

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**  
**College of Veterinary Medicine**  
**Clinicopathological Study in Sudanese Sheep and Goat**  
**Infested by External Parasite**

دراسة لبحث الآثار السريرية المرضية في الضأن و المعز السوداني المصابة  
بالطفيليات الخارجية

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(وَمَا أُوتِيتُمْ مِّنَ الْعِلْمِ إِلَّا قَلِيلًا)

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*Dedication*

*TO OUR MOTHERS, FATHERS,  
BROTHERS, SISTERS AND FRIENDS TO  
ALL OF THEM WITH LOVE*

## **Acknowledgement**

First of all, our sincere thanks to Allah who gave us the power and patience to begin and complete our study

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## ABSTRACT

The study was conducted to investigate clinical signs and lesions, hematological profile, serum copper (Cu), Iron (Fe), total protein and albumin concentrations of sheep and goats with lice and ticks infestations. Twenty sheep and goats (2 to 4 years of age) were used. Fourteen animals were from infested (positive) group and six from non infested (negative) group.

Blood samples were collected from jugular vein. Blood was placed into EDTA tubes for hematological examinations. Serum was isolated for other serobiochemical analysis. Cu and Fe were determined by analytical methods for atomic absorption spectrophotometry. Total protein and albumin were measured by an enzymatic method using commercial kits and spectrophotometer. Clinical signs and lesions were observed and recorded.

The clinical signs of the affected animals were multiple. These included scratching, in extreme cases, affected animals rubbed on any solid object, decreased feed intake, decreased weight gains and milk production, paleness of mucus membranes. The skin showed rough coat, varying degrees of hair loss, scaling, thickening and wrinkling. The present study revealed that there were no significant differences ( $P>0.05$ ) in hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total leukocytic count (WBCs) between infested)

and non infested sheep and goats. However there was significant difference ( $P<0.05$ ) in total erythrocytic count in infested animals ( $8.4\pm 2.5 \times 10^6 \text{ mm}^3$

when compared to none infested animals ( $10 \pm 1.8 \times 10^6 \text{ mm}^3$ ). The serobiochemical result showed that the mean value of serum Cu concentration was significantly ( $P<0.01$ ) decreased in the positive group ( $0.13\pm 0.04 \text{ mg/ml}$ ) as compared to the negative group ( $0.42\pm 0.05 \text{ mg/ml}$ ). The mean value of serum Fe concentration was significantly ( $P<0.01$ ) decreased in the positive group ( $57.86\pm 2.17 \text{ mg/ml}$ ) as compared to the negative group ( $77.50\pm 2.495 \text{ mg/ml}$ ). The mean value of serum total protein, albumin and globulins concentrations were significantly ( $P<0.01$ ) decreased in the positive group ( $5.7\pm 0.20$ ,  $2.71\pm 0.13$ ,  $2.89\pm 0.28 \text{ g/dl}$  respectively) as compared to the negative group ( $7.79\pm 0.23$ ,  $3.48\pm 0.33$ ,  $4.310\pm 0.18 \text{ g/dl}$  respectively).

The result showed that infestation of lice and ticks adversely affect sheep and goat health.

**Key words:** Ticks, Lice, Sheep, Goat, Hematology, Clinical, Infetsion

## المستخلص

أجريت هذه الدراسة لبحث الأعراض السريرية والآفات المرضية، التغيرات في مستويات مكونات الدم، و مستوى النحاس، الحديد، تركيز البروتين الكلي، الزلال و القلوبوليين في مصل دم الضأن والماعز المصابة بالقمل و القراد. أستخدم في هذه الدراسة عشرون رأس من الضأن والماعز التي تتراوح أعمارها من سنتين إلى أربع سنوات. أربعة عشر من الحيوانات كانت من المجموعة المصابة) الموجبة (وست من مجموعة السيطرة) السالبة).

تم جمع عينات الدم من الوريد الوداجي، و وضعت في أنابيب تحتوي على مضاد للتجلط (EDTA) و ذلك لإجراء إختبارات مكونات الدم. تم فصل المصل لإجراء الإختبارات البايوكيميائية و التي شملت قياس مستوى النحاس و الحديد بواسطة قياس شدة موجات الطيف للإمتصاص الذري، و قياس مستوى البروتين و الزلال بالطريقة الإنزيمية. تمت ملاحظة و تسجيل الأعراض السريرية والآفات المرضية.

الأعراض السريرية التي تمت ملاحظتها هي نقصان في الوزن و إنتاج الحليب، شحوب الأغشية المخاطية، حك و فرك الحيوانات المصابة لأجسامها بواسطة الأجسام الصلبة. كما ظهرت درجات متفاوتة من تساقط و خشونة الشعر، سماكة الجلد و تجعده و وجود قشور عليه .

أظهرت الدراسة الحالية عدم وجود فروق معنوية ( $P > 0.05$ ) في متوسطات مستوى كل من الهيموغلوبين (Hb) ، الحجم الكلي لكريات الدم الحمراء (PCV) ، متوسط حجم كرية الدم الحمراء (MCV) ، متوسط محتوى الهيموغلوبين في كرية الدم الحمراء (MCH) ،

متوسط تركيز الهيموغلوبين في كرية الدم الحمراء (MCHC) و العدد الكلي لكريات الدم البيضاء (WBCs) بين الضأن والماعز

المصابة وغير المصابة. بينما كان هناك فرق معنوي ( $P < 0.05$ ) في العدد الكلي لكريات الدم الحمراء في الحيوانات المصابة ( $8.4 \pm 2.5 \times 10^6$  مل<sup>3</sup>) بالمقارنة مع الحيوانات غير المصابة ( $1.8 \pm 10 \times 10^6$  مل<sup>3</sup>). أظهرت النتائج البايوكيميائية أن قيمة متوسط تركيز النحاس في المصل انخفضت بشكل معنوي ( $P < 0.01$ ) في المجموعة الموجبة ( $0.13 \pm 0.04$  mg/ml) مقارنة بالمجموعة السالبة ( $0.42 \pm 0.05$  mg/ml) وكذلك انخفض متوسط تركيز الحديد في المصل ( $P < 0.01$ ) في المجموعة الإيجابية ( $57.86 \pm 2.17$  mg/ml) مقارنة بالمجموعة السالبة ( $77.50 \pm 2.495$  mg / ml). كما وجد انخفاض معنوي لتراكيز البروتين الكلي، الزلال و القلوبولين ( $P < 0.01$ ) في المجموعة الإيجابية ( $5.7 \pm 0.20$ ) ،  $2.71 \pm 0.13$  ،  $2.89 \pm 0.28$  جم / ديسيلتر على التوالي (مقارنة بالمجموعة السالبة  $7.79 \pm 0.23$  ،  $3.48 \pm 0.33$  ،  $4.310 \pm 0.18$  g / dl على التوالي).

أستنتجت من هذه الدراسة أن الإصابة بالقمل والقراد تؤثر سلبا على صحة الضأن والماعز.

**الكلمات المفتاحية:** قراد، قمل، ضأن، معز، اختبار دم، اختبار سريري، اصابة



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## INTRODUCTION

Small ruminants (sheep and goats) have a unique role in smallholder agriculture as they require small investments; faster growth rates, have shorter production cycles, and greater environmental adaptability as compared to large ruminants. Sheep and goat play an important economic role and make a significant contribution to both domestic and export markets through provision of food (meat and milk) and non-food (manure, skin and wool) products (Nesradin *et al.*, 2017).

Ectoparasites commonly ticks; mites and lice limit production in the sheep and goat industry significantly in Sudan. Geographical location, housing conditions and species play a role in which parasites are likely to be a problem. Ectoparasites feed on body tissue such as skin, wool and hair. The wounds and skin irritation produced by these parasites result in discomfort and irritation to the animal. Some Ectoparasites feed on blood causing blood-loss anemia, especially in young animals. In addition to these ectoparasites can transmit diseases from sick to healthy animals. They can reduce weight gains and milk production. In general, infested livestock cannot be efficiently managed to realize optimum production levels (Gregory, 2011; Tewodros *et al.*, 2012; Kaufman *et al.*, 2012).

Hematology and serum biochemistry of infected animals is very sensitive indicators for the degree of the parasitic infection severity. Therefore, the objectives of this study were:

- 1- Hematological examination.
- 2- Estimation of total protein and albumin in serum.
- 3- Investigation of the levels of copper and iron in serum.
- 4- Clinical observation.

# CHAPTER 1

## LITERATURE REVIEW

### 1.1. Livestock population of Sudan

The livestock population of Sudan, amount to 114 millionhead of which the sheep and goats comprise about 80 million head(According to the Ministry of Livestock, Fisheries and Rangelands of the Sudan 2016) which greatly contributes inSudan economy ([Nazik \*et al.\*, 2017](#)).

Sheep and goats are farmed throughout the world for meat, fibre, milk and leather. These small ruminants are very susceptible to external parasites, which has significant implications for their health and welfare as well as the quality and value of the end products for which they are farmed ([Bates, 2012](#)).

### 1.2. External Parasites of Sheep and Goats

Arthropod pests limit production in the sheep and goat industry in many ways. External parasites feed on body tissue such as blood, skin, and hair. The wounds and skin irritation produced by these parasites result in discomfort and irritation to the animal. Parasites can transmit diseases from sick to healthy animals. They can reduce weight gains and milk production. In general, infested livestock cannot be efficiently managed to realize optimum production levels ([Kaufman \*et al.\*, 2018](#)).

### 1.2.1. Lice

Lice are external parasites which spend their entire lives on the sheep or goat. Both immature and adult stages suck the blood or feed on the skin. Goat lice are host specific and only attack goats and their close relatives, such as sheep (Butler, 1985).

There are 4 kinds of biting lice and 5 kinds of sucking lice that can attack sheep and goats (Durdan, 2002).

#### 1.2.1.1. Biting Lice

*Bovicolacrassipes* (the Angora-goat-biting louse) and *Bovicolalimbata* are the two major species of biting lice. *Bovicolacaprae* and *Bovicolaovis* (goat-biting louse and sheep-biting louse) are of lesser significance. All four species live on the skin surface feeding on bits of hair and other skin surface debris. Egg hatch requires 9 to 12 days, and the entire life cycle averages 1 month. The biting lice of goats are world-wide in distribution with winter-time populations being most severe. In Florida high populations have been observed year round.

#### 1.2.1.2. Sucking Lice

Five species of sucking lice attack sheep and goats. The following are of importance:

1. **African blue louse:** They are found on the body, head, and neck. Heavy populations have caused the death of the host.
2. **Foot louse:** This louse prefers the feet and legs of goats and sheep. Populations peak in the spring, and at that time the lice may affect the belly area as well. Scrotum infestations on bucks are common. Lambs

seem to have the highest infestations. Egg hatch for this species of louse takes longer than the other species. Therefore, retreatment should be applied after 3 weeks for most insecticides.

3. **Goat-sucking louse:** Populations are dispersed over the animal's body. These lice are also found on sheep.
4. **The face and body louse and the long-nosed cattle louse:** These are minor pests.

### **1.2.1.3. Transmission of lice:**

Lice are generally transmitted from one animal to another by contact. Transmission from herd to herd is usually accomplished by transportation of infested animals, although some lice may move from place to place by clinging to flies (phoresy). Lice are most often introduced to herds by bringing in infested animals. Louse populations vary seasonally, depending largely on the condition of the host. Most sucking and biting lice begin to increase in number during the fall and reach peak populations in late winter or early spring. Summer populations are usually minimal, causing no obvious symptoms. Animals under stress will usually support larger louse populations than normally found ([Lloyd, 2002](#)).

### **1.2.1.4. Clinical signs of Louse-infested animals**

Louse-infested animals may be recognized by their dull, matted coat or excessive scratching and grooming behavior. Sucking lice pierce the host's skin and draw blood. Biting lice have chewing mouthparts and feed on particles of hair, scab and skin exudations. The irritation from louse-feeding causes animals to rub and scratch, causing raw areas on the skin or loss of hair. Weight loss may occur as a result of nervousness and improper



nutrition. Milk production is reduced about 25 percent. Also, the host is often listless and in severe cases the loss of blood to sucking lice can lead to anemia (Butler, 1985).

#### **1.2.1.5. Control of louse infestations**

Control of louse infestations is needed whenever an animal scratches and rubs to excess. Louse control is difficult since pesticides do not kill the louse egg. Since eggs of most species will hatch 8 to 12 days after pesticide application, retreatment is necessary 2 weeks after the first pesticide application (Kaufman *et al.*, 2018).

#### **1.2.2. Ticks**

Ticks may be divided into two major groups namely the soft ticks (Argasids) and the hard ticks (Ixodids). Hard ticks can further be divided into three (one host, two host and three host ticks) depending upon the number of hosts involved in their life cycle. Ticks that commonly infest sheep have 4 distinct life stages: egg, larva, nymph and adult. A larval or seed tick feeds on small vertebrate animals then drop off the host and molts to the nymph. The 8 legged nymph feeds on small vertebrate animals, drops to the ground and molts to the adult. The adult attaches to a third host, (dog, human, and sheep) drops to the ground and lays eggs (1000-5000) and dies. This is termed a 3 host life cycle and usually takes 3 years (Gregory Johnson, 2011; Kaufman and Butler, 2012).

Different tick species have different locations of attachment. The location can indicate the type of tick (Kufman *et al.*, 2012).

**Site of tick attachments on animals**

Tick species	Common sites of attachment
<i>Haemaphysalis</i>	Ear, limbs, dewlaps, neck, tail, axial, groin and abdomen
<i>Boophilusmicroplus</i>	Ear, limbs, dewlaps, abdomen and chest
<i>Boophilusdecoloratus</i>	Abdomen, limbs, dewlap and groin
<i>Ambylommavariegatum</i>	Under the tail, margin of the anus, limbs and groin
<i>Rhipicephalusevertsi</i>	Neck, under the tail and around the anus
<i>Hyaloma a. anatolicum</i>	Chest, abdomen, neck, udder and scrotum

## Description of ticks affecting sheep and goats

<i>DESCRIPTION</i>	<i>COMMON SPECIES</i>	<i>EFFECT ON HOST</i>	<i>COMMENTS</i>
<p>-Obligate ectoparasites</p> <p>-Have long mouth parts</p> <p>- divided into 2 main families: Argasidae and Ixodidae</p> <p>-Argasids(soft ticks) do not have hard chitinous plates on the dorsal surface of their bodies</p> <p>-Ixodes(hard ticks) have dorsal plates</p> <p>-Have peripheral sensory organs on legs, body, mouthparts which enable them to communicate with other ticks</p>	<p><i>-Demodexcaprae</i></p> <p><i>-Ixodesholocyclus</i></p> <p><i>Rhipicephalussanguineus</i></p> <p><i>-Rhipicepalusmicroplus</i></p> <p>-</p> <p><i>Boophilusdecoloratus</i></p>	<p>-Transmit tick borne diseases eg theileriosis, babesiosis, anaplasmosis , heart water</p> <p>-skin,udder and ear damage</p> <p>-reduced growth</p> <p>-low milk production</p> <p>-predispose animals to secondary attacks from other parasites</p>	<p>-more prevalent when temperature and rainfall is high</p> <p>-cause greatest economic loss</p> <p>-about 35 tick species are found in Southern Africa</p> <p>-belong to phylum arthropoda</p> <p>-soft ticks usually feed on one host</p> <p>-bites from hard ticks are usually painless</p> <p>-Ixodid's life cycle has 4 stages:egg, larvae, nymph, adult</p> <p>-have an instant response to chemical eg CO2 and NH3 which indicate presence of host</p>

### **1.2.2.1. Clinical signs of tick-infested animals**

The effects of ticks are various including reduced growth, milk and meat production, damaged hides and skins, transmission of tick-borne diseases of various types and predispose animals to secondary attacks from other parasites such as screw worm flies and infection by pathogens such as *Dermatophilos congolensis*, the causative agent of streptothricosis. Other losses directly attributable to ticks include skin damage that greatly lowers value of the skin. In addition to these ticks create ear infections if not controlled. They cause skin irritation, wool destruction, and even anemia if not controlled.

### **1.2.2. 2. Control of ticks infestations**

Treat lambs when the ewes are treated, but spray lightly and use extreme caution when treating lambs less than 3 months of age. Spraying or dipping once a year will usually keep ticks under control. Treat all bucks and replacement ewes before adding them to the flock. Dipping does a more thorough job than spraying, but spraying can provide good control. High-pressure sprayers are more convenient and usually more effective for treating large flocks. An adequate job can be done with low-pressure sprayers (40–100 lb) if 1 to 2 pounds of household detergent are added to each 100 gallons of water (Hinkle, 2017). Also can use some traditional methods of external parasite control, regular removal of moist bedding, hay and manure along with preventing the accumulation of weed heaps, grass cuttings and vegetable refuse is very helpful. Effect and incidence of ectoparasites can usually be reduced by improving nutrition, hygiene of animal houses and by occasional spraying or dipping (ESGPIP, 2010).

### **1.2.3. Prevalence of ectoparasite infestations in sheep and goats**

The result of [Adang et al., 2015](#)) revealed nonstatistically significant differences in the prevalence of ectoparasite infestations between sheep and goats, in age of sheep and goats, in sex and age of sheep. His study concluded that ectoparasites are common to both sheep and goats in Gombe and could affect their health and productivity as well as their economic and market value.

### **1.2.4. Ticks infest sheep and goats in the Sudan.**

Very little work has been carried out on ticks infesting small ruminants in the Sudan. Of some 70 tick species recorded in the Sudan, 34 species of different genera were collected from sheep and goats, two belonging to the genus *Amblyomma*, seven to the genus *Hyalomma*, 22 to the genus *Rhipicephalus* and three to the genus *Boophilus*. Nevertheless, their distribution, the seasonal abundance and population dynamics are poorly studied. The variable climatic conditions of the country and the importance of the animal wealth in the national economy are all factors that call for more efforts to study the tick problem in this country ([Osman, 1997](#)).

### **1.2.5. Effect of ectoparasite on hematology profile:**

Previous study showed hematological changes in animals heavily infested with ectoparasite. The haematological findings revealed significant reduction in haemoglobin, packed cell volume, total erythrocyte count ([Biswal et al., 1988](#)). [Springell, \(1971\)](#) reported reduction in haematocrit and haematological values as compared to those calves free from tick infestation. An increase in

number of eosinophils and lymphocytes in ticks infested cattle was also reported by (Gebelhoff, 1974; William *et al.*, 1977). Decrease in neutrophils and monocytes in ticksinfested cattle was also reported by Maske, (1993). Lower Hb, TEC and marked leucocytosis is due to anaemia because of blood sucking ability of parasites and haemorrhage (Soulsby, 1982).

#### **1.2.6. Effect of ectoparasites on blood trace element:**

External parasitic infestations or ectoparasitosis are a cause of severe health problems in livestock that may be accompanied by a decrease in blood trace element and mineral levels (Emerson *et al.*, 1984). Trace minerals participate in many important catalytic, enzymatic, and structural functions of higher vertebrates, and their concentrations in mammals depend on several environmental and biological conditions (Underwood, 1977). In animals, hair and feathers are known matrices for excretion of trace elements. Zinc, copper, and calcium have been measured in the Van region of Turkey, 36–50% of commercially important animal species have been found to suffer from lice (*Bovicola caprae*, *Linognathus africanus*, *Linognathus ovillus*, and *Linognathus pedalis*) infestations (Deger, *et al.*, 1994; Deger, *et al.*, 2002).

#### **1.2.7. Effect of ectoparasites on blood protein:**

The results of Khalid *et al.*, (2018) illustrated the effect of ectoparasites such as lice and ticks on some biochemical parameters of blood serum, where there was a significant decreasing ( $P < 0.05$ ) in total protein

concentration, He attributed the reason of low concentration of total protein to the lack discomfort and loss of appetite for food.

# **CHAPTER TWO**

## **MATERIALS AND METHODS**

### **2.1. Experimental Animals and design**

Proposed study was conducted on 20 sheep and goat from farms at Hilut Ku Ku - Khartoum Bahry Province - Sudan, average age (2-4 years) including 14 infested animals with ectoparasite and 6 animals none infested with ectoparasite.

Clinical inspection of each sampled animal was performed visually and by multiple fleece partings, followed by physical examination of skin, inspection, and palpation of the skin across all parts of the animal for the presence of parasites and gross lesions indicating the clinical form of infestation by ectoparasites. Animals found with ectoparasites were considered as positive.

### **2.2. Blood samples**

#### **2.2.1. Blood Collection**

Jugular blood samples (5ml) were collected from selected goat and sheep into clean dry vials containing the sodium salt of ethylene diamine tetra acetic acid (EDTA) as anticoagulant for haematological analysis. Five ml of blood without addition of anticoagulant was allowed to clot at room temperature for one hour, kept overnight at 4 c°, centrifuged at 3000 rpm for 15 minutes. Clear unhaemolyzed serum was separated and then stored at -20 c° until used.



## **2.2.2. Haematological examinations**

The methods described by Jain, (1986) were used for the determination of the haematological examinations.

### **2.2.2.1. Leukocytic series**

#### **2.2.2.1.1. Total white blood cells (WBC) count**

WBC was counted in an improved Neubauer haemocytometers (Hawksley and Sons Ltd, England) using Turk's solution as a dilution fluid (glacial acetic acid 1 ml, 1% aqueous gentian violet 1 ml, distilled water up to 200 ml ).

#### **Principle**

WBC count is facilitated by destruction of erythrocytes in a blood sample by means of acetic acid.

#### **Procedure**

The pipette was filled with blood to the 0.5 mark and then filled with the diluting fluid to the 11 mark on the stem distal to the bulb. The dilution of blood obtained was 1:20. Cover slip was pressed on the surface of the haemocytometer. The diluted blood was mixed thoroughly and the counting chamber was filled carefully, and then the cells were allowed to settle. Under low power (x10 objective) of the bright field microscope (KYOWA, Japan), the number of white blood cells was counted in each of the 4 large corner squares. The calculation was made as follows:

Total cells count = N

Volume = 4 mm x 0.1 mm (depth of cell) = 0.4 cu mm

Dilution = 1: 20

Total WBC count ( $\times 10^3 / \mu\text{l}$ ) =  $N \times 1/0.4 \times 20 = N \times 50$

## **2.2.2.2. Erythrocytic series**

### **2.2.2.2.1. Haemoglobin (Hb) concentration**

Hb concentration was determined by the cyanomethoemoglobin technique using a haemoglobinmeter (Evans Electro Selenium Ltd, England).

#### **Principle**

The method was based on the conversion of haemoglobin by means of Drabkin's solution (0.2 g potassium cyanide, 0.2 g potassium ferricyanide and 1g sodium bicarbonate per liter distilled water) to cyanomethoemoglobin.

#### **Procedure**

Five ml of Drabkin's solution were pipetted into three test tubes labeled as test tube, blank tube and standard tube. 0.02 ml of blood was placed in the test tube; 0.02 ml of standard was placed in standard tube and 0.02 ml distilled water were placed in the blank tube. The tubes contents were mixed and the optical density (O.D) was read against blank at a wave-length of 540 nm. Haemoglobin concentration (C) was then calculated according to the following equation:

$$C \text{ (g/dl)} = \frac{\text{O.D of sample}}{\text{O.D of standard}} \times \text{standard}$$

### **2.2.2.2.2. Packed cell volume (PCV)**

The PCV was measured in duplicate using plain capillary tubes. The capillary tubes were filled with blood to about 3/4; and one end was sealed by cristaseal. Then the tubes were centrifuged at 3000 r.p.m for 5 min in a

micro-haematocrit centrifuge (Hawksley, London) and the PCV as percentage of whole blood was determined using a reader.

### **2.2.2.2.3. Total Red blood cells (RBCs) count**

RBCs were counted in an improved Neubauer haemocytometers (Hawksley and Sons Ltd, England) using formal citrate (1% formal saline was added to 3% aqueous trisodium citrate) as a diluting fluid.

#### **Principle**

The addition of formal-citrate solution to a blood sample destroys leucocytes and thus makes it easier to count the remaining erythrocytes.

#### **Procedure**

The pipette was filled with blood to 0.5 mark. Then it was held in vertical position in the diluting fluid and by applying suction, the blood sample and the fluid reach exactly 101 mark. The dilution of blood obtained was 1: 200. The blood and the diluting fluid were mixed thoroughly and left for 2 min before counting. The cover slip was pressed on the haemocytometer surface tightly. The diluted blood was filled the space between the counting chamber and the cover slip, taking care that no fluid flows into surrounding moats, and then the cells were allowed to settle for 5 min. Using high power (x40) of the bright field microscope (Kyowa, Japan), all the red cells were counted in 5 squares in the corner and middle of the central area.

#### **Calculation**

Total cells counted = N

Volume =  $\frac{1}{5} \times \frac{1}{10}$  mm (depth of cell) = 0.02 cu mm

Dilution = 1: 200

Red cells per cu mm =  $N \times \frac{1}{0.2} \times 200 = N \times 10.000$

#### **2.2.2.2.4. Mean corpuscular values**

##### **2.2.2.2.4.1. Mean corpuscular volume (MCV)**

The MCV was calculated from RBC count and PCV values as follows:

$$\text{MCV (Femtoliter)} = \frac{\text{PCV\%} \times 10}{\text{RBC count (x } 10^6 / \mu\text{l)}} \quad (\text{FL})$$

##### **2.2.2.2.4.2. Mean corpuscular haemoglobin (MCH)**

The MCH was calculated from RBC and haemoglobin values as follows:

$$\text{MCH (Picograms)} = \frac{\text{Hb (g/dl)} \times 10}{\text{RBC count (x } 10^6 / \mu\text{l)}} \quad (\text{pg})$$

##### **2.2.2.2.4.3. Mean corpuscular haemoglobin concentration (MCHC)**

The MCHC was calculated from Hb and PCV values as follows:

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)} \times 100}{\text{PCV\%}} \quad (\text{g/dl})$$

### **2.3. Biochemical examinations**

#### **2.3.1. Determination of serum copper and iron concentration**

The method used for the determination of serum copper and iron, were obtained from Perkin-Elmer Analytical methods for Atomic Absorption Spectrophotometry.

For the determination of serum copper, the serum sample was diluted with an equal volume of deionized water and then analyzed for copper by atomic absorption spectrometer (Perkin- Elmer model-3110, USA), using air/ acetylene, fuel rich flame (air 5.0 liter min<sup>-1</sup> , C<sub>2</sub>H<sub>2</sub> 1.0 liter min<sup>-1</sup>) with copper lamp at wavelength 324.8 nm.

For determination of serum iron 1 ml of serum sample was diluted with an equal volume of a 20% (W/V) trichloroacetic acid (TCA) solution in a test tube. The tube was capped loosely, mixed and heated in a heating block at 90 °c for 15 min. Then it was cooled and centrifuged. Then the supernatant was analyzed for iron by atomic absorption spectrophotometer (Perkin-Elmer model-3110, USA) using air/ acetylene, fuel rich (air 5.0 liter min<sup>-1</sup>, C<sub>2</sub>H<sub>2</sub> 1.0 liter min<sup>-1</sup>) with iron lamp at wavelength 324.8 nm.

### **2.3.2. Determination of serum total protein concentration**

Total serum protein concentration was determined by the Biuret reagent using a commercial kit (Linear Chemical - Spain).

#### **Principle**

The method is based on the Biuret reaction in which a chelate is formed between Cu<sup>2+</sup> ion and the peptide bonds of the protein in alkaline solution to form a violet coloured complex. The intensity of colour produced is proportional to the concentration of protein in the sample.

#### **Kit reagent**

Working reagent: Tartrate (15 µ/l), NaI (100 µ/l), KI (15µ/l), CuSO<sub>4</sub> (5µ/l).

#### **Procedure**

Three test tubes were labeled as test, standard and blank. 100µl serum was placed in the test tube, 100µl standard's solution was placed in the standard tube. 5 ml of working solution were added to the three test tubes. Then the tubes were mixed and incubated for 20 minutes at 37 °c. The optical density (O.D) of sample and standard were read using a spectrophotometer (Jenway 6505 U.V/VIS) at a wave length 540 nm against reagent blank solution and serum total protein concentration (C) was calculated as follows:

$$C \text{ (g/dl)} = \frac{(\text{O.D}) \text{ Sample}}{(\text{O.D}) \text{ Standard}} \times \text{Standard concentration}$$

### **2.3.3. Determination of serum albumin concentration**

Serum albumin concentration was determined according to Bromocresol green method (BCG) using commercial kit (Linear Chemical-Spain)

#### **Principle**

The measurement of serum albumin is based on its quantitative binding to the indicator 3, 5, 5, 5tetra bromo cresol (Bromocresol green, BCG).

#### **Kit reagent**

Reagent /R1 (BCG): Succinato buffer pH 4.2 (75µ/l), Bromocresol (15 µ/l), Brij (0.57ml/l).

Reagent/R2 (standard): Bovine albumin 5g/100ml.

#### **Procedure**

Three test tubes were labeled as test, standard and blank. 20 µl serum was placed in the test tube, 20µl standard solution (R2) was placed in the standard tube. 4 ml of reagent (R1) were added to the three test tubes. Then the tubes were mixed and incubated for 10 minutes at 37 °c. The optical density (O.D) of sample and stander were read using a spectrophotometer (Jenway 6505 UV/VIS) at a wave length 640 nm against reagent blank solution and serum albumin concentration (C) was calculated as follows:

$$C \text{ (g/dl)} = \frac{(\text{O.D}) \text{ Sample}}{(\text{O.D}) \text{ Standard}} \times \text{Standard concentration (5)}$$

#### **2.3.4. Determination of serum total globulins concentration:**

Serum globulins concentration was obtained by subtracting serum albumin concentration from that of serum total protein concentration.

#### **2.4. Statistical analysis**

Data were analyzed with SPSS (Statistical package for social sciences) statistical software, version 19. Data are presented as the mean  $\pm$  standard deviation (SD). Analysis of variance (ANOVA), T-test were used. A level of P value of less than 0.05 was considered statistically significant.

## CHAPTER 3

### RESULT

#### 3.1. Clinical signs

Sheep and goat in the positive group showed multiple clinical signs. These included scratching themselves by their teeth, hind hooves and horns. In extreme cases, affected animals rubbed on walls of shelters, fence posts and any solid object. Decreased feed intake, resulting in decreased weight gains and milk production. Their skin showed rough coat, varying degrees of hair loss, scaling, thickening and wrinkling. In addition to these the eyes mucus membranes of the affected animals appeared pale (Figures 1-7). The negative animals group appeared normal.



**Figure 1:** Goat (positive group) showing rough hair coat and emaciation.





**Figure2:** Goat (positive group) showing loss of hair coat and emaciation.



**Figure 3:** Sheep (positive group) showing loss of coat and emaciation.



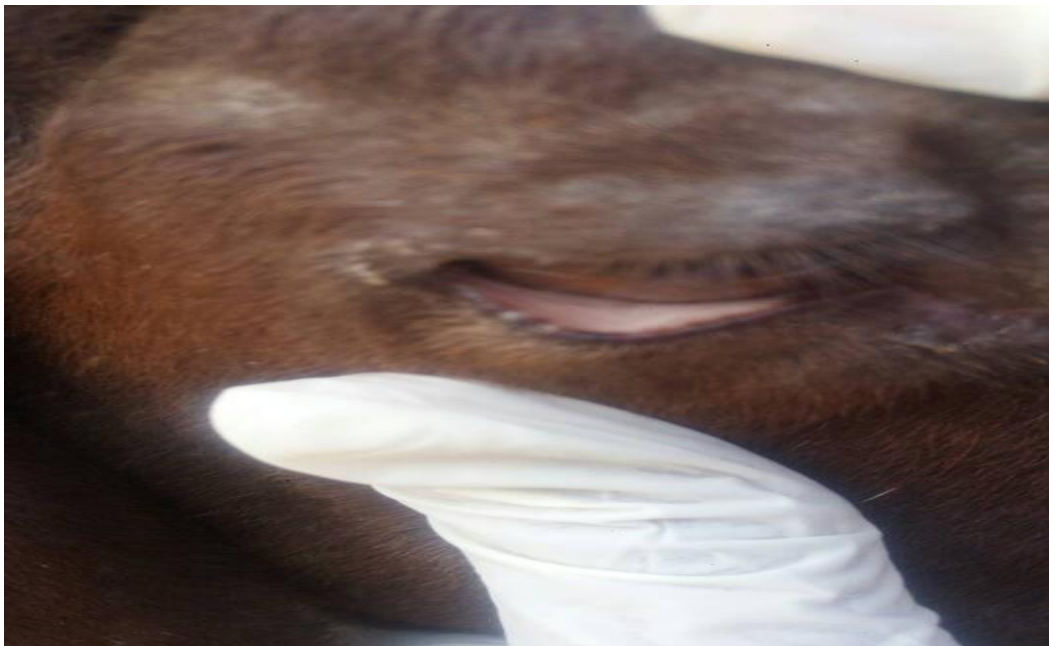
**Figure 4:** Sheep (positive group) showing ticks in its ear.



**Figure 5:** Sheep (positive group) showing ticks near its eye.



**Figure 6:** Goat (positive group) showing ticks in the anal region and tail



**Figure 7:** Goat (positive group) showing paleness of eye mucus membrane.



**Figure 8:** Goat (positive group) showing alopecia, crusts, scales and lice.

### **3.2. Haematological findings**

The haematological findings are shown in table (1). The overall Hb concentrations, RBCs counts, PCV, MCV, MCH, MCHC and WBCs values showed no significant differences ( $P>0.05$ ) between negative and positive groups.

**Table (1): The overall mean ( $\pm$  SD) haematological values of sheep and goat**

<b>Groups</b> <b>Parameters</b>	<b>Negative group</b>	<b>Positive group</b>	<b>Level of significance</b>
<b>RBC (X 10<sup>6</sup> mm<sup>3</sup>)</b>	10 $\pm$ 1.8 <sup>a</sup>	8.4 $\pm$ 2.5 <sup>b</sup>	*
<b>Hb ( gm/dl )</b>	9.0 $\pm$ 1.1	8.4 $\pm$ 9.9	NS
<b>PCV ( % )</b>	27.25 $\pm$ 4.57	25.05 $\pm$ 3.29	
<b>MCV ( m3 )</b>	27 $\pm$ 12.2	34.01 $\pm$ 8.4	
<b>MCH ( pg )</b>	9 $\pm$ 1.28	10 $\pm$ 3.2	
<b>MCHC ( % )</b>	33.5 $\pm$ 1.32	33 $\pm$ 3.1	
<b>WBCs (X 10<sup>3</sup>)</b>	8.1 $\pm$ 1.7	7.6 $\pm$ 2.6	

Values are means  $\pm$  standard deviation (SD)  
(animals No = 20).

Different superscripts are significantly ( $P < 0.05$ ) different

NS: not significant ( $P > 0.05$ )

### **3.3. Biochemicals analysis**

#### **3.3.1. Copper concentration in serum of sheep and goat**

Mean value of copper concentration in serum is shown in table (2). There was significant decrease ( $P < 0.01$ ) of copper concentration in serum of the positive group as compared to the negative group.

### **3.3.2. Iron concentration in serum of sheep and goat**

Mean value of iron concentration in serum is shown in table (2). There was significant decrease ( $P<0.01$ ) of iron concentration in serum of the positive group as compared to the negative group.

### **3.3.3. Total protein concentration in serum of sheep and goat**

Mean value of total protein concentration in serum is shown in table (3). There was significant decrease ( $P<0.01$ ) of total protein concentration in serum of the positive group as compared to the negative group.

### **3.3.4. Albumin concentration in serum of sheep and goat**

Mean value of albumin concentration in serum is shown in table (3). There was significant decrease ( $P<0.01$ ) of albumin concentration in serum of the positive group as compared to the negative group.

### **3.3.5. Globulin concentration in serum of sheep and goat**

Mean value of globulin concentration in serum is shown in table (3). There was significant decrease ( $P<0.01$ ) of globulin concentration in serum of the positive group as compared to the negative group.

**Table (2): Serum copper and iron concentrations of sheep and goat**

	<b>Serum Cu concentration (mg/ml)</b>	<b>Serum Fe concentration (mg/ml)</b>	<b>Level of significant</b>
<b>Negative group</b>	0.4220±0.0595 <sup>a</sup>	77.50±2.495 <sup>a</sup>	**
<b>Positive group</b>	0.1323±0.0376 <sup>b</sup>	57.86±2.172 <sup>b</sup>	

Results are mean ± SD (animals No = 20). Means in the same column with different superscripts are significantly ( $P < 0.01$ ) different.

\*\* : significant ( $P < 0.01$ )

**Table (3): Serum protein concentrations of sheep and goat**

	<b>Serum total protein concentration (g/dl)</b>	<b>Serum albumin concentration (g/dl)</b>	<b>Serum globulin concentration (g/dl)</b>	<b>Level of significant</b>
<b>Negative group</b>	7.79±0.235 <sup>a</sup>	3.48±0.332 <sup>a</sup>	4.310±0.18 <sup>a</sup>	**
<b>Positive group</b>	5.7±0.201 <sup>b</sup>	2.71±0.132 <sup>b</sup>	2.89±0.284 <sup>b</sup>	

Results are mean ± SD (animals No = 20). Means in the same column with different superscripts are significantly ( $P < 0.01$ ) different.

\*\* : significant ( $P < 0.01$ )

## CHAPTER 4

### DISCUSSION

The present study investigated the effect of lice and ticks on sheep and goat health.

The major clinical manifestations of lice and ticks infestation in sheep and goats were alopecia, rough coat, irritation, papulo-crustous dermatitis, scaling, thickening and wrinkling skin. These lesions and signs are attributed to the irritation and hypersensitivity reaction to the antigens present in the lice saliva (Taylor *et al.*, 2007). Also infested animals were emaciated due to the action of parasites as a stressor stimulate the primary stages of stress in decrease feed intake.

The hematological findings revealed no significant ( $P > 0.05$ ) differences between infested and non infested group of goats and sheep in all blood parameters studied except of significant reduction in total RBCs count of infested group as compared of none infested group . Our finding are in disagreement with the findings of (Ajithet *et al.*, 2017; Iqbal *et al.*, 2018) who found a significant decrease in hemoglobin, PCV and TEC levels in goats affected with lice infestation. Differences may have been attributable to low sample sizes in our study. However, MCV, MCH and MCHC were not found to be significantly variable in the infested and non-infested group. This may be attributed to the normocytic normochromic anemia in the infested goats of the study area.

Serum copper and iron concentration of the infested group were significantly ( $P < 0.01$ ) lower than that in none infested group. Because of interference in feeding, many essential nutrients, especially minerals have been found to be deficient in affected animals (Nair, 2007). In a report,



copper and zinc were found to be decreased in lice infested goats and both the minerals play an important part in erythropoiesis and play an important part in the immunity to infections (Iqbal *et al.*, 2018). Lice feed on human blood, and heavy and chronic lice infestation can lead to chronic blood loss with resultant iron deficiency anaemia (Althomali *et al.*, 2015; Guss, *et al.*, 2011).

The results of biochemical parameters of serum in this study showed a significant decrease ( $P<0.01$ ) in the values of total protein, albumin and globulin concentrations which are in matching with the findings of (Khalid *et al.*, 2018) and disagree with the findings of (Sharma *et al.*, 1990) who found that total serum protein (TSP) was higher in infected goats than normal goats. Our findings suggest a physiological response to parasite feeding.

## **CONCLUSION**

The results of the present study indicate that there were significant alterations in serum copper, iron, total protein and globulin of sheep and goats infested by lice and ticks.

## **RECOMMENDATIONS**

- 1- Treatment and control programs of ectoparasites should be encouraged.
- 2- More studies should be done to investigate the effects of ectoparasites in animal health.
- 3- Further investigations of others trace elements that have link with ectoparasites infestation should be determined.
- 4- Nutritional supplementation of minerals is required to ectoparasites infested animals.
- 5- Extensive surveys should be done to establish normal range of sero biochemical levels in Sudanese sheep and goats.

## REFERENCES

- Adang, K.L., Ayuba, J. and K.P. Yoriyo.** (2015). Ectoparasites of Sheep (Ovisaries L.) and Goats (Capra hirus L.) in Gombe State, Nigeria. Pakistan Journal of Biological Sciences 18 (5): 224-231, 2015.
- Ajith, Y., Dimri U, Singh, S.K., Gopalakrishnan, A., Devi, G., Verma, M.R, et al.,** (2017). Lice induced immuno-oxidative wreckage of goats. Vet Parasitol; 242:24-30.
- Althomali, S.A., Alzubaidi, L.M., Alkhaldi, D.M.** (2015). Severe iron deficiency anaemia associated with heavy lice infestation in a young woman. BMJ Case Rep. 2015 Nov 5; 2015.pii: bcr2015212207. doi: 10.1136/bcr-2015-212207.
- Bates, P.** (2012). External Parasites of Small Ruminants (A Practical Guide to their Prevention and Control). Hardback / 256 Pages / 9781845936648. formerly VLA, UK.
- Biswal, B., Misra, S.C. and P.C. Bisoi.** (1988): Jour.Vet. Parasitol. 2(1):9-13.
- Butler, J. F.** (1985). Lice affecting livestock. *In*: Livestock Entomology (R. E. Williams, R. D. Hall, A. B. Broce and P. J. Scholl, eds.), pp. 101–127. Wiley, New York.
- Deger, S., S. Tasci, Y. Akgül, and M. Alkan.**(1994). Ectoparasitic dermatitis in domestic animals in Van region, Y. Y. -. Vet Fak. Derg.5, 155–161 (1994).
- Deger, y., S. Dede, and S. Deger.** (2002). Serum Copper, Zinc, and Calcium Concentrations in Lice-Infested Sheep. Biological Trace Element Research Vol. 88.

**Durden, L. A.** (2002). Lice (Phthiraptera), *In: Medical and Veterinary Entomology*, (G. R. Mullen and L. A. Durden, eds.), pp. 45–65. San Diego, CA: Elsevier Science

**Emerson1, K. C., C. Masser, and J. J. O. Whitaker.** (1984). Lice (Mallophaga and Anoplura) from mammals of Oregon, *Northwest Sci.* 58, 153–161 (1984).

**ESGPIP** (2010) Control of external parasite of sheep and goat, Ethiopian Society of Animal Production (ESAP). Technical bulletin 41: 2-11.

**Gebelhoff, E.,** (1974). Effect of tick infestation with *B. decoloratus* and *H.a. excavatum* on blood picture of calf. Dissertation, Frele Univ. Berlin, PP.55.

**Gregory Johnson.** (2011). Managing Ectoparasites on Sheep, Montana State Univ., Extension Pub., 300-1111SA.

**Guss. D.A.1, Koenig, M., Castillo, E.M.** (2011). Severe iron deficiency anemia and lice infestation. *J Emerg Med.* 2011 Oct; 41(4):362-5. doi: 10.1016/j.jemermed.2010.05.030. Epub 2010 Jul 24.

**Hinkle, N.C.** (2017). Sheep and goats: External parasite control. In *Georgia Pest Management Handbook* (pp. 95–96). Athens: University of Georgia College of Agriculture & Environmental Sciences.

**Iqbal, R.M, A.Q Mir, R. Waseem, S.A. Beigh, S.A. Hussain, S.U. Nabi and H.U., Kaufman P.E., P.G. Koehler and J.J. Butler** (2012). External Parasites of Sheep and goats, Univ. of Florida, Extension Pub., ENY-273, Revised- May 2009, Reviewed- June 2012

**Kaufman, P.E , Koehler P. G. , and Butler, J. F.** (2018). External Parasites of Sheep and Goats. ENY-273/IG129: <http://edis.ifas.ufl.edu>

**Khalid Thamir Mattar AL-Shaibani, Hiba Riyadh Jameel Al-abo**

**Lloyd, J. E.** (2002). Louse flies, keds and related flies (Hippoboscoidea), *In: Medical and Veterinary Entomology*, (G. R. Mullen and L. A. Durden, eds.), pp. 349–362. San Diego, CA: Elsevier Science.

**Maske, D.K.**(1993): *Jour.vet.Parasitol.*,7(2): 130-131.

**Nair SS. Nauriyal DS.** (2007). Diagnostic significance of haematological changes associated with various canine dermatoses. *Intas-polivet* ; 8(1):68-72.

**Nazik, Z. Eisa, Salih, A, Babiker and Hamid, S. Abdalla** (2017).Effect of Natural Gastrointestinal Parasitic Infection on Fattening Performance of Sudan Desert Sheep. *Journal of animals science and livestock production*. Vol.1 No.1:005. DOI: 10.21767/2577-0594.100005

**NesradinYune, NejashAbdelaYune and Abdela.** (2017). Epidemiology and Economic Importance of Sheep and Goat Pox: A Review on Past and Current Aspects. *J Vet SciTechnol* 2017, 8:2.

**Osman, A.M.** (1997). Ticks infesting sheep and goats in the Sudan.*Parassitologia*; 39(2):139-42.

**Sharma, R.L., Peer, F,U., &Bhat, T.K.** (1990). Antiparasitic activity of Ivermectin against Natural Psoroptes communes var. ovis infestation in Kashmir Merino Sheep. *Indian Vet. J.* 67:264–66

**Soulsby, E.J.L.,**(1982). *Helminths, Arthropodes and Protozoa of Domesticated Animals*,7th ed the ELBS and Baillieve, Tindal cass LTD, London, pp.766.

**Springell, P.H.** (1971). *Austral.J.Biol. Sci.*24: 381-389.

**Taylor, M.A., Coop, R,L., Wall, R.L.**(2007). *Veterinary Parasitology*, 3rd ed. Blackwell Publishing, Oxford. 2007, 586-593.

**Tewodros Fentahun, FasilWoldemariam, MershaChanie and Malede Berhan** (2012). Prevalence of Ectoparasites on Small Ruminants in and Around Gondar Town. American-Eurasian Journal of Scientific Research 7 (3): 106-111.

**Underwood, E. J.** (1977). Trace Elements in Human and Animal Nutrition.4th ed., Academic, New York (1977).

**William, R.E., Hair, J.A. and Bhekner, R.G.,** (1977). J.Econ.Ent.,70:229-233.