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Histopathological, Biochemical and Hematological Values in Rabbits Infested by the Camel Tick *Hyalomma dromedarii* (Acari: Ixodidae)

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Abstract: This study was carried out to investigate the effects of the *Hyalomma dromedarii* infestation on biochemical, histopathological and hematological values of rabbits and the effects of three degrees of temperature on the females performance of tick. In hemogram and leukogram measurements; PCV, RBCs, MCV and MCH in groups infested by adult tick were significantly decreased in comparison to that of larvae and nymph infested group. It was found that the total leukocytic count was significantly higher in groups infested with immature (Larvae and nymphs) and adult stages than control group. AST and ALT were significantly higher in the group infested by immature ticks compared to that in the group infested by adulte ticks. Concernning histopathological changes, liver of rabbits infested by *Hyalomma dromedarii* in mature and immature stages showed dilated, congested, edematous portal vein and dilated bile duct. Signs of degeneration in the form of pyknosis, karyolysis were also observed. Dilated, congested blood sinusoid and vacuolar degeneration could be observed. The kidney of rabbit infested by *Hyalomma dromedarii* in mature and immature stages showed vacuolar degeneration in some tubular cells. Some glomeruli were degenerated with wide urinary space. Interstitial, interglomerular hemorrhage and cell debris in lumen of some tubules were seen. The effects of three degrees of temperature (24, 30 and 37°C) on females tick performance were studied. The results showed that, egg mass had a significant reduction between the three degrees of temperature (24-37 °C). Oviposition period was gradually decreased from 24 to 37°C ($P<0.01$).This indicated that rabbits which were infested by adult stage were more dramatically affected in all parameters than rabbits infested by immature stages of *H. dromedarii*.

Key words: MCV · RBCs · PCV · Neutrophils · AST · ALT

INTRODUCTION

Ticks are arthropods (Arachnida belonging to Acari), ectoparasites and blood-sucking of vertebrates (Mammals, birds and reptiles). They may act as vectors, intermediate hosts and reservoirs of a wide variety of infectious agents. There are three families of ticks: Nutalliellidae, Argasidae (Soft ticks) and Ixodidae (Hard ticks). This last one is the most diverse, with at least 692 described species in the world [1] and with great importance in human and animal (Veterinary) health [2]. The life cycle of a tick comprises three growth stages: larva, nymph and adult (Male and female). In this cycle, animals (Wildlife, livestock and/or companion animals) can act as reservoirs or amplifier hosts and humans are accidental hosts. In favorable conditions, it takes from months (i.e. *Hyalomma* spp.) to 1-3 years (i.e. *Ixodes ricinus*) for the tick to hatch from the egg, go through all three stages, reproduce and then die [3, 4]. Ticks normally feed on more than one host. *H. dromedarii* is widely distributed in North Africa, the northern regions of West, Central and East Africa, Arabia, Asia Minor, the Middle East and Central South Asia. Camels are the principal hosts of the adults, with some records from cattle and goats, immature stages infest hares, burrowing rodents [5]. This fact gives them a high potential for pathogens

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transmission such as bacteria, protozoa or viruses. Tick-bites may cause diseases by different pathogenic mechanisms. They may cause anemia in animals on which they parasite (In some cases there are hundreds of ticks feeding on a unique animal), preventing them to get fatter and causing important economic losses. In addition, their bites cause inflammation, hyperemia, edema, hemorrhage and thickening of the skin due to the induction of allergic reactions [6]. Further, blood feeding ectoparasites can cause severe anemia and other biochemical changes [7-9]. Consequently, ticks cause serious economic losses resulting from weight reduction, low milk production and paralysis that eventually leads to death in livestock [10, 11]. Ticks may also cause paralysis by inoculation of neurotoxins, allergic reactions and local injury with subsequent risk of super-infection by skin bacteria. Nevertheless, the most important mechanism of disease transmission is through the inoculation of pathogenic microorganisms.

In the current study, the biochemical; hematological and histopathological changes on rabbits infested by different stages of *H. dromedarii* and also the effects of three degrees of temperature on the female performance of ticks were investigated.

MATERIALS AND METHODS

Ticks: Engorged females of *H. dromedarii* were collected from the ground of Camel markets in Burkash village, Giza governorate, Egypt. Identification of females was confirmed in the laboratory according to Hoogstraal [12] and Estrada-Pena, *et al.* [13]. The females were incubated at a constant temperature of 24± 2°C with a relative humidity of 75 ± 5% in permanent darkness to obtain eggs and larvae as previously described by Patrick and Hair [14]

Animals: Thirteen local breed rabbits aged 5-6 months and with initial weights ranging from 1.25 to 1.50 kg were used in this experiment. Rabbits were housed individually in galvanized cages kept in well-ventilated pens. Each cage had a stainless steel nipple for drinking water and a feeder. Feed and water was offered ad libitum. All rabbits were kept under the same management, hygiene and environmental conditions during the experimental period. Rabbits were divided into three groups. The first group (Five rabbits) was used for feeding of immature stages (Larvae and nymph). The second group (Five rabbits) was used for feeding of adult stages and the third group (Three rabbits) was used as a negative control (No tick feeding on them).

Infestations and Blood Samples

Immature Stages (larvae & Nymph): One thousand (Two hundred larvae for each rabbit) laboratory-reared, 3 day-old unfed larvae and nymphs were fed on five local breed rabbits. After six days, from attachment of larvae, blood samples were collected (In the early morning before the diet was administered). and the same group of rabbits were used for the feeding of nymphs (*H.drommedarii* have two host). After feeding of nymphs, rabbits were scarified. Blood samples were collected in both hebarnized tube to study the hematological parameters and in nonhebarnized tube to study the biochemical parameters. Blood was centrifuged at 2000 rpm for 15 min. Sera were collected in Eppendorph tubes and stored at -20°C till use.

Adult Stages: Two hundred (Forty adult tick; 20 male & 20 female) for each rabbit, laboratory -reared, 7 day-old unfed adult stage were fed on five local breed rabbits. After one week, from attachment of adult tick, engorged females were collected. After feeding of adult tick, rabbits were scarified. Blood samples were collected as above mention.

Hematological Studies: The blood samples were analyzed for hemoglobin (Hb) as cyanomethemoglobin method according to Kuwahara [15] using kits, Packed Cell Volume (PCV) was determined by microhematocrit method according to Schalm, *et al.* [16]. Total erythrocytes and total leukocytes were counted by Neubauer's haemocytometer. Differential leucocytic count was performed as cross sectional method according to Schalm, *et al.* [16]. The erythrocytic indices were determined as color index. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated using the formula of Jan [17].

Biochemical Studies: Separated serum samples were analyzed for alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST/GPT) by colorimetric method according to Winn-Deen [18] using Linear Chemicals.S.L. kits. Total protein was determined by coloremetric method (Biuret reagent) according to Cannon, *et al.* [19] using SPECTRUM kits (BioMerieux, SA).

Pathological Studies: Histopathological studies were conducted on the liver and kidneys of all groups.

Histological Processing: Tissue specimens were collected from the liver and kidney, immediately after

sacrifice of the animal groups at the end of the experiment and these tissues were then fixed in 10% formol saline. The specimens were dehydrated, cleared and embedded in paraffin blocks. Paraffin sections of 5 µm thickness were prepared, stained with H&E and examined microscopically to detect histopathological alterations. Drury and Wallington [20] and Banchroft, *et al*. [21].

Females Performance: Biological parameters of *H. dromedarii* females were studied. Pre-oviposition, oviposition, weights of egg masses, hatching period were recorded under three degrees of temperature (24,30,36°C).

Statistical Analysis: All data were subjected to statistical analysis including the calculation of mean and standard errors. Differences between the control and treated groups were tested for significance using a one-way analysis of variance followed by Duncan's multiple range test. Differences were considered significant at $P<0.05$ level, Snedecor and Cochran [22] using SPSS version 10 computer program download from http://www.spss.com. The mean values (±SE) of the hematological and biochemical parameters were calculated and the effect of tick infection on these parameters was analyzed using T- test according to Snedecor and Cochran [23].

RESULTS

Hematological Parameters

Hemogram: Comparing the blood measurements of Hb, PCV, RBCs, CI, MCV, MCH and MCHC of rabbits infested by different stages of ticks and the control group, considerable variability was observed (Table 1). Results showed that Hb was significantly lower after nymph and adult tick infestation compared to the control. PCV was significantly decreased after larvae infestation and also after the feeding of mature stage. RBCs, CI and MCH were significantly decreased in larvae infested group. However, MCHC was significantly lower after feeding of immature (Larvae and nymphs) stage.

Comparing the effects of larvae and nymph infestation with that of adult tick, results revealed that PCV, RBCs, MCV and MCH in groups infested by adult tick were significantly lower than that of larvae and nymph of the infested group.

Leukogram: Table 2 shows the leukogram in larvae, nymph and adult tick infested rabbits. Comparing the leukogram measurement in different groups with control group, it was found that total leukocytic count was significantly higher in larvae and adult groups also after feeding of immature (Larvae and nymphs)stages compared to control. While Lymphocyte count (L) was significantly decreased in larvae, nymph and adult infestation compared to control group, neutrophil (N) was significantly increased in larvae infested group and also in groups after both immature (Larvae and nymphs) and mature infestation compared to control. N/L ratio was significantly higher in larvae infestation and after immature infestation compared to control. Finally, acidophil was significantly increased in larvae and adult group also after feeding of both immature (Larvae and nymphs) stage compared to control. Comparing the effects of larvae and nymph infestation with that of adult, results revealed no significant differences.

Biochemical Parameters: Comparing measured biochemical parameters of rabbits in different infested groups and control group (Table 3), it was found that AST was significantly higher in groups infested by larvae, adult ticks and also after the feeding of immature stage compared to control. ALT was significantly higher in groups infested by larvae and adult ticks compared control group.

Regarding biochemical parameters within the two infested groups, results showed that AST was significantly higher in group infested by adult ticks than that in group infested by larvae. Also, AST was significantly higher after feeding of immature stage (Larvae and nymphs) than mature stage.

Table 1: Hemogram of Rabbits infested by different stage of *H. dromedarii* ticks

Parameter	Control	Larvae infestation	Nymph Infestation	Adult infestation	After feeding of immature stage	After feeding of mature stage
Hb (gm/dl)	14.754±1.358	10.934±0.393	9.990*±1.170	9.847*±1.443	10.140±3.090	12.225±0.392
PCV%	36.130±2.400	30.00*±1.220	30.00±3.240	34.667^{a}±0.667	36.00±6.00	30.330*±1.760
RBCs x10^{6} cells/mm^{3}	5.358±0.245	3.966**±0.231	4.565±0.338	6.137^{b}±0.730	4.440±0.360	4.727±0.393
CI	1.184±0.048	1.248±0.096	0.802*±0.075	0.915±0.005	1.030±0.390	1.090±0.076
MCV (fl)	71.740±7.37	81.710±5.73	75.830±3.44	60.980 ^{b}±1.18	82.700±4.02	70.720^{A}±2.52
MCH (Pg%)	27.080±1.11	28.52±2.23	18.35*±1.70	20.880 ^{a}±2.18	23.56±1.87	24.96±1.72
MCHC %	39.620±2.80	35.070±2.34	33.180±3.13	38.010±1.15	27.500*±4.00	32.560±1.08

Values that have * and ** were significantly different from control at p □ 0.05 and p □ 0.01 respectively.
Values that have a or b were significantly different for adult and larvae infestation at p □ 0.05 and p □ 0.01 respectively.
Values that have A were significantly different for feeding mature and immature at p □ 0.05.

Table 2: Leukogram of Rabbits infested by different stage of *H.dromedarii* ticks

Parameter	Control	Larvae infestation	Nymph Infestation	Adult infestation	After feeding of immature stage	After feeding of mature stage
Total leukocyte	6.722±0.328	5.632±0.545	8.912*±0.545	8.133*±0.571	11.100**±2.951	8.898*±0.729
Lymphocyte (%)	71.50±2.43	56.83**±2.70	57.17*±2.59	59**±3.24	69.00±3.43	64.33±3.14
Neutrophil (%)	18.17±2.30	16.83±2.86	25.33*±1.07	17.17±3.31	27.33*±3.39	26.50*±2.60
N/L ratio	0.337±0.044	0.450*±0.035	0.328±0.042	0.327±0.063	0.480*±0.037	0.400±0.056
Acidophil(%)	3.83±0.72	4.67±1.05	6.17**±1.01	7.17**±1.14	5.57*±0.74	5.57*±0.74
Monocyte(%)	3.33±0.94	4.54±0.76	5. 67±0.47	5.16±0.01	4.66±0.66	4.57±0.64

Values have * and ** were significantly different from control at p □ 0.005 and p □ 0.01 respectively.

Table 3: Biochemical parameters of Rabbits infested by different stage of *H. dromedarii* ticks

Parameter	Control	Larvae infestation	Nymph Infestation	Adult infestation	After feeding of immature stage	After feeding of mature stage
AST (µl/100ml)	113.71 ±3.49	142.20** ±6.39	119.60[a] ±5.90	135.33 ** ±2.85	146.00 ** ±7.42	122.00[A] ±5.86
ALT (µl/100ml)	22.10 ±3.06	28.252 ±7.50	39.375 ** ±4.83	35.067 * ±5.23	24.450±5. 082	24.567 ±6.17
Total protein (gm/100ml)	7.45 ±1.24	5.40 ±1.17	5.44 ±1.02	4.50± 0.96	5.99±.710	6.44 ±.739

Values have * and ** were significantly different from control at p □ 0.05 and p □ 0.01 respectively.
Values have a, b were significantly different for adult and larvae infestation at p □ 0.05 and p □ 0.01 respectively.
Values that have A were significant of feeding mature than immature at p □ 0.05.

Table 4: Female of *H. dromedarii* performance at three degrees of temperature.

	Biological parameter			
Temperature (°C)	Egg mass (g)	Pre-oviposition period (day)	Oviposition period (day)	Pre-hatching period (day)
24	0.5402±0.0387 [a]	8.80±1.16	15.33±3.28 [a]	19.33±0.333[a]
30	0.4301±0.0330 [b]	6.33±1.20	8.00±0.58 [b]	5.33±0.882[b]
37	0.4460±0.0270 [b]	11.20±1.66	5.75±1.31 [b]	6.6±0.882[b]
F value	3.195	2.516	6.594	107.46
P value	0.046	IN	0.025	0.000

IN = Insignificant
Superscript letters represent significant differences between three degrees of temperature

Histological Results of Liver: The liver of control rabbit revealed normal characteristic architecture (Figs.1A & 1B).

Concerning the liver of rabbit infested by *H. dromedarii* in mature stage, it showed dilated, congested, edematous portal vein and dilated bile duct. Signs of degeneration in the form of pyknosis, karyolysis were observed. Dilated, congested blood sinusoid and vacuolar degeneration could be observed (Fig 2).

Concerning the liver of rabbit infested by *H. dromedarii* in immature stage, dilated, congested, edematous portal vein, dilated bile duct and cellular infiltration around it were seen. Vacuolar degeneration, dilated and congested blood sinusoid could be observed. Signs of degeneration in the form of pyknosis, karyolysis and karyorhexis were seen. Vacuolar degeneration and fatty changes were also seen (Figs 3,4).

Histological Results of Kidney: The normal histological structure of the kidney was observed in the control rabbits (Fig 5A&B).

The kidney of rabbit, infested by *H. dromedarii* in mature stage, showed vacuolar degeneration in some tubular cells. Some glomeruli are degenerated with wide urinary space. Interstitial, interglomerular hemorrhage and cell debris in lumen of some tubules were seen (Fig 6).

In the case of kidney of rabbit infested by *H. dromedarii* in immature stage, it showed inter glomerular and interstitial hemorrhage. Fatty and vacuolar degeneration could be observed. Edema in interstitial tissue and in lining epithelium were also seen (Figs7,8). Sings of degeneration in the form pyknosis, karyolysis were also observed. Glomerular lobulation and thickening in the lining epithelium with narrowing in the lumen were also seen (Fig 9).

Females Performance of *H. Dromedarii* at Three Degrees of Temperature: Observations made on egg mass and incubation period of *H. dromedarii* female ticks are shown in Table 4. It was clear that the mean of egg mass was significantly reduced from 24°C to 37°C ($P<0.05$). Incubation period showed that it significantly decreased from 24°C and 30, 37 °C (Table 4). The data in table 4 revealed no significant difference between pre-ovipositiosn period of female ticks of *H. dromedarii* at 24, 30 and 37°C.Oviposition period was gradually decreased at 24 to 37°C ($P<0.01$).

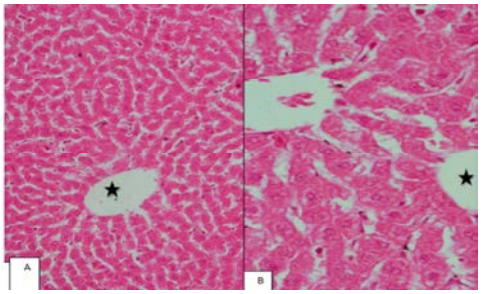

Fig. 1: Two views of a section in liver of control rabbit showing normal histological structure of hepatic lobules and central vein (A): (Hx&Ex100) & (B): (Hx&Ex400).

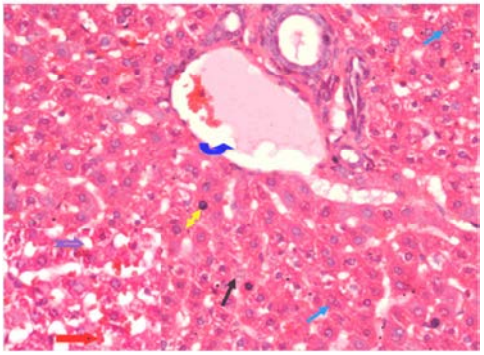

Fig. 2: Section of the liver of rabbit infested with *H. dromedarii* in mature stage showing dilated, congested, edematous portal vein, dilated bile duct (blue arrow). Signs of degeneration in the form of pyknosis (yellow arrow), karyolysis (black arrow). Dilated, congested blood sinusoid (red arrow) and vacuolar degeneration (purple arrow) at left of figure. (Hx&Ex400).

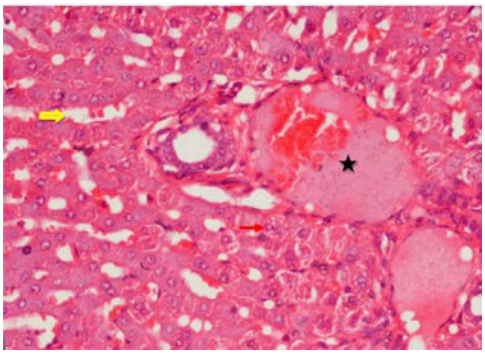

Fig. 3: Section of the liver of rabbit infested by *H. dromedarii* in immature stage showing dilated ,congested, edematous ,vacuolated portal vein, dilated bile duct and cellular infiltration around (star).Dilated and congested blood sinusoid(yellow arrow).(Hx&Ex400).

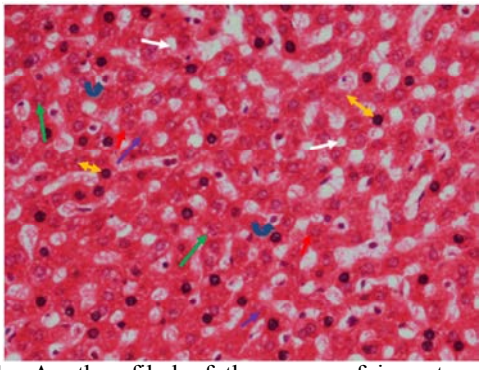

Fig. 4: Another filed of the group of immature stage showing signs of degeneration in the form of pyknosis (yellow arrow), karyolysis (purple arrow) and karyorhexis (green arrow), vacuolar degeneration (white arrow) and fatty changes(curved blue arrow). (Hx&Ex400)

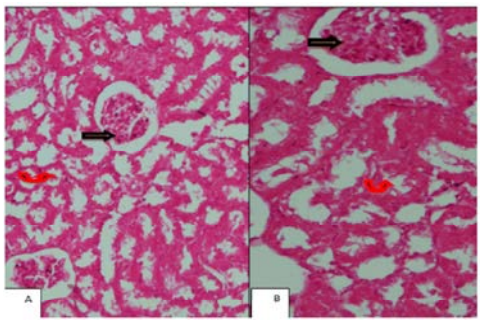

Fig. 5: Section of the kidney of control rabbit showing normal appearance of glomerulus (black arrow) and renal tubules (red curved arrow) (A):(Hx&Ex100)&(B): (Hx&Ex400)

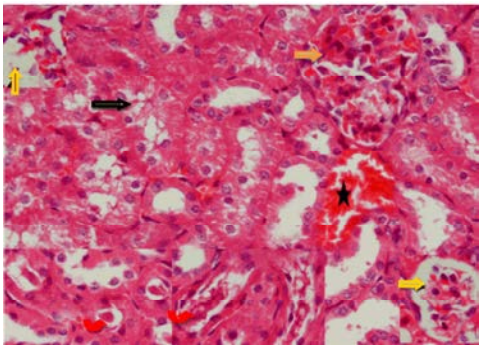

Fig. 6: Section of the kidney of a rabbit infested by *H. dromedarii* in mature stage showing vacuolar degeneration in tubular cells (black arrow), interstitial(star) and interglomerular hemorrhage (yellow arrow).Cell debris in lumen of some tubules (red curved arrow). Some glomeruli are degenerated with wide urinary space (yellow arrow) (Hx&Ex400)

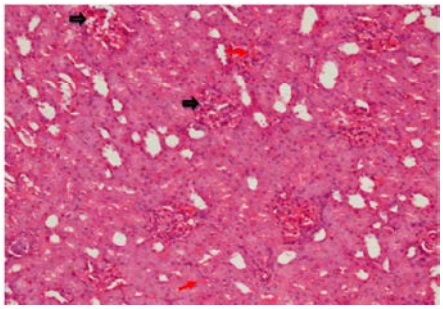

Fig. 7: Section of the kidney of rabbit infested by *H. dromedarii* in immature stage showing inter glomerular (black arrow), interstitial hemorrhage (red arrow). (Hx&Ex100)

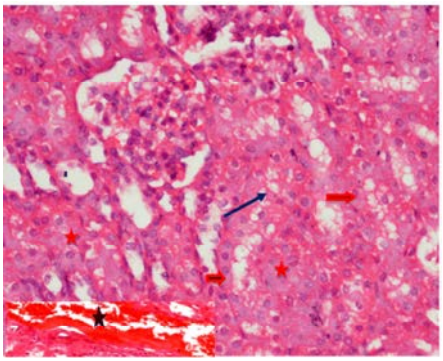

Fig. 8: High power of pervious figure of the kidney of rabbit infested by *H. dromedarii* in immature stage showing fatty and vacuolar degeneration (blue arrow), edema, congestion, in interstitial tissue (star) and in lining epithelium (red arrow) .(Hx&Ex400)

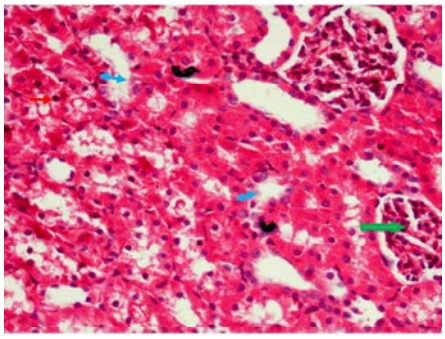

Fig. 9: Another field of the kidney of the rabbit infested by the immature stage showing signs of degeneration in the form pyknosis (red arrow), karyolysis (light blue arrow).Vacuolar degeneration (yellow arrow) glomerular lobulation (green arrow) were seen. Thickening in the lining epithelium with narrowing in the lumen was observed (black curved arrow).(Hx&Ex400).

DISCUSSION

It has been established that ticks are capable of concentrating their blood meal by eliminating excess water, therefore allowing for a significant increase in the quantity of red blood cells that may be withdrawn from a host by tick parasitism. In this investigation, results revealed that PCV, RBCs, MCV, RBCs and MCH in group infested by adult tick were significantly decreased compared to that of larvae and nymph infested group. However, Hb, CI and MCHC were significantly lower after immature (Larvae and nymphs) infestation. This results indicated that, the two groups of rabbit infested by tick stages (Mature and immature) were affected. But blood measurements of group of rabbits infested by adult stage of ticks were more affected than the other group. It would, therefore, seem probable that the anemic condition existing in animals, heavily infested by ticks is primarily the result of blood removal from the host. Supporting evidence of this premise is established when comparing the severity of animal blood loss and the number of feeding ticks. Those animals supporting the largest number of ticks demonstrated the greatest reduction of PCV, Hb and RBC [11]. This results in preventing the animals to get fatter and causing important economic losses. Similar hematological reductions of tick– infested have also been reported by other investigators Hair,*et.al*.[11] They showed that severe anemic conditions were developed in the fawns infested by high levels of *Theileria-infected* and non-infected ticks, while a subacute anemia was developed in the infested animals with low tick numbers. In the two high tick infestation treatments, the PCV, Hb and RBC were reduced below 65% of the initial blood measurements, while the same factors for the animals receiving low tick numbers were reduced to about half of their starting values.

This experiment demonstrated that, acidophil, neutrophil and N/L ratio were significantly increased in the two groups of rabbits compared to control group. These results were induced by sucking ticks feeding long time (Two weeks for immature stages and one week for adult stages) on rabbits and producing numerous saliva toxic secretions and this made the cellular immune system active. Our results are compatible with Habeeb [9]. She found that the leukocyte cells, esinophils (Anti-allergy) increased in rabbit sera if female ticks of *Ornithodoros savignyii* feeding was repeated daily when the basophil (Carrying histamine) appeared.

In addition, AST and Alt revealed an elevation and highly significant increase in the two groups of infested rabbits compared to the control. Activity of AST and ALT

showed consistent elevation above their normal values, thus probably indicating changes in liver and kidney cells. It is a fact, that AST enzymes is intracellular bonded and that the increase in their circulating levels in serum might be indicative to cellular destruction, Harwood and James [24]. The same results were shown by Habeeb and Zaki [25], they reported that AST revealed an elevation and highly significant increase in infested chicken with *Argas persicus* tick during d15 and 30 of experiment.

In this investigation our results which were obtained from biochemical and hematological studies were supported by histopathological studies in liver and kidney of rabbits infested by mature and immature tick of *H. dromedarii.*

Temperature and humidity are two main factors which have an effect on the life cycle of *H. dromedarii.* Some studies found that all biological parameters of *H. dromedarii* were affected by this two factors [3,4]. In this study, it was shown that all parameters of females performance were significantly reduced at 24°C in comparison to 30, 37°C except at pre-oviposition period. The results obtained by Elghale and Hassan (3) are compatible with our results in all parameters except pre-oviposition period. They found that pre-oviposition periods ranged between 9.8 and 11.7 days in the shade but were longer in the sun in December (14.7 days).

It was concluded that rabbits infested by adult stage were more dramatically affected in all parameters compared to immature stages (Larvae and nymphs) except in histopathological studies that showed no significant changes in liver and kidney between the two groups of rabbits infested by mature and immature stages of ticks.

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