy animals**. Ochi (2004)** showed significant elevation in the antibody titers in the sera of the immunized rabbits compared with control.

In this study, the challenge of larvae infestation with *Hyalomma a. anatolicum* in both experimental rabbits (control and immunized rabbits) using whole crude

extract of larvae, showed that all rabbits attempted to groom the infested ear-pinnae, particularly in the early stages of feeding. When removed the ear-bags after three days observed congestion, papules and edema in the site of the infested area, this may be explained by the cellular infiltration at the attachment site and showed the individual engorged, abnormality fed-rejected and highest number of larvae were dead on to ear-bags in post-immunized rabbits. In pre-immunized (control) rabbits high number of engorged larvae still sucking blood, low number of fed-rejected and abnormality dropped larvae to ear-bags were presented. The abnormal rejection of larvae may be due to their inability to gain entrance to blood vessels as a result of the host immunological reactions. **Brown (1982)** found that the transfer of peritoneal exudates cell from immunized or immune serum to naive Guinea-pigs resulted in significant rejection of larval and adult (35.1%) of *Amblyomma americanum* tick associated with cutaneous basophil responses indicating humoral and cell-mediated immune response.

The antigen is located on the surface of the digested cells, which line the ticks gut. The feeding tick takes in antibody to Bm86. Binding of antibody to the protein on the tick gut leads eventually to lysis of the tick's gut cells **(Tellam *et al.*, 2002).** Antibodies carry out a variety of functions including neutralization, complement fixation (classical pathway), and facilitating leukocyte interaction with target cells including microbes (**Brossard and Wikel, 2004)**

The another mechanism after feeding and dropping from the host, the digestion of hemoglobin in the gut proceeded and completed by the 13th day, about 10% of the hematin is released from the hemoglobin and transferred to the eggs and subsequently larvae, the hematin converted in the gut to hemoprotein (ferrous conjugated with protein) called hemixodovin which differs from hemoglobin obtained from the host. the role of host immunity is to prevent binding the hematin with gut protein and subsequently increase the level of free hematin (non-conjugated) leading to persistent of the female long time of feeding or dropped off readily before complete growing (low body weight) and produced low number of immature eggs **(Vaz *et al.*, 1996).** Immune mechanism may further reduce feeding success by enhancing inflammatory reactions **(Wikel and Allen, 1982).** Edema reduces blood flow or induces blood formation, as in the skin of cattle resistant to *Boophilus miroplus*, but not in susceptible animals (**Tatchell** and **Moorhouse, 1968).**

Ochi (2004) revealed that larval antigens are more effective agent than the nymphal antigens against the larvae, nymphs and adults of the homologous ticks, because the unfed tick instars constitutes much more tissues than those of the nymphs partly occupied by blood. This can eventually be reflected on the quantity of the protective protein antigen. **Brossard and Wikel (2004**) found that after the third infestation, the number of engorged larvae in rabbits was significantly reduced due to the development of immune response of the challenged animals**.**

High protective effect against tick infestation using purified 37 kDa larval antigen **(Das *et al.,* 2005)** and demonstrated that the larvae of *Hyalomma a. anato/icum* are an important source of biological material for isolation of protective antigens. Only the larval extract of *Hyalomma anatolicum* showed significant protection against ticks challenge in immunized rabbits. This may be due to the presence of higher immunoprotective antigen concentrations in larval extract **(Moshaveri-nia *et al.,* 2008).** Three infestations of rabbits with adult of *Hyalomma dromedarii* induced significant immunity expressed as an inhibition of fertility of the ticks and laying fewer eggs than ticks fed on non immune animals (control) (**Habeeb *et al.*, 2009).**

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions:

Relying on the experimental results of this study, the following conclusions can be emerged:

1- Four species of ticks belonged to two genera, *Hyalomma anatolicum anatolicum*, *H. marginatum, Rhipicephalus turanicus* and *R. sanguinus* were observed from different zones of the studied areas.

2- All species were distributed more in March, April, May and June. This related to humidity and ambient temperature.

3- The pathological observations (clinical observation and hematological) were severely obvious among sheep infested with *Hyalomma spp.*

4- The type of anemia in sheep infested with *Hyalomma spp*. and *Rhipicephalus spp.* was normocytic normochromic anemia.

5- All observed tick were attached to the uncovered body sites with wool of sheep.

6- No Larval and Nymphal stages were observed in this study. This might be that there species were belonged to more than one host tick.

7- Whole crude larval extract of *Hyalomma anatolicum anatolicum* antigen can display a good immune stimulant for inducing cellular and humoral antibodies in immunized rabbits.

RECOMMENDATIONS

B. Recommendations:

According to previously mentioned conclusions, the following points of view can be recommended:

1- Strategic tick control should be designed by taking in to account the seasonal dynamics of tick infestation.

2- Similar immunological studies should be carried out on sheep and goats.

3- Comparative studies between biological methods (genetic mutation) and by using the chemical acaricide for control of tick.

4- Trial to obtain specific gene from tick for vaccine preparation against tick infestation.

5- Preparation of the different crude extracts of ticks body (Salivary gland or Malpighian tubules) as trials for antigens sources and using it for immunization.

6- More specific method for identifying monoclonal antibody of IgM and IgG class against tick integens using ELISA.

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APPENDIXES

Instruments and equipment

- Arçelk, 2031D, Turkey.
- Autoclave, Lab Tech, Korea.
- Biohit, mechanical pipettors, 50-100 µl, 100-1000 µl, 1000-5000 µl, Finland.
- Biolabo reagents kits, albumin BCG method, France.
- Biovision microscope- 103B. Altay, China.
- Dissecting microscope compound, digital, Motic education, China.
- Dissecting microscope, Motic-Education, China.
- Electric shaving machine, Wahl, Hungary.
- Electric shaving machine, Wahl, Hungary.
- Electronic analytical balance, Mettler-Toledo, Switzerland.
- Haemometer, Marienfeld/ Sahli, Germany.
- Hemocytometer, Germany.
- Hot plate magnetic stirrer, Bibby, UK.
- Humidity-Temperature meter, Concord-France.
- Incubator, WTC-binder, Germany.
- Micro-Hematocrit centrifuge, 1200rpm, Taiwan.
- Peptone broth, Hi. Media, India
- Radial immunodiffusion plates, IgG RID, Italy.
- Spectrophotometer, Apel, PD- 303, Japan.
- Speed centrifuge, Triup international group, Italy.
- UV/ Visible-Spectrophotometer, Eppendorf, bioPhotometer, Germany.
- Vernier, Electronic digital calliper, Lezaco- 0-150mm, China.
- Water-bath, Daihan. Lab. Tech. Co.Ltd, Korea.

Appendix (1): Conversion table (diameter of precipitated rinrelated to concentration of IgG mg/dl (IgG-RID plate kits)

DIAMETER (mm)	mg/dl*	DIAMETER	mg/dl*
4	175,4	7,6	1594,7
4,1	202,9	7,7	1646,6
4,2	231,2	7,8	1699,3
4,3	260,0	7,9	1752,7
4,4	289,6	8	1806,7
4,5	319,9	8,1	1861,4
4,6	350,8	8,2	1916,8
4,7	382,4	8,3	1972,9
4,8	414,7	8,4	2029,7
4,9	447,6	8,5	2087,1
5	481,3	8,6	2145,2
5,1	515,7	8,7	2204,0
5,2	550,7	8,8	2263,5
5,3	586,3	8,9	2323.6
5,4	622,7	9	2384,5
5,5	659,7	9,1	2446,0
5,6	697,4	9,2	2508,2
5,7	735,9	9,3	2571,0
5,8	774,9	9,4	2634,6
5,9	814,7	9,5	2698,8
6	855,1	9,6	2763,7
6,1	896,2	9,7	2829,3
6,2	938,0	9,8	2895,5
6,3	980,5	9,9	2962,5
6,4	1023,7	10	3030,1
6,5	1067,5	10,1	3098,5
6,6	1112,1	10,2	3167,5
6,7	1157,3	10,3	3237,2
6,8	1203,2	10,4	3307,5
6,9	1249,7	10,5	3378,5
7	1297	10,6	3450,2
7,1	1344,9	10,7	3522,6
7,2	1393,5	10,8	3595,7
7,3	1442,7	10,9	3669,5
7,4	1492,7	11	3743,8
7,5	1543,4	11,1	3818,9

IFCC: International Federation of Clinical Chemistry.

Appendix (2): Metrological data of the duration (March, 2009- February, 2010) of different zone in Sulaimani governorate.

Monthly	Zone- I			Zone- II			Zone- III		
	Average Max		Total	Average Max		Total	Average Max		Tot
	RH%	Temp/ C°	Rainfall/mm	RH%	Temp/ C°	Rainfall/mm	RH%	Temp /C°	Rainfall/ mm
March 2009	48.01 90	10.4 21.2	23.9	59 95	11.4 22.3	79	35.2 90	15.33 24.2	28.7
May 2009	53 92	14.7 25.7	16.3	40.8 95	15.3 25.7	78.8	39.7 95	18.8 28.1	26.1
April 2009	49 83	21.9 28.2	3.1	21.43 77.1	23.90 31.4	1.5	29 64.1	25.13 33.1	0
June 2009	27 77.2	24.2 35.8	0	13.58 31.1	29.25 40.6	0	18.9 24.1	32.4 41.9	0
July 2009	26 68.2	29.1 38.2	0	12.2 28	30.2 41.2	0	16.3 21.9	33.1 43	0
August 2009	21.4 46.3	32.9 40	0	12 24.8	32.4 43.2	0	146. 18.4	35.9 44.6	0
September 2009	55 87	27.9 36.8	6	22.8 85	29.1 37.2	11.3	21.22 82	31.3 40.4	3.2
October 2009	56.7 88.2	21.8 32.3	2.5	26.19 98	22.23 33.1	14	25.04 90	26.2 35.2	13.5
November 2009	71.2 98	12.6 24.6	167	63.9 95	12.7 24.1	246	68 98	19.1 29.1	155.5
December 2009	67.3 93.2	8.7 13.2	29.4	72.5 95	9.3 17.3	44.2	36.41 88.5	16.9 25.1	32.7
January 2010	74.4 98	6.8 15.2	256.4	60 95	9.6 18.2	149.8	57.1 92	10.5 16.3	23.3
February 2010	73.2 98	8.9 19.3	101.9	64.4 98	10.1 20.7	187.7	56 97	13.13 22-6.1	118.2
Total			605.6			812.3			401.2

Appendix (3): Distribution of tick species and sex according to months and seasons in different zones.

Months	Zone-I								Zone-II								Zone-III							
	Rhipicephalus. spp				*Hyalomma.* spp				*Rhipicephalus.* spp				*Hyalomma.* spp				*Rhipicephalus.*spp				*Hyalomma.* spp			
	R. s		*R. t*		*H. a*		*H. m*		*R. s*		*R. t*		*H. a*		*H. m*		*R. s*		*R. t*		*H. a*		*H.m*	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
March	0	2	8	15	2	3	0	2	0	3	5	7	5	17	0	4	1	3	1	9	17	44	3	3
April	1	2	11	37	2	7	1	6	1	5	12	26	9	27	1	6	4	7	3	8	23	66	5	13
May	2	4	19	38	6	9	3	6	2	2	11	22	14	32	4	11	4	9	6	7	19	73	4	12
Total/ Spring	3	8	38	90	10	19	4	14	3	10	28	55	28	76	5	21	9	19	10	24	59	183	12	28
June	3	4	5	19	4	11	3	6	0	4	8	17	14	27	2	5	3	7	0	3	13	37	3	1
July	0	2	3	13	1	4	2	4	0	1	7	5	13	19	1	0	1	4	0	3	4	14	4	2
August	1	0	2	7	1	4	0	1	0	1	2	0	4	4	0	0	1	0	2	0	6	8	4	0
Total/ Summer	4	6	10	39	6	19	5	11	0	6	17	22	31	50	3	5	5	11	2	6	23	59	11	3
September	1	0	0	0	1	7	0	0	1	0	1	0	4	0	0	0	0	0	1	0	3	1	0	0
October	1	0	0	0	1	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0	3	0	0	0
November	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Total/ Autumn	2	0	0	0	2	8	0	0	1	0	2	0	6	0	0	0	0	0	1	0	7	1	0	0
December	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
January	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
February	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	5	5	16	0	0
Total/ Winter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	5	5	16	0	0
Total. Overall	9	14	48	129	18	46	9	25	4	16	47	77	65	126	8	26	14	33	15	35	94	259	23	31
χ^2 cal. P<0.01	281.6 **																							

R. s = Rhipicephalus sanguineus; R. t = Rhipicephalus turanicus; H. a.a = Hyalomma anatolicum anatolicum; H. m = Hyalomma marginau

Appendix (4): Normal hemograms value in sheep Coles (1986).

Parameters	Units	Value
PVC (haematocrit).	%	27-45
Hemoglobin (HgC).	g/dl	9-15
Red Blood Cells (RBCs).	10^6/µl	9-15
White Blood Cells (WBCs).	10^3/µl	4-12
Mean corpuscular Volume (MCV).	Fl (femtoliter)	28-40
Mean Corpuscular Hemoglobin (MCH).	Pg (picogram)	8-12
Mean Corpuscular Hemoglobin Concentration (MCHC).	g/dl	31-34

Appendix (5; A and B): Evaluation for determination of serum immunoglobulin concentration

5A- Glutaraldehyde coagulation test reading:

Rabbit		Glutaraldehyde coagulation	Immunoglobulin concentration
Control	R1	Complete coagulation	More than 600 mg/dl
	R2	Complete coagulation	More than 600 mg/dl
	R3	Complete coagulation	More than 600 mg/dl
	R4	Semisolid coagulation	400-600 mg/dl
	R5	Complete coagulation	**More than 600 mg/dl**

Rabbit		Glutaraldehyde coagulation	Immunoglobulin concentration
Immunized rabbit	R1	Complete coagulation	More than 600 mg/dl
	R2	Complete coagulation	More than 600 mg/dl
	R3	Complete coagulation	More than 600 mg/dl
	R4	Complete coagulation	More than 600 mg/dl
	R5	Complete coagulation	More than 600 mg/dl
	R6	Complete coagulation	More than 600 mg/dl
	R7	Complete coagulation	More than 600 mg/dl
	R8	Complete coagulation	More than 600 mg/dl
	R9	Complete coagulation	More than 600 mg/dl
	R10	Complete coagulation	**More than 600 mg/dl**

5B-Sodium sulfite concentration test:-

Rabbit		Sodium sulfite concentration			Immunoglobulin concentration
		14%	16%	18%	
Control	R1	cloudiness	Flakes noted	Flakes noted	500- 1500 mg/dl
	R2	cloudiness	Flakes noted	Flakes noted	500- 1500 mg/dl
	R3	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R4	cloudiness	Flakes noted	Flakes noted	500- 1500 mg/dl
	R5	cloudiness	Flakes noted	Flakes noted	**500- 1500 mg/dl**

Rabbit		Sodium sulfite concentration			Immunoglobulin concentration
		14%	16%	18%	
Immunized rabbit	R1	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R2	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R3	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R4	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R5	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R6	Cloudiness	Flakes noted	Flakes noted	500- 1500 mg/dl
	R7	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R8	Flakes noted	Flakes noted	Flakes noted	> 1500 mg/dl
	R9	cloudiness	Flakes noted	Flakes noted	500- 1500 mg/dl
	R10	Flakes noted	Flakes noted	Flakes noted	**> 1500 mg/dl**

دراسة عن الوبائية القراد الصلب (Ixodidae) في الأغنام ومحاولة تمنيع الارانب ضد القراد الأناضولي الأناضولي *Hyalomma anatolicum anatolicum* في محافظة السليمانية-أقليم كردستان العراق.

أطروحة مقدمة الى

كلية الزراعة/ جامعة السليمانية

كجزء من متطلبات نيل درجة دكتوراة فلسفة

في العلوم الزراعية

الأنتاج الحيواني

(علم الحشـرات)

من قبل

بهـــزاد حمة صالـــح مصطفـــــى

بكالوريوس-الطب البيطري 1992م

ماجستير- صحة الحيوان 2006م

بأشراف

الأستاذ الدكتور محمد عبد العزيـز قـــادر

جماد الأول 1432 هـ **نيسان** 2011 م

الخلا صـــــــة

دراسة عن الوبائية القراد الصلب (Ixodidae) في الأغنام ومحاولة تمنيع الارانب ضد القراد الأناضولي الأناضولي *Hyalomma anatolicum anatolicum* في محافظة السليمانية- كردستان العراق

أجريت هذة الدراسة للفترة من أذار 2009 لغاية شباط 2010 في ثلاثة مناطق (1، 2 و3) مختلفة في محافظة السليمانية لبيان انتشار القراد(Ixodidae) في الأغنام المصابة. أن نسبة أنتشار الأصابة في الاغنام في المناطق الثلاثة كان 298 (11.8 %) في محافظة السليمانية، وكانت نسبة الاصابة في المنطقة الاولى (منطقة بشدر) 85 (10.1 %) وفي المنطقة الثانية (مركز السليمانية) 94 (1 .11 %) وفي المنطقة الثالثة (منطقة كرميان) 119 (3. 14 %). نسبة الاصابة كانت اعلى في الأشهر، أذار و نيسان ومايس و تموز في جميع المناطق. بينما لم تكن هناك اصابة بين شهري تشرين الثاني وشباط، ولكن ظهر الأصابة في المنطقة الثالثة. تم تشخيص جنسين *Hyalomma* و *Rhipicephalus* واربعة انواع من القراد. *Hyalomma marginatum*, *Hyalomma anatolicum anatolicum*, *Rhipicephalus turanicus*, *Rhipicephalus sanguineus*

كانت هناك نوعين أكثر شيوعا" ضمن الأغنام المصابة وهما *Hyalomma anatolicum anatolicum* في المنطقة الثالثة(منطقة كرميان) 353 (70 %) و *Rhipicephalus turanicus* في المنطقة الأولى(بشدر) 177 (4 ،59%).

وجدت النوع *H. a anatolicum* في شهر أذار 61(75.3%)، ونيسان 89 (69%) ومايس 92 (68.7%) وتموز50 (74.6%) بالتعاقب في المنطقة الثالثة (كرميان). وجدت ايضا" بان *H. a. anatolicum* و *R. turanicus* في شهر أذار 22 (53.6%)و12 (29.3%)، وفي شهر نيسان 36(41.4%) و38 (43.7%)، وفي شهر مايس 46 (46.9%) و 33(% 33.7)، وفي شهر تموز 41(53.2%) و25(% 32.5) في المنطقة الثانية. بينما *R. turanicus* كانت عالية الأنتشار في نيسان 48 (71.6%) وفي مايس 57 (65.5%) في المنطقة الأولى

بطريقة المعادلة الخطية ، النسبة المئوية اللأغنام المصابة في أي منطقة من المناطق الثلاثة مقارنة" مع عدد القراد كانت مميزة وكان عاليا" في المنطقة الثالثة.

Number of ticks(X) ×0.23 + 3.10 ولوحظ بان نسبة الأناث الى الذكور كان 1:2.

تم دراسة موقع وجود القراد والتصاقة بأ جسا م الأغنام، و اعلى نسبة للقراد كانت في الأذن 492(42.0 %) وتحت الذيل 208 (17.7 %). الأعراض السريرية كانت أكثر ضراوة في الأغنام المصابة بنوع *Hyalomma spp* من نوع *Rhipicephalus spp* والتي شملت فقر الدم، فقدان الشهية، المشاكل الجلدية ومنها خدوش في الجلد وفقدان المطاطية و الصلع و البقع الأحتقانية.

الفحوصات الدموية أظهرت وجود فروقات معنوية عند مستوى $p \leq 0.01$ للقيم الدموية لكل من الكريات الدم الحمراء الكلي ، خلايا الدم المرصوصة(المضغوطة) و تركيز الهيموكلوبين في الأصابات بجنس *Hyalomma spp.* عند مقارنتها مع *Rhipicephalus spp.* هيموكلوبين الخلايا الجزئي في الأغنام المصابة ب *Hyalomma spp.* اختلفت معنويا" عند مستوى $p \leq 0.05$ عند المقارنة بالأصابة ب *Rhipicephalus spp.* . العدد الكلي للكريات الدم البيضاء، حجم الخلايا الجزيئي ، تركيز هيموكلوبين الخلايا الجزئي لم تظهر اي فروق معنوية عند أجراء هذة المقايس لدى الأغنام المصابة بكلا الجنسين.

كما شملت الدراسة تربية الأناث المحتقنة بالدم من النوع *Hyalomma anatolicum anatolicum* في المختبر لبيان وقت وضع البيض وتطورالبرقات، حيث كانت الفترة (27 ± 1.32) يوم و (2.26 ± 22.4) يوم بالتعاقب. وتم استخدام اليرقة المفقسة لتحضير الخلاصة، وتم تحضير المصل الممنع في الأرانب بواسطة الحقن تحت الجلد ثلاث مرات بأستخدام مستخلص اليرقات الخام الكامل(WCL).

التفاعل المناعي الخلوي كان واضحا" ومعنويا" بزيادة معامل البلعمة ضد البكتريا *Staphylococcus aureus* في الأرانب الممنعة (0.61 ± 7.2).

ظهرت فرط الحساسية الجلديةالشديدة ومن النوع المتأخرفي الأرانب الممنعة عند حقنها بمستخلص اليرقات الخام الكامل(WCL). وتم قياسها بعد 2، 4 ، 6، 12، 24، 36، 48، 72، 96، ساعة و 7 أيام وجدت هناك فروق معنوية ($p \leq 0.01$) في زيادة تثخن الجلد

8.70± 0.64, 11.10± 0.26, 10.2± 0.38, 7.90± 0.52, 4.80± 0.47, 3.80 ± 0.34
2.60 ± 0.19, 3.20 ± 0.30, 4.90± 0.24 6.70 ± 0.49, مليمتير بالتعاقب.

الأستجابة المناعية الخلطية الكمية (تركيز اللأميونوكلوبيولينات) للمستضدات تم تقديرها بواسطة التفاعلات الكيمياوية(أختبارglutaraldehyde coagulation كان أكثرمن 600 ملغم/100 مل و أختبار Sodium sulfite concentration كان أكثرمن 1500 ملغم/100 مل) في مصل الأرانب الممنعة.

تم قياس تركيز الكلوبيولينات المناعية النوعية بواسطة Radial immuno-diffusion plates kits مع وجود فروق معنوية ($p \leq 0.01$) للأميونوكلوبيولين من نوع – ج في الأرانب قبل الحقن 1.068 ± 0.050 ملغم/ 100 مل مقارنة بالأرانب المحقونة (الممنعة) 2.758 ± 0.220 ملغم/ 100 مل.

كانت هناك رفض واضح للأرانب الممنعة لألتصاق اليرقات النامية على أذانها عند مقارنتها مع الأرانب غير الممنعة.

تۆیژینه وەیەك دەربارەی بڵا وبوونەوە (Ixodidae) لە نێو مە ڕدا وهەوڵدان بە بەرگریکردنی کەرۆیشك دژی گەنەی *Hyalomma anatolicum anatolicum*

لە پارێزگەی سولیمانی- هەرێمی کوردستانی عێراق

تێزەیەکە

پێشکەش بە کۆلێجی کشتوکاڵ/ زانکۆی سلێمانی وەك بەشێك لە پێویستییەکانی بەدەستهێنانی پلەی دکتورای فەلسەفە لە زانستی بەروبومی ئاژەڵیدا (مێرووزانی)

لەلایەن

بە هـــــــــزاد حەمە صالح مصطفی

بەکالۆریوس لە پزیشکی ڤیتێرنەریدا / 1992

مـاســـــتەر لە دروستـــــی ئـــاژەڵ / 2006

بە سەرپەرشتی

پـــرۆفسۆر دکتۆر محـــــەمەد عەبدولعەزیـــــز قـــــادر

پوختـــه

تۆ ێژینه وەیەك دەربارەی بڵا وبوونەوەی گەنه (Ixodidae) **لە نێو مە ڕدا وهەوڵدان بە بەرگریکردنی**
کەرۆیشك دژی گەنەی *Hyalomma anatolicum anatolicum*
لە پارێزگە ی سولیمانی- هەرێمی کوردستانی عێراق

ئە م تۆێژینه وە یە، لە نێوان مانگی مارس (2009) **و فێبروەری** (2010)**، لە سێ نێوچەی جیاواز** (I، II، III) **، لە پارێزگە ی** (**سو لیمانی**) **کراوە ، بۆ ئەوەی بڵابوونە وەی گە نە** (Ixodidae) **لە نێو مە ڕە تووشبووەکاندا دەسنیشان بکات**.

ڕێژەی بڵابوونەوی تووشبوونی مەڕەکان لە سێ نێوچەکەی پارێزگەی (**سولیمانی**) 298(11.8 ٪) . **ڕێژەی تووشبوون لە یەکەمین نێوچە دا** (**پشدەر**) 85 (10.1 ٪) . **لە دووەمین نێوچە** (**مەلبەندی سولیمانی**) 94 (11.1 ٪). **لە سییەمین نێوچەش** (**گەرمیان**) 119(14.3 ٪). **وە ڕێژەی تووشبوون لە مانگە کانی** (**مارس، ئە پرێل، مای و یولیو**) **دا لە هەموو نێوچەکاندا بەرزبوو. بەڵام لە مانگەکانی** (**نۆڤەمبەر و فێبروەریی**) **دا ، تووشبوون نەبوو. کەچی لە سییەمین نێوچەدا، تووشبوون دەرکەوت**.

دوو ڕەگەزی Hyalomma **و** Rhipicephalus **و چوار جۆر گەنە** *Hyalomma anatolicum anatolicum* ، *Hyalomma marginatum* ، *Rhipicephalus turanicus* ، *Rhipicephalus sanguineus* **دە ستنیشانکران**.

دوو جۆر لە نێوان مەڕە تووشبووەکاندا *Hyalomma anatolicum anatolicum* **لە سییە مین نیوچە** (**گەرمیان**) **زۆر بڵاوبوون** 353 (70٪) ، *Rhipicephalus turanicus* **لەیەکەمین نێوچە** (**پشدەر**) 177 (59.4 ٪) . **هە روەها جۆری** *H. anatolicum anatolicum* **لە مانگی ئازار دا** 61 (75.3 ٪) ، **مانگی نیسان** 89 (69 ٪). **مانگی مایس** 92 (68.7 ٪) **و مانگی حوزەیران** 50 (74.6٪). **لە سییە مین نێوجە** (**گەرمیان**) **بینرا . هەروەها** *Hyalomma anatolicum anatolicum* **وە** *Rhipicephalus turanicu* **لە مانگی ئازاردا** 22 (53.6%) **و** 12 (29.3%) **بوون، وەلە مانگی نیسان** 36 (41.4%) **و** 38 (43.7%)**، وە لە مانگی مایس** 46 (46.9%), 33(33.7%) **، وەلە مانگی حوزەیران** 41 (53.2%), 25 (32.5%) **لەدووەمین نێوچە یەك لە دوای یەك هاتوون . بەڵام** *Rhipicephalus turanicus* **لە مانگی نیسان** 48 (71.6٪) **وە لە مانگی مایس** 57 (65.5٪) **زۆر بەرزبوو لە یەکەمین نێوچە دا .**

لە ڕێی هاوکێشە یەکی هێڵداریەوە، ڕێژەی سەدی مەڕە تووشبووەکان لە هەر یەکێ لە ئەو سێ نێوچە یە دا، گەر لەگەڵ ژمارەی گەنەکاندا بەراوردبکەین لە سییەمین نێوچەدا

Number of ticks (X) 0.23 +3.1 **لە بەر چاو و بەرزبوو. ڕێژەی مێکان بو نێرەکانیش** (1 : 2) **بوو**.

شوینی پیکەوەلکانی گەنەکان بەلاشەی مەڕەکا نـەوە تۆیژرایـەوە، بـەرزترین ڕێـژەی گەنـە لـە گوێچکەیاندا بوو 492 (42.0 ٪) وە لە ژێردووگیشیاندا 208 (17.7٪) بوون
نیشـانەکانی تووشـبوون لـە مـەڕە تووشـبووەکاندا بـە جـۆری *Hyalomma. spp* لـە جـۆری *Rhipicephalus. spp* زۆرتربـوو ، کـە لـە کـەم خـۆینی ، کـەم خـواردن، گـرفتی پێـست وەك ڕووشاندن، ووشك بوون، ڕووتاندنەوە و پەیدابوونی پەڵەی سوورهەڵگەڕاودا خۆی دەبینی.
پیشکینەکانی خۆین ئەوەی دەرخست: جیـاوازی بەرچـاو لـە ئاسـتی ($P \leq 0.01$). لـە بـڕەکـانی خۆین لە خرۆکە سوورەکانی خۆین، خانەکانی خوێن بەستراو، وخەستی هیمۆگلوبین، لە ڕەگەزی *Hyalomma. spp* هەبوو، کـاتێ لەگـەڵ *Rhipicephalus. spp* بـەراوردکران. هیمۆگلـوبین خانەی بەشەکان لە مەڕە تووشبووەکان بە *Hyalomma. spp* بەشیوەیەکی واتـادار جیاواز بـوو لە ئاستی ($P \leq 0.05$) کاتی لەگەڵ *Rhipicephalus. spp* بەراوردکرا.
لە ژمارەی گشتی خرۆکە سپیەکانی خۆین ، قەبـارەی خانـە لاوەکییـەکان، خەسـتی هیمۆگلـۆبین خانەی لاوەکیی، هیچ جورە جیاوازییەکی ئەوتۆی تێدا دەرنەکەوت، کاتی ئەم پێوانانەمان لـە هەردوو ڕەگەزەکەدا ولە نێومەڕە تووشبووەکاندا کرد. هـەروەها تـۆیژینەوەکـە، پـەروەردەکردنی مێـی بـە خـۆین لێـدراو Engorged female لـە جـۆری *Hyalomma anatolicum anatolicum* گرتەوە، ئەمەش لەم تـاقیگە بوو، بـۆ ئـەوەی کـاتی هێلکـەدانان(1.32 ± 27) ڕۆژ و هـەڵهاتنی گـەراکان (2.26 ± 22.4) ڕۆژ دیاربیکـەین . گـەرا هـەڵاتووەکان بـۆ ئامادەکردنی پوختەیەك بە کار هینران، هەروەها immunized serum لە کەرویشکە کاندا ئامادە کرا بەهۆی دەرزلیدانەوە لە ژیر پیست و بـۆ مـاوی 3 جـار ، بـە بـەکارهێنانی Whole Crude Larval Extract (WCL) ، کـارلێکی بـەرگری خانـەکان ئاشکراو واتـادارېوو، ئەمـەش بـە زیـادکردنی هۆکـاری (phagocytic index) دژە بـەکتیریا *Staphylococcus aureus* لە کەرویشکانەی بەرگریدارەکاندا (0.61 ± 7.2) بوو.
خرۆکەیـەکی (Hypersensitivity) پێسـت زۆر لـە جـۆری دواکـەوتوو لـە کەرویشـکە بەرگریدارەکاندا دەرکەوت، کاتی گیراوەی (WCL) لێدرا. دوای (2 ,4، 6, 12، 24، 36، 48، 72، 96) کاژێر و (7) ڕۆژ پیوانەکرا، جیاوازییەکی بەر چاو ($P \leq 0.01$) لە زیادبوونی

هەڵئاوسانی پێستدا هەبوو 0.34 ±3.80, 0.47 ±4.81 , 0.52 ±7.90, 0.38 ± 10.20, 0.30± 3.20, 0.24 ± 4.90, 0.49 ± 6.70, 0.64 ± 8.70, 0.26 ± 11.10, و ± 2.60 0.19 ملیمیتر.

وەڵامدانەوەی بەرگریکارانەی تێکەڵەی بڕەکان (خەستی ئەمیۆنۆگلۆبیۆلینات) دژەکارەکان، بەهوی کارلێکی کیمیایی (تاقیکردنەوەی Glutaraldehyde coagulation) دیاربیکرا و لە 600 ملغم / 100 مل زیاتربوو. تاقیکردنەوەی خەستی Sodium sulfite لە 500 ملغم/ 100مل لە (serum) ی کە رۆشکە بەرگریدارەکاندا زیاتربوو. خەستی (گلۆبیۆلینات)ی جۆری بەرگریدار بەهۆی (Radial immuno-diffusion plates kits) وە پێورا، لە سەر ئاستی($P\leq 0.01$) جیاوازی واتاداری بینرا، ئەمینۆگلۆبیۆلین لە جۆری-G لە کەرۆشکەکاندا پێش دەرزیلێدان (0.050 ± 1.068 ملغم/ 100مل) بوو، لەگەڵ کەرۆشکە لێدراوەکان (بەرگریداوەکان) بە (0.220 ± 2.758 ملغم/ 100مل) بەراوردکرێن.

لە ئە نجامدا، ڕەتکردنەوە یەکی ئاشکرا لە کەرۆشکە بەرگریدارەکان بۆ پێوەلکاندنی گەراگەشەکردووکان لە سەر گوێچکەیان هەبوو، گەر لەگەڵ کەرۆشکە نا بەرگریدارەکاندا بەراوردیکەین.