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Histological Evaluation of Some Skin Lesions of Tick Bites in Sheep

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Abstract

In naturally infested sheep, two genera and four species of Ixodidae, two genera and two species of mites, two genera and two species of lice, three genera and three species of myiasis, and three genera and three species of fleas. Highly infested, as described in sheep, with Arachnida at 55%, comprising 46% Hard ticks and 9% Mites. The overall number of skins refused to the tanning industry was 100 (21.21%) in sheep. Histological and blood values analysis of the skin of sheep repeatedly infested with the *Hyalomma anatolicum anatolicum* was studied in vitro. Infiltration of neutrophils, macrophages, and lymphocytes adjacent to the tick bite site was observed. In this review, the presence of ectoparasites affected both the red and white blood cell series of hematological parameters. Among the main parameters analyzed, hematocrit, red blood cells, hemoglobin, and lymphocytes showed reductions, while eosinophils, neutrophils, and basophils increased in infested animals as a result of the host immune response. A t-test, $p < 0.05$, in this study reveals a statistically significant difference in blood values between individuals with naturally infested ectoparasites. This study highlights the species of Ixodidae and other ectoparasites (mites, myiasis, fleas, and lice) that cause various patterns of cutaneous lesions (as observed through histopathology) in sheep, which affects their industry.

Keyword: Sheep, Hard ticks, Ixodidae, Skin infestation, Inflammation, Dermal changes, Histopathology.

I. Introduction

Ticks, especially hard ticks (members of the Ixodidae family), are blood-sucking ectoparasites that serve as vectors for the transmission of pathogenic microorganisms, including viruses, bacteria, and protozoans, causing severe infectious disorders in animals and humans (**Gratz, 1999; de la Fuente *et al.*, 2008**). Ixodid ticks insert their mouthparts into the host skin and take a blood meal for several days, resulting in an increase in body weight up to 200-fold. During blood feeding, tick saliva containing a wide range of bioactive substances is injected into host animals to promote successful blood sucking (**Nuttall, 2019**). During salivation, pathogenic microorganisms can be transmitted from pathogen-infected ticks to host animals (**Embers & Narasimhan, 2013; Yamaji *et al.*, 2018**). Apart from tick-transmitted infectious diseases, some people who have experienced tick bites suffer from repeated episodes of systemic anaphylaxis after eating red meat or being treated with monoclonal antibodies for cancer therapy. (**Platts-Mills and Commins, 2013; Cabezas-Cruz *et al.*, 2019**). Thus, tick infestations and tickborne diseases constitute a growing burden to human and animal health worldwide. Basophils constitute fewer than 1% of blood leukocytes, and their pathophysiological roles in allergic reactions remain mostly unclear. However, basophil infiltration into the skin has been demonstrated in some human skin diseases (**Gratz, 1999; de la Fuente *et al.*, 2008**). Recent studies have shown that basophils play a crucial role in the development of IgE-mediated chronic allergic reactions, acting as initiators in mice (**Kazimirova and Stibraniova, 2013**). Basophils also respond when challenged by the mosquito (**James & Rossignol, 1991**).

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II. Materials and Methods

Sheep Isolation.

In this study, 100 suspected sheep infested with ectoparasites were isolated from various sheep flocks in different regions in Sulaymaniyah province.

Tick Collection and Identification.

Recently, fully engorged female ixodid ticks were collected from different flocks of sheep using tissue forceps and cotton and then soaked in ethanol (Figure 1). They were placed individually into clean universal glass vials of dimensions (2.5×8.0) cm. The tick samples were brought to the laboratory, cleaned, and kept in 70% ethanol at room temperature. Ticks were identified, based on morphological features according to (Hoogstraal, 1956), which represented, using a dissecting microscope (Dissecting microscope, Motic Education, China.), magnifying-hand lens and the binocular microscope (Altay, Biovision-103B, China), and identification of collected tick's samples referenced by Iraqi museum of entomology (Mustafa, 2011).

Figure 1: A and B. Site of tick infestation in sheep (Mustafa, 2011).

A B

Duration of bite from hard ticks.
Tissue biopsies are taken according to the site of tick infestation. All skin biopsies obtained from the skins of sheep from the Suleimani slaughterhouse were immediately fixed in 10 % buffered formalin for at least 48 hours before processing to prevent postmortem changes. We examined 100 sheep infested with hard tick bites. The Skin biopsies from skin lesions with tick bites were examined by hematoxylin-eosin staining and immunostaining. Staining for basophils was performed using Gamze stain at 10%. The numbers of infiltrating eosinophils and basophils were enumerated in 5 high-power fields.

Blood Sampling and blood examination of sheep.
Three milliliters of blood were drawn from the jugular vein of infested sheep with ectoparasite; the area was prepared aseptically after clipping and soaking with alcohol, and deposited into a disposable clean plastic tube with anticoagulant (DMD-Dispo, S.A.R. Limited Liability Company) used for estimation of hematological parameters, which were undertaken as described by **Coles (1986)**. These parameters include: total erythrocyte count (RBC × 10^6/µl), total leukocyte count (WBC × 10^3/µl), and packed cell volume (PCV%).

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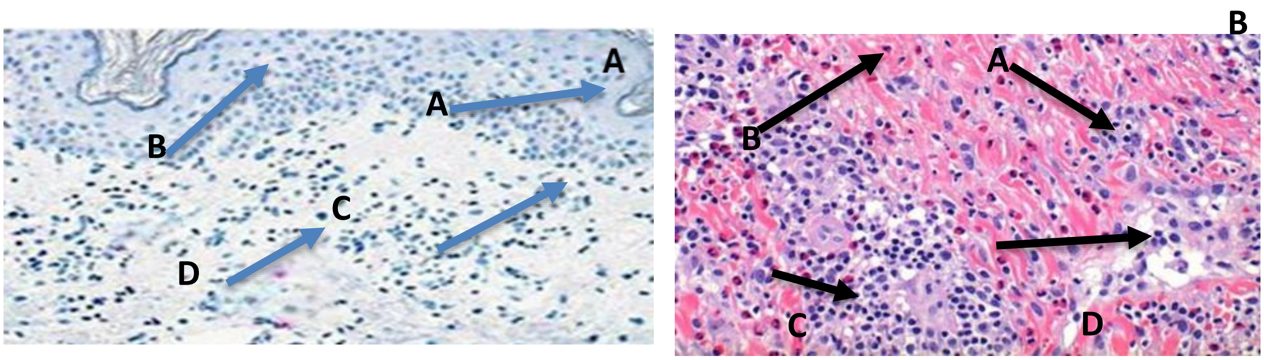
Skin scraping examination.

Skin scraping examination: scrapings were taken from different parts of the skin to detect mange mites, with 1 cm² areas of skin scraped. The scrapings were placed in test tubes containing 5 mL of distilled water and 10% KOH, then heated until hairs and epidermal scales dissolved. The mixture was centrifuged at 2,500 rpm for 3 minutes. The sediment was suspended in distilled water and centrifuged again. After decanting the supernatant, the sediment was suspended in saturated sucrose solution and centrifuged. Finally, samples from the top were examined under a microscope to identify mange mites (**Urquhart *et al.*, 1996**). After administering local anesthesia with 2% lidocaine at the skin biopsy sites, 4×4 mm skin biopsies were taken from 20 sheep and 25 goats, typically affected by mange mites, lice, ticks, and sheep kids, and fixed in 10% neutral-buffered formalin.

Figure 2: Hematoxylin-eosin staining showed infiltration of A: Esinophils, B: Basophils. Severe infiltration by C; Neutrophils, D: lymphocytes observed in the dermis. H.E., obj 20x.



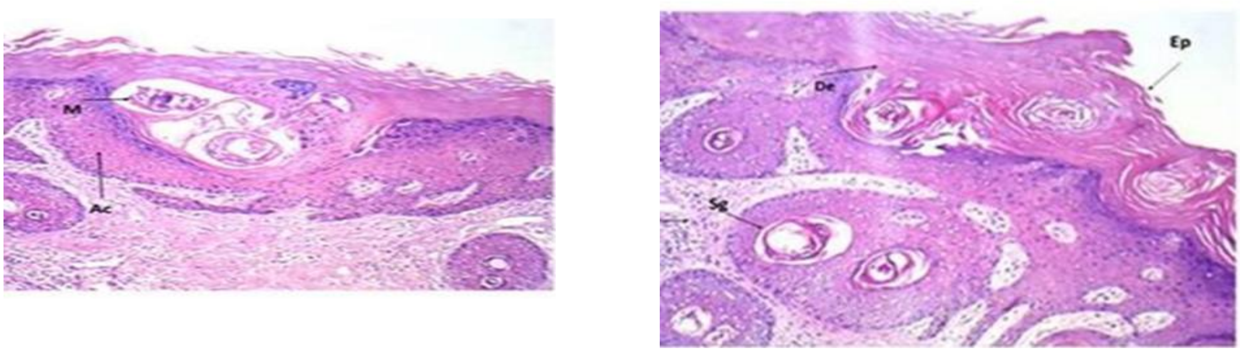


Figure 3: Sarcoptic mange in sheep. Histological aspect of the skin with *Sarcoptes scabiei*, in which the idiosoma (body of mite) (M) is associated with predominantly orthokeratotic hyperkeratosis (Hk) and acanthosis (Ac). X100; (B): Destruction of dermis (De) and epidermis (Ep), hyperplastic changes in sebaceous glands (Sg), and infiltration of neutrophils, eosinophils, lymphocytes, and a few macrophages (arrow). **Atteya *et al.* (2020)**

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Histopathology (Histological analysis).

The tissues were processed and stained with hematoxylin and eosin (H&E) stain for histopathological examination, as described by **Lloyd et al. (1993)**, using a microtome technique to observe and evaluate the histological sectioning of the skin bite site. Subsequent microscopic examination was performed to detect the blood parameter's reaction at the bite site (**Hlatshwayo *et al.*, 2004**).

Statistical analysis:

The association test between blood parameters and ectoparasite infestation in naturally infected sheep using a t-test ($p < 0.05, 0.01$). A descriptive analysis was conducted on some of the data.

III. Results

In this study, researchers identified several genera and species of ectoparasites in 100 suspected sheep, including two genera, Hyalomma and Rhipicephalus, and four species, including *Hyalomma anatolicum*, *Hyalomma marginatum*, *Rhipicephalus turanicus*, and *Rhipicephalus sanguineus* of Ixodidae. Two genera include Sarcoptes and Psoroptes, and two species are included: Sarcoptes scabiei and *Psoroptes ovis*, both of which are mites. Two genera, Damalinia and Linognathus, and two species are included: *Damalinia ovis* and *Linognathus stenopsis*, both of which are lice. Three genera include: Old World screwworm Chrysomya, Blue bottle fly Calliphora, and green bottle fly Lucilia, and three species include: *Chrysomya Bezziana*, *Calliphora vicina*, and *Lucilia Sericata* of myiasis. Three genera are included: Ctenocephalides, Pulex, and Xenopsylla, and three species are listed: *Ctenocephalides canis*, *Pulex irritans*, and *Xenopsylla cheopis*, which are types of fleas, according to Table 1.

Table 1. Identification of some ectoparasites on the skin among infested sheep.

Ectoparasite	Genera	Species
Ticks	- Hyalomma	- Hyalomma anatolicum.
		-Hyalomma marginatum
	-Rhipicephalus	-Rhipicephalus turanicus
		-Rhipicephalus sanguineus
Mite- Scabies	Sasrcoptes	-Sarcoptes scabiei
	-Psoroptes	-Psoroptes ovis
Myasis	- Old world screwworm/ Chrysomya	-Chrysomya Bezziana
	Blue bottle fly/ Calliphora	-Calliphora vicina
	Green bottle fly/ Lucilia	-Lucilia Sericata
Lice	- Damalinia	- Damalinia ovis
	- Linognathus	- Linognathus stenopsis
Fleas	Ctenocephalides	- Ctenocephalides canis
	Pulex	-Pulex irritans
	Xenopsylla	-Xenopsylla cheopis

Table 2 shows that the levels of erythrocytes and neutrophils were increased when sheep were highly infested with hard ticks, mites, myiasis, lice, and fleas, compared to those lightly infested with other ectoparasites. Testing with a t-test, $p < 0.05$, reveals statistical significance in the blood values of naturally infested ectoparasites.

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Table 2. The values of blood cells in infested sheep with some ectoparasites.

Blood parameters %	Normal Blood values %	Bite site by Arthropoda/Insects species% %										t-critical value
		Tick		Mite		Myasis		Lice		Fleas		
		L.I	H. I	L.I	H. I	L.I	H. I	L.I	H. I	L.I	H. I	
Erythrocyte	9-15- 10^6/ml	8.6	7.9	8.8	8.6	9.4	9.1	9.6	9.4	9.7	9.5	3.182
Hematocrit	27-45 %	28	25	26.5	29	31	27	32	31	38	38	
Neutrophile	10-50 %	37	54	42	49	46	57	48	59	44	51	

L. I = Low infestation H. I = High infestation

These results show the evaluation of some blood values which response to the arthropod antigen. So, two skin biopsies were taken from the shaved area with a scalpel blade at the tick attachment points for histopathological studies, according to Table 3, which shows that the level of basophils, eosinophils, neutrophils, and monocytes is increased at the skin bite site, especially when the sheep were heavily infested with hard ticks. In mite-infested and myiasis areas, high values in basophiles are noticed. Testing with a t-test, $p < 0.05$, reveals statistical significance between blood values with naturally infested ectoparasites.

Table 3. The values of blood cells in the bite site of the skin by some ectoparasite in sheep.

Blood parameters / %	Normal Blood values %	Bite site by Arthropoda/Insects species% %										t-critical value
		Tick		Mite		Myasis		Lice		Fleas		
		L. I	H. I	L. I	H. I	L. I	H. I	L. I	H. I	L. I	H. I	
Basophile	0-3	4	6	3	7	1	3	3	4	1	4	3.182
Eosinophile	0-10	6	12	4	8	3	5	6	7	7	5	
Lymphocyte	45-55	38	44	38	39	41	56	44	45	45	46	
Monocyte	0-6	0.5	0.7	0.5	0.7	0.6	0.8	0.4	0.5	0.3	0.5	

L.I = Low infestation H. I = High infestation

The total number of skins refused 21 (21%) in sheep through the suspected sheep, as the following; that the most rejected skins were infected with myiasis 64%, mite 44% and in mixed infection 27.3%; hard tick with lice 33%, Ticks, lice, and fleas 14%, and Mite and myiasis 50% among sheep. Identified that the high-infested sheep were with Arachnida 55%, followed by the infested with insects 34%, and 11% in sheep mixed infested with ectoparasites, according to Table 4.

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Table 4. Effects of ectoparasites on skin quality in sheep.

Arthropods/ Insect	Sheep infested /100	Sheep infested /100			
		Number of skin accepted/ Degree purity			No. of skin refused %
		Good	Very Good	Excellence	
Arachnida					
Ticks	46%	23	15	2	6 (13%)
Mite- Scabies	9%	3	1	1	4 (44%)
Overall	55 (55%)	26	16	3	10 (18.2%)
Insects					
Myasis	11%	4			7 (64%)
Lice	17%	13	2	1	1 (6%)
Fleas	6%	-	2	4	0%
Overall	34 (34%)	17	4	5	8 (23.5)
Mixed infested					
Ticks and lice	3%	2	-	-	1 (33%)
Ticks, lice, and fleas	6%	4		1	1 (14%)
Mite and myiasis	2%		1	-	1 (50%)
Over all	11 (11%)	6	1	1	3 (27.3)
Overall	100%	49	21	9	21 (21%)
		79 (79%)			

IV. Discussion

In this study identified two genera and four species of Ixodidae; two genera and two species of mites; two genera and two species of lice; three genera and three species of myiasis, and three genera and three species were identified in fleas, according to morphological features, depending on the keys mentioned by, **Hoogstraal, (1956) and Walker *et al.*, (2003).**

In the present study, it is shown that the levels of erythrocytes were increased when sheep were highly infested with hard ticks, mites, myiasis, lice, and fleas, compared to those lightly infested with other ectoparasites. according to A t-test, $p < 0.05$, reveals statistical significance between blood values in naturally infested ectoparasites. This finding disagrees with the result of **Gurbuz and Semista (2013**), which states a significant decrease in the total WBC count in sheep infested with *P. ovis* compared to healthy (control) sheep. Hematological changes in sheep infested with a high load of *B. ovis* and non-infested controls, observing the systemic effects of this parasite on the animals. The absence of a significant difference in Packed cell volume values between the two groups confirms that the parasite does not feed on blood (**Fourie and Horak, 2000**). On the other hand, the simultaneous decline in PCV of both groups may indicate a low nutritional profile of the feed provided, as no internal parasites or blood parasites were detected that could have contributed to the fall in PCV. This is consistent with **Coles' (1986)** findings, which suggest the absence of significant anemia development.

Differential leukocyte counts in this experimental study unequivocally revealed that eosinophilia is the only leukocyte change associated with *Bovicola ovis* infestation in sheep. At the same time, the proportion of other WBC constituents was similar between the two experimental groups when testing with a t-test, $p < 0.05$. This finding is in agreement with that of **Aatish *et al.* (2007)**, who reported significantly increased eosinophil numbers in sheep infested with mange mites compared to healthy sheep. An increase in the number of circulating eosinophils has frequently been associated with parasitic infestations. This could be due to allergic reactions caused by lice or their products, inflammatory reactions, or the activation of the immune system **(Nelson *et al.*, 1977).**

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In this study, the biopsy results from all suspected sheep infested with hard ticks demonstrated similar histologic features. In all infested sheep, remnants of embedded tick parts were surrounded by a zone of dermal necrosis. The study shows that the levels of basophils, eosinophils, neutrophils, and monocyte is increased at the skin bite site, especially when the sheep were heavily infested with hard ticks. In mite-infested and myiasis areas, high values in basophiles are noticed in heavily infested areas. The absence or presence of basophils in tick bite lesions suggests that basophils might be involved in the development of acquired resistance to tick bites, this results agreement with described that the basophil infiltration into the skin is considered to be accompanied by eosinophil infiltration **Mukai *et al.* (2005),** shows the infiltration of basophils at tick-feeding sites of mice occurred during the second, but not the first, tick infestation. Microscopically, tick bites have caused necrotic lesions in the epidermis and dermis at the attachment sites, characterized by the presence of eosinophils, macrophages, and lymphocytes **(Shaukat *et al.*, 2016).**

During this evaluation, the results show the response of specific blood values to the arthropod antigen. Skin biopsies were taken from the shaved area, using a scalpel blade, at the tick attachment points for histopathological studies. This shows that the level of basophils, eosinophils, neutrophils, and monocytes is increased at the skin bite site, especially when the sheep were heavily infested with hard ticks. In mite-infested and myiasis areas, high values in basophiles are noticed. Lymphocytes with numerous eosinophils are the rule, with scattered superficial neutrophils seen in excoriated or impetiginized cases, these results agree with**, Ito *et al.* (2011)** which revealed that the basophil infiltration into the skin is often accompanied by eosinophil infiltration; the number of basophils in the skin and the ratio of tissue basophils to eosinophils vary among different skin diseases In the dermis, just below the tick insertion site, there was coagulation necrosis, fibrin deposition, and an inflammatory infiltrate composed of mixed cells, neutrophils, lymphocytes, plasma cells, macrophages, and a few eosinophils (**Melo *et al.*, 2023)**. Collected a higher number of ectoparasites in infested sheep, but did not indicate a high prevalence of infestation. Skins were refused because they might be attributed to various important factors, including favorable climatic conditions, malnutrition, especially during the long dry season, poor husbandry systems, poor awareness among farmers of the effects of ectoparasites, and inadequate animal health services in the study area (**Sarkar *et al.*, 2010**). This agreement with **Hagos *et al.* (2013)** indicated a statistically significant association between cockle and scratch, both on pickled sheep and wet blue goat skins. A similar observation was noted on pickled sheep and wet blue goat skins (**Yisak, 2000**). The effect of these parasites on sheep skins during the spring season showed a slight increase, which may be because these ectoparasites are in the growth and reproduction stage. These results contrast with those of **Sayyad *et al.* (2016)**, who found that the seasonal ectoparasitic infestation during the study was higher in the summer season than in the winter. Skins Variations in climate and animal feeding are significant factors in determining the quality and impact on the quality of the skin, concerning the substance of the skin, and exposure to parasitic damage. Highland skins are thinner, less greasy, and much stronger in fiber structure than other skins (**Amsalu *et al.*, 2000**). The irritation caused by lice leads to scratching (James and Moon, 1998, 1999) and rubbing, which can cause skin damage (**Ward and Armstrong, 2000**), resulting in alopecia, a condition similar to our observation. On histological sections, epidermal lesions such as hyperkeratosis and diffuse dermatitis are characteristic of skin lesions caused by lice. **Halligan and Johnstone (1992**) described excess collagen fiber deposition in the dermis due to lice infestation, a finding similar to the present study. Skin lesions caused by ticks have been described by several authors (**Wall and Shearer, 1997**). On the subcutis, tick bites left bleeding points to which the mouthparts of the ticks were attached (**Asp and Tauni, 1988**). Louse infestation is the primary cause of reduced hide and skin quality, which affects the tanning industry and, in turn, impacts the country's economy. A large number of parasites infect domesticated animals and are responsible for production loss (**Shaukat *et al.*, 2016**). Moreover, the leather industry remains constrained by the poor quality of raw materials, a lack of efficient market structure, weak extension services, competition from local/rural tanning industries, and a lack of price incentives for producing high-quality raw materials (**Mahmud, 2001**). The overall number of skins refused to the tanning industry was 4.61% in sheep (**Mustafa, 2019**). Skin quality is primarily defined by the absence of damage to the grain layer of the skin, and finished leather is related to some defects that hides and skins acquire during the animal's life, as well as during the slaughtering, storage, and transportation stages (**Kidanu, 2001**).

Conclusion

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Infestation of nymphs and adults of Ixodidae on sheep skin causes significant keratinization of the sheep skin, resulting in the production of leather with a low percentage of elongation. Histologically, all cases hard ticks adhered to the epidermis with in the dermis, just below the tick insertion site, there was coagulation necrosis, fibrin deposition, and inflammatory infiltrate composed of mixed cells (neutrophils, lymphocytes, plasma cells, macrophages, and few eosinophils) This study reinforces the different patterns of cutaneous lesions caused by tick, mite, fleas, infestation in sheep.

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