

Thesis Ref. No. \_\_\_\_\_



**EXPERIMENTAL STUDY ON SHEEP INFESTED WITH *Bovicola ovis* AND  
*Melophagus ovinus* OF PATHOLOGICAL CHANGES, PROCESSED SKIN  
DEFECT AND EFFECT OF TREATMENT IN IMPROVING SKIN QUALITY**

MSc Thesis

By

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of Pathology and Parasitology

October, 2015  
Bishoftu, Ethiopia

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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical veterinary pathology

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Pathological Changes, Processed Skin Defect and Effect of Treatment in Improving Skin  
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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Getachew Kebebew entitled: Experimental Study on Sheep Infested With *Bovicola Ovis* and *Melophagus Ovinus* of Pathological Changes, Processed Skin Defect and Effect of Treatment in Improving Skin Quality and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Tropical veterinary pathology.

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## **STATEMENT OF THE AUTHOR**

First, I declare that this thesis is my original work and all that sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirement for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and it is deposited at the University/College library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for award of any academic degree, diploma or certificate.

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## ACRONYMS

AAU	Addis Abeba University
ADARDO	Ada‘a District Agricultural and Rural Development Office
ANOVA	Analysis of Variance
CSA	Central Statistical Authority
CVMA	College of Veterinary Medicine and Agriculture
ETB	Ethiopian Birr
FAO	Food and Agricultural Organization
GDP	Gross Domestic Product
NAHDIC	National Animal Health Diagnostic and Investigation Center
NBE	National Bank of Ethiopia
QASE	Quality and Standard Authority of Ethiopia
SD	Standard Deviation
TWBC	Total White Blood Cell
UNIDO	United Nations Industrial Development Organization
USD	United State Dollar
WBC	White blood cell

## SUMMARY

Experimental study was conducted from January, 2015 to August, 2015 on sheep experimentally infested by *B .ovis* and *M. ovinus* with the objective of identifying specific pathological changes they induce, the type of defect at pickle and their effect on skin quality and effect of treatment on resumption of skin to normal state. For this purpose, thirty indigenous sheep of Arsi origin approximately 18 months age were bought from market. They were treated against infection and parasites and kept for a month to stabilize them before the commencement of the experiment. The sheep were then grouped in to five groups each with six individual sheep as non-infested control, *B .ovis* infested untreated, *B .ovis* infested treated, *M. ovinus* infested untreated and *M. ovinus* infested treated. *B .ovis* and *M. ovinus* were manually collected from donor sheep; identified, counted and then infested at 500 *B .ovis* and 50 *M. ovinus* per sheep to the respective treatment groups. All groups were managed under similar condition and followed for 95 days and Sheep within the infested treated groups were treated with diazinon and followed for additional 30 days. The total WBC count was increased in *B .ovis* and *M. ovinus* infested groups compared to non-infested control group. However, the statistically significant was only for groups infested by *M. ovinus* ( $P < 0.05$ ). In all infested groups the differential leukocyte count was increased; nevertheless, the significant difference was only for eosinophil ( $P < 0.05$ ). The common gross lesions noticed were skin discoloration with excreta, crust formation, macules and erythema for *M. ovinus* and *B .ovis* with slight difference in intensity of lesion. Microscopically, hyperkeratosis and acanthosis were more severe in groups infested by *M. ovinus* than *B .ovis*. The density of inflammatory cells infiltrating dermis was severely intensive in groups infested by *M. ovinus* than *B .ovis*. In groups that were treated, the gross pathological lesions except skin discoloration were totally disappeared within 30 days; nonetheless, the microscopic lesions persisted with a decrease in number of inflammatory cells specifically that of eosinophils. At pickle stage *M. ovinus* was characterized by large elevated cockles and *B .ovis* by extensive but small cockles. All processed skins from *B.ovis* and *M. ovinus* infested groups degraded and lied toward the stages of moderate

grades (5 or 6) and less which is very poor. In treated group 50% of the processed skins from *B .ovis* were improved in quality and were lied to grades 1 to 4 (very good to good) but only 33% of skin from *M. ovinus* were improved and lied to 1 to 4 grades. It could be concluded that infestation by *B .ovis* and *M. ovinus* cause severe to mild gross pathological lesions however severe microscopic lesions to sheep skin and reduced the quality of processed skin from top grades to low grade. Definitely treatment improves skin quality but not in all affected skin, and varies between *B .ovis* and *M. ovinus*.

**Keywords:** *B.ovis*, Cockle, Ethiopia, Experiment, Lesion, *M. ovinus*, Sheep, Skin,

## 1. INTRODUCTION

In Ethiopia, a livestock sector contribute for 12% of the total Gross Domestic Product (GDP), and provides livelihood for 65% of the population and also it accounts for 37–87% out of the total household cash income. For many years not only the export of livestock but also the export of processed and semi-processed skins and hide constitutes Ethiopia's second largest export commodity by accounting for 12–15% of the total export earnings next to coffee (Solomon *et al.*, 2003 and FAO, 2005).

Sheep represent the most important segment of the Ethiopian livestock system. The national sheep population is estimated at 25.5 million (CSA, 2008). Annually, 16.6 million pieces of skins are produced in the country, based on the off take rates of 33% for sheep (Tadesse, 2005). Although large numbers of sheep are slaughtered per annum, the production of high quality skins remains very low (Zewdu, 1995; Kassa, 2005).

The exportation of skin and hide is the largest foreign exchange earns in Ethiopia and the leather industry is one of the fourth growing economic sectors. However, this sector and the country as whole lost revenue due to a decline in quality and fall in export prices (Pittards, 1999; NBE, 2000). The majority of leather defects in the factory are due to damage at pre slaughter stage of production on the live sheep, a considerable portion of which are due to skin diseases caused by ectoparasites (Kassa *et al.*, 1998). In Ethiopian tanneries, 35% of sheep skins have been downgraded and rejected due to defects caused by external parasites (Kassa, 2005).

Ectoparasites cause skin lesions or defects in all layers of the skin. However, the extent and type of gross and microscopic skin lesions may vary with the specific causes of skin diseases. Characteristic gross skin lesions in animals affected by ectoparasites include encrustation, papules, nodules, and areas of superficial erosions and ulcerations (Wall and Shearer, 1997). Microscopically, epidermal lesions in the skin of animals affected by ectoparasites include hyperkeratosis, acanthosis, and spongiosis. The main dermal lesions

are vasculitis, follicular lesions, and aggregates of inflammatory cells. Various forms of collagen pathologies have been also described (Jones *et al.*, 1997).

Almost all commercial tanneries have indicated rejections of 20 to 24% of purchased skins from sheep and goat, which has resulted in a loss of \$6.9 million. Therefore, this creates a serious problem for competition in international markets through the export of processed skin (Belachew, 2004).

Skins infested with *Bovicola ovis/biting* lice and *Melophagus ovinus*/sheep ked were observed to have cockle (–Ekek” in Amharic) which is a series of pimple-like bumps on the skin of the sheep in areas where these parasites fed (Asegedech *et al.*, 1999 and Tefera and Abebe, 2007 a, b). Heath *et al.* (1995b) added that there is a direct correlation between louse (*B. ovis*) scores and cockle, high louse scores being associated with a more severe degree of cockle. Lice infestations particularly that of *B. ovis* had been known to greatly damage the grain of skin (Heath *et al.*, 1995a and Mullen and O’Connor, 2002). *M. ovinus* are the most economically important pests of sheep (Wall, 2007; Mersha, *et al.*, 2010; Berhanu *et al.*, 2011; Kumsa *et al.*, 2012). Other reports showed that heavily sheep ked infested animals become weak and unthrifty and show weight loss, anemia, wool staining and reduced resistance to disease (Abadi, 2000 and Wall, 2007).

In Ethiopia, keds and lice are considered as the major causes of cockle and are visible on the skin surface of affected animals (Kassa, 1998; Ermias, 2000). It is an allergic skin hypersensitivity reaction due to lice infestation and this defect appears on the grain side of semi-processed and crust leather after pickling that cannot be detected when the skin is examined raw or unprocessed. It results in huge economic loss to tanneries and the country at large since the damage is recognized after a lot of cost is incurred on the processing after which the damaged skins have to be rejected or downgraded (Kassa, 2006). Studies conducted at Sebeta tannery on sheep skin on routine production system indicate that 89% of cockle in pickled sheep pelts (Yisehak, 2000). According to Tefera and Abebe (2007b), about 70.8% of the pickled sheep pelt were downgraded and rejected

due to cockle caused by both lice and sheep ked. As elicited by Demissie *et al.* (2000), the estimated economic loss due to drop in quality of sheep and goat skin is around 14 million USD per year.

Previous studies conducted in Ethiopia did not experimentally characterize and stereotype gross and microscopic skin lesions caused by specific ectoparasites. Most of the reports on skin defects were from the field cases where animals are naturally parasitized by mixed species of ectoparasites. Moreover, the effect of treatment on resumption of skin to its normal composition and departure of defects on processed skin was not evaluated.

Therefore, this study is formulated with the following objectives:

- To characterize specific pathological changes on live animals skin caused by *B. ovis* and *M. ovinus* infestation
- To observe effect of treatment on the restoration of skin to its natural composition on live animals as well as it's clearing of defects from processed skin.
- To identify defects and skin quality degradation on processed skin caused by *B. ovis* and *M. ovinus* infestation

## 2. LITERATURE REVIEW

### 2.1. Sheep Biting Lice (*Bovicola ovis*)

#### 2.1.1. *Biology and infestation of sheep biting lice*

The sheep body louse (*Bovicola ovis*, formerly called *Damalinia ovis*) is a pale yellow insect 1.5 to 2 mm long with brown transverse stripes on the abdomen and a broad, red-brown head. It is a chewing louse and feeds on skin scurf, lipid and sweat gland secretions, superficial skin cells and skin bacteria. Male *B. ovis* are smaller than female *B. ovis* and have more pointed abdomens (Sinclair *et al.*, 1989; Wall and Shearer, 1997).

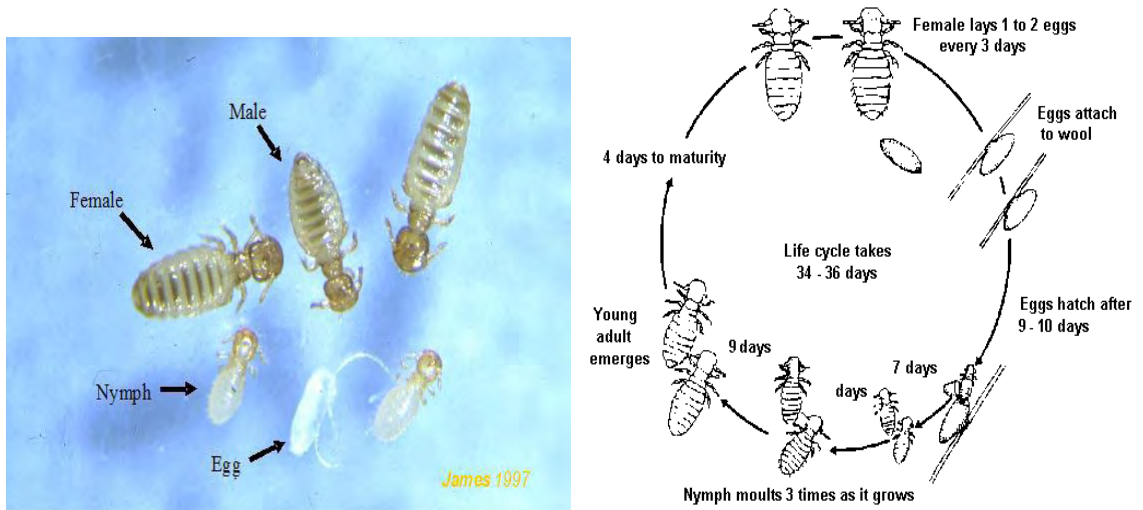
Lice usually are unable to survive for more than 1-2 days off their host and tend to remain with a single host animal throughout their lives. Most species of louse are highly host specific and many species specialize in infesting only one part of their host body (Wall and Shearer, 1997) and transfer to new hosts is by body contact, particularly under condition of close confinement (Sewell and Brookesby, 1990; Peter, 1995). To allow them survive as permanent ectoparasites, lice show a number of adaptations which enable them to maintain a life of intimate contact with their hosts.

#### Life cycle of sheep biting lice

Females cement individual eggs to wool fibres, most within 12 mm of the skin. For egg laying to occur the temperature must be in the range  $37.5C \pm 2.5C$  and a fibre of suitable diameter must be available. A detailed description of the egg laying behaviour of *B. ovis* was given by Murray (1960). Mated and unmated *B. ovis* females deposit approximately the same number of eggs, but unlike cattle lice (*B. bovis*), which can reproduce without mating, *B. ovis* eggs will not hatch if mating has not taken place.

## Common biting lice species

The important species in sheep and goats are found in the genus *Damalina* and *Linognathus*. There are a number of morphologically similar host specific species, the species that affect small ruminants are *Damalina ovis* on sheep; *Damalina caprae*, *Damalina limbata* and *Damalina crassiceps* on goats. The sheep chewing lice, *Damalina ovis*, is one of the most common lice found on sheep (Bay and Harris, 1988; Wall and Shearer, 1997).



**Figure 1:** Morphology of sheep biting lice, *Bovicola ovis* and its life cycle (James, 1999)

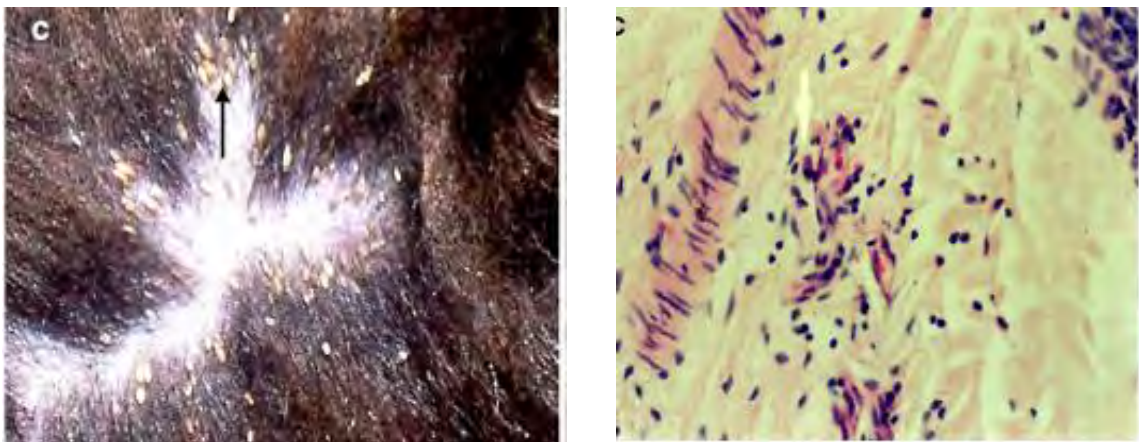
### 2.1.2. Common gross and microscopic skin lesions induced by lice

All species cause irritation of the skin and stimulate scratching, rubbing, and licking leading to restlessness, damage to the fleece and skin and reduction in milk production. The saliva and feces of the lice contains substances, which are capable of causing allergies, giving rise to severe irritations, followed by the skin thickening and sometimes self trauma (Peter, 1995).

In lice infestation, the pathological changes were not that much pronounced. The site of attachment of lice was red and slightly elevated. In some cases, pinpoint hemorrhages

were noticed (Sarkar *et al.*, 2010). The irritation caused by lice leads to scratching (James and Moon, 1998; James and Moon, 1999) and rubbing that could damage the skin (Asp and Tauni, 1988; Heath *et al.*, 1996; Ward and Armstrong, 2000) resulting in alopecia.

According to Mersha *et al.* (2010), histologically skin lesions such as Perifolliculitis, perivasculitis, hyperkeratosis and diffuse dermatitis characterize lesions caused by lice. Halligan and Johnstone (1992) described excess collagen fiber deposition in the dermis due to lice. Skin biopsies from sheep and goats infested by lice shows mild parakeratotic hyperkeratosis, crusts, and infiltrated with eosinophils. Initially, very small acellular crust was formed and latter contain large number of necrotized epidermal cells. Perivascular or diffuse dermatitis with eosinophils, plasma cells, and lymphocytes were encountered in the dermis. Lice infestation has caused extensive fibroblast cells and elastic fibers proliferation in the dermis (Mersha *et al.*, 2010).



**Figure 2:** Gross and microscopic skin lesions in sheep affected with lice. Sheep heavily infested with *B. ovis* and eggs (nits) glued to the wool (left) and Perifolliculitis in the dermis of sheep with pediculosis (right) (Mersha *et al.*, 2010).

#### Production loss from lice

Infestation with sheep lice can reduce clean wool cut by up to 1 kg per head (Wilkinson *et al.*, 1982, Niven and Pritchard, 1985). Lice also reduce yield, cause fleeces to become

coated and yellow and result in increased losses during processing. In New Zealand, sheep lice have been shown to cause a defect in sheep leather known as cockle (Heath et al. 1995b). This is manifest as multiple, sometimes discolored, lumps visible in sheep leather after processing. Infestation with *B. ovis* does not affect fiber diameter and, contrary to popular belief, does not cause reduction in body weight. The effect of lice is usually a function of their density. A small number of lice may present no problem and in fact may be a normal part of skin fauna. However, they have massive potential for increase (Wall and Shearer, 1997). Reduction in the value of the wool clip is economically the most important consequence of ovine pediculosis, but additional hazards in warm countries is that the fleece and skin damage by the rubbing and soiled by louse feces, is an attractant for blow flies strike (Urquhart *et al.*, 1996).

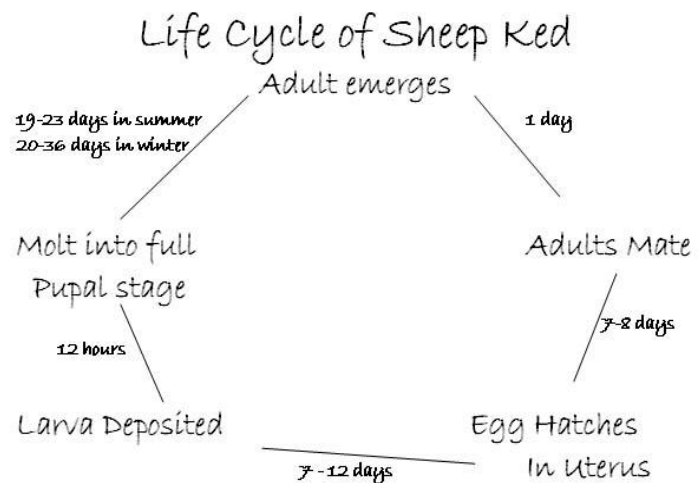
## **2.2. Sheep Ked (*Melophagus ovinus*)**

### **2.2.1. Biology and infestation of sheep ked**

Keds are brown wingless biting flies about 6-7mm in length. Sheep ked live their entire life cycle on the host but able to move from one host to another rapidly within the flock (Sewell and Brockesby, 1990). Adult keds are permanent ectoparasites and feed on the blood of sheep and sometimes goats (Bay and Harris, 1988; Wall and Shearer, 1997). The parasite is mainly seen in colder and wetter areas and infestations may be lost when sheep are moved to hot dry areas. Keds are most active in cooler months and are common on coarse rather than fine woolen breeds (Radostits *et al.*, 1994). Although the degree of infestations usually encountered cause only irritations (Radostits *et al.*, 1994), the resulting scratching, biting, and rubbing themselves against fences, stones, shrubs, and other things causes severe damage to the skin and wool (Urquhart *et al.*, 1996). Furthermore, both sexes are blood feeders and feed several times each day and therefore very heavy infestations may cause severe anemia (Sewell and Brockesby, 1990; Urquhart *et al.*, 1996).

### 2.2.2. Life cycle of sheep ked

A single egg is ovulated at a time. The egg hatches inside the body of the female and the larva is retained and nourished within the female during its three larval stages, until it is fully developed and ready to pupariate (Bay and Harris, 1988; Wall and Shearer, 1997). The single larva is deposited on the host and pupates within a few hours. The larva is attached to the wool fiber some distance above the skin and many larvae and pupae are removed during shearing. The young keds usually emerges in 20-22 days but this period may be prolonged for up to 36 days in winter. The complete life cycle takes 5-6 weeks in optimum warm conditions. Young female may mate several hours after emergence but their ovaries are not mature until they are 6-7 days old, and the first larvae is not deposited until they are 12-14 days old (Bay and Harris, 1988). The female ked live for 4-5 months and lays 10-15 larvae during this time (Radostits *et al.*, 1994) thus build up of infestations is slow.



**Figure 3:** Adult sheep ked morphology and its life cycle (Bay and Harris, 1988).

#### Production loss from sheep ked

Sheep keds have been reported to cause a 2 lb. reduction in carcass weight, a 20% reduction in clean, dry weight of fleece and a 7% loss in clean fiber. Heavily-infested sheep itch and

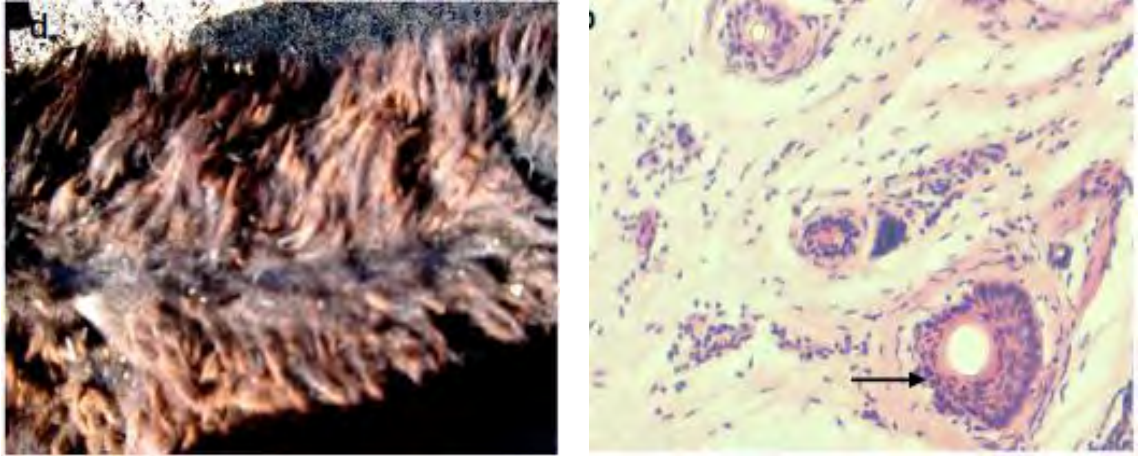
have a dirty unthrifty appearance. The feeding by the ked causes firm, hard nodules to develop in the grain layer of the sheepskin. This causes a defect in the skin known as “Cockle”, which reduces the value of the skin because of a decrease in tinsel strength of the leather (John, 2002).

#### Common gross and microscopic lesions induced by sheep ked

Sheep keds causes a wide spread pruritis, exacerbated by scratching and biting (Legg *et al.*, 1991). Small (2005) confirmed that, skin lesions caused by sheep keds were initially characterized by the presence of wheals and papules centered up on a puncture wound, which were then covered by scales and crusts. As Mersha *et al.* (2010) described, skin lesions caused by sheep ked characterized by scaly crust and the wool of affected sheep was ragged and stained with the excreta of sheep ked.

The characteristic microscopic skin lesions caused by sheep ked was the presence of many empty capillaries in the dermis (Mersha *et al.*, 2010). The findings of Heath *et al.* (1995a) have indicated that arteriolar vasoconstriction and fibrinoid degeneration of the tunica media of capillaries and arteries may be responsible for the detection of empty capillaries. Various types of dermal pathologies varied from follicular lesions to perivascular and/or diffuse dermatitis was described in other studies (Jones *et al.*, 1997).

Sheep keds causes a rash called cockle on the skin, which is the principal cause of skin rejection at tanneries (Tefera and Abebe, 2007a). Skin puncture made by blood sucking keds cause development of “cockles” in the processed sheep skin (Health *et al.*, 1995b; Bayou, 1998). Cockle is an inflammatory response of the skin to the presence of sheep keds and their saliva, which is recognized after the wool or hair has been removed from the skin. Staining of the wool by the feces of the keds reduces its value and gives it a peculiar musty odor (Radostitis *et al.*, 1994; Wall and Shearer, 1997).



**Figure 4:** Gross and Microscopic skin lesions in sheep affected with sheep ked. Unattractive, damaged, and ragged wool due to sheep ked infestation and Extensive inflammation in the reticular layer consisting of eosinophils (arrow), lymphocytes and plasma cell infiltrates due to sheep ked infestation (Mersha *et al.*, 2010).

### 2.3. Processed Skin Defects Caused by Lice and Ked

The tanning industries receive and process 14.3 million skin annually (Mohammed, 2000). Pickled and wet blue skins are the major export forms. Ethiopian highland sheep skin has international reputation for its unique natural substance of fineness, flexibility, strength, and compactness of texture. It is suitable for production of high quality dress gloves, sport gloves and garments (Asfaw, 2002).

Skin is the outer covering of small animals such as sheep, goats and calf in raw and /or untanned form (FAO, 1995). Skin quality is primarily defined by the absence of damage to the grain layer of the skin (Hadly, 2001). The quality of finished leather is related to number of surface and structural defects hide and skin acquire during the life of the animal, slaughtering, storage and transportation stages (Chakole, 2001). Top quality leathers, known as Aniline, are produced from hides and skin having few or no visible defects. Aniline leathers are generally soft, full in substance, and visibly display the attractive grain patterns of the natural skin and are produced by direct drum dyeing of skins without passing grain correction process (Chakole, 2001).

Aniline leathers are produced by direct drum dyeing of leathers to increase resistance to stains, heat and mechanical damage without passing through grain correction process (Chakole, 2001). Defects like “kek” are detected only after removal of the hair/wool in tanneries, due to this raw skin selected as top grades may end up into down grades and rejects. Low quality finished leathers are produced from hides and skins suffering from different defects; most of them are finished covering their defects with pigment or synthetic coating materials. The grain correction, which requires special skill and the chemicals used, will definitely increase the cost of leather production (Chakole, 2001).

Cockle is a nodular condition of the skin arising in response to infestation with *D. ovis* and is possibly a hypersensitivity reaction on the part of some sheep to antigen of louse origin (Heath *et al.*, 1996). It is detectable after depletion of the hair or wool where they present as numerous small raised nodules on the grain surface of the pelt (Dempsy *et al.*, 1972 cited by Pfeffer, *et al.*, 1996). A similar lesion known as “kek” was associated with keds and lice infestations on Ethiopian sheep pelt (Bayou, 1998; Yishak, 2000) & in goat pelts with mange and louse infestations (Abdulhamid, 2001).

“Ekek” in sheep and mange in goats are causes of down grading and rejection of sheep and goat skins (Bayou, 1998). According to Chakole (2001) in the past 10-15 years defects from the diseases and other causes have reached very significant level especially in sheep and goats skin dropping aniline leather (Grade 1-3) and increasing rejections. Tanneries reported that 35% of sheep skin and 56% of goats’ skin are rejected due to external parasites. And out of the reject groups of the processed skin, about 80-90 % defects were believed to be due to external parasites. According to Chakole (2001) the estimated economic loss due to drop in quality of sheep and goat skin is around US \$14 million per year.

### **3. MATERIALS AND METHODS**

#### **3.1. Description of the Study Area**

The experimental study was carried out at the College of Veterinary Medicine and Agriculture, Addis Ababa University (CVMA, AAU), Bishoftu from January, 2015 to August, 2015. Bishoftu town is found at 47 km Southeast of Addis Ababa, East Showa zone, Oromia regional state, located at 9°N latitude and 40°E longitude at an altitude of 1850 meters above sea level. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February and the mean annual maximum and minimum temperature is 26°C and 14°C respectively, with mean relatively humidity of 61.3% (ADARDO, 2007).

#### **3.2. Experimental Animals**

Thirty indigenous Arsi breed male sheep approximately 18 months of age were bought from Asella and Sagure local markets. Before transportation animals were treated with long acting oxytetracycline (Alamycin LA, 200mg/ml at 1ml per 10kg body weight). On arrival at College of Veterinary Medicine and Agriculture at Bishoftu, the animals were ear-tagged, screened for hemoparasite and internal and external parasites, dewormed with albendazole (Albenda-QK, 300mg), and sprayed by Diazinon at 0.6: 1000ml dilution ratio. In this condition the animals were acclimatized for a month. Donor sheep or sheep positive for ectoparasites of interest were purchased and kept in separated house at another location as source of parasites for infestation of experimental animals.

#### **3.3. Experimental Animals Grouping and Infestation Methods**

The experimental animals were divided into five groups with six animals per group by randomized block design technique to minimize variation between groups. Sheep in group I comprised non-infested control, group II and III were infested by *Bovicola ovis* and group IV and V were infested with *M. ovinus*.

The *B. ovis* were collected manually from their sites of attachment on the donor (Annex 1b), counted and identified. Subsequently, five hundred parasites were deposited at the base of the hairs at five different sites (neck, shoulder/wither, thoracic, flank and ramp on both sides) (Heath, *et al.*, 1996) and the hairs on the sites were fixed by a scotch tape to prevent falling down and facilitate dispersion along the skin surface (Annex 1c).

The *M. ovinus* were collected manually from their sites of attachment on the donor, counted and identified. After that, fifty parasites were deposited at the base of the hairs at five different sites (neck, shoulder/wither, thoracic, flank and ramp on both sides) (Olaechea *et al.*, 2007).

All groups were followed for 95 days for pathological lesion development after infestation; soon after 95 days follow up, sheep in group III and V were treated by Diazinon (0.6: 1000ml dilution ratio) and followed for additional 30 days.

### **3.4. Animal Management and Feeding**

Throughout the experiment animals were fed ad-libitum with hay and water and supplemented with concentrates and mineral licks. All protocols and procedures of animal management were according to the guideline set by the animal research and ethics review committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University.

### **3.5. Clinical Examination, Sample Collection and Sample Processing**

For hematological analysis about 4 ml of blood sample was collected weekly during the study period from jugular vein of all the experimental animals using ethylene diamine tetra acetic acid (EDTA) coated vacutainer tubes (Annex 1d). Hematological parameters measured were total and differential WBCs count. Total WBC counts were carried out manually using the improved Hemocytometer. A stained thin smear was examined in order to determine the percentage of each type of leukocyte (differential counts) present according to their staining reactions, shape of the nucleus, and presence or absence of

granules in their cytoplasm (Coles, 1986). The details of procedures of these laboratory techniques are indicated in (Annex 3).

Visual inspection of the skin and wool was conducted every week to detect parasites and lesion. Biopsy materials of the skin was collected every two weeks from the affected part and fixed in 10% buffered neutral formalin solution for histopathological studies. After inducing local anesthesia (2% Lidocaine) at biopsy sites (Annex, 1e), skin punch biopsies about 5 mm in diameter was taken post-infestation from the leading edge of the lesion at the interface between normal and lesioned skin (Annex, 1f). The obtained specimen was put in the universal bottle containing 10% buffered neutral formalin (McNeilly *et al.*, 2010; Bancroft and Harry, 1994). Histopathological technique was conducted according to Talukder (2007). The tissue was trimmed, fixed in 10% buffered formalin, dehydrated in ascending grades of alcohol, cleared with xylene and impregnated with molten paraffin wax. Then after it was sectioned at 5 $\mu$ m thickness, spread on warm water bath and tissue was attached to albumenized glass slides. The slide was incubated at 60<sup>0</sup>c to melt and deparaffinize the wax. The sectioned tissue is then deparaffinized in three changes of xylene, hydrated in descending grades of alcohol and stained with hematoxylin and eosin. Finally the sample was examined under microscope using 10x, 40x and 100x magnification and photomicrographs was taken (Annex, 6).

### **3.6. Processed Skin Examination**

After all required data and samples were collected from the experimental animals, the animals were euthanized, flayed and the skins' of animals was taken to the Colba tannery for processing and grading based on the defects on processed skin. Accordingly, sheep skins were examined to identify the skin damage as cockle at the tannery. Each skin was sorted and examined for defects in natural light by skin selectors (Annex 9f), and distribution of defects (cockle) on each skin was recorded, and the skins was graded as per the criteria given by QSAE (2001).

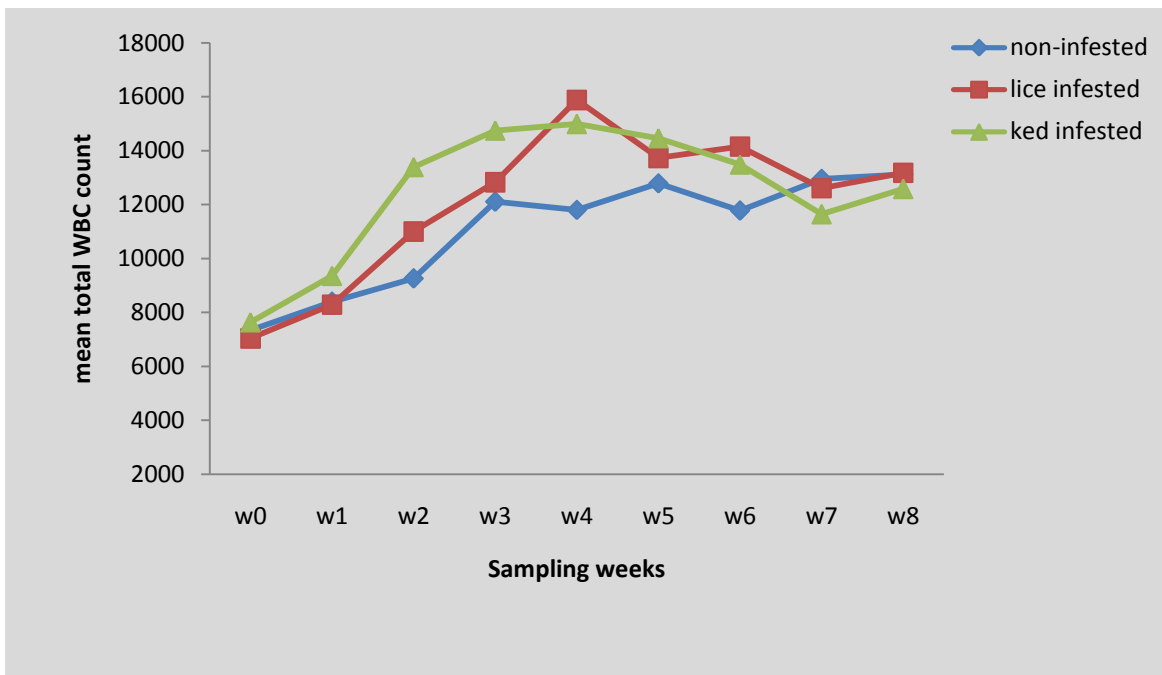
### **3.7. Data Management and Analysis**

The collected data were coded and entered into excel spreadsheet and subjected to analysis using SPSS version 20 statistical software as outlined by Steel and Torrie (1981). Arithmetic mean and standard deviation (SD) were determined and classified by treatment group's infestation status for white blood cells parameters. The statistical significance difference between various treatment groups was determined using the ANOVA for hematology parameters data, cockle distribution extent and skin grade. Where results were significant Post Hoc multiple comparisons test were made to establish differences between treatment groups. Data on pathological changes was summarized using simple descriptions. Difference between groups was considered significant when p-value is less than 0.05 (95% confidence level).

## 4. RESULT

### 4.1. Result of Total WBC Count

The mean total WBC counts of *B. ovis* and *M. ovinus* infested groups were higher compared to the non-infested control (Table 1). The total WBC count increased from the day of infestation and peak level was registered on week four post infestation for *B. ovis* infested and week three for *M. ovinus* infested sheep group. Thereafter, both *B. ovis* and *M. ovinus* infested groups' demonstrated gradual decline with increasing at the week eight whereas non-infested control group showed little increment in their total WBC with fluctuating pattern (Fig. 5). Overall mean WBC count registered for control group was  $11.06 \times 10^3 \pm 2.8 \times 10^3 / \mu\text{l}$  and for the *B. ovis* and *M. ovinus* infested groups were  $12.08 \times 10^3 \pm 4.3 \times 10^3 / \mu\text{l}$  and  $12.47 \times 2.9 \times 10^3$ , respectively.



**Figure 5:** Mean total WBC count in experimentally infested and non infested sheep

## 4.2. Result of Differential WBC Count

Because of the variation in the mean WBC count between the *B. ovis* as well as *M. ovinus* infested and non-infested control groups at pre-infestation point, the data of relative differential WBC were transformed to absolute differential WBC count to show the changes in relation to total white blood cell count.

Lymphocyte count had increased up to the third week for *M. ovinus* infested and fourth week for *B. ovis* infested. Then after, both *B. ovis* and *M. ovinus* infested groups demonstrated a gradual decline until week seven followed by a rise up at week eight (Fig. 6a). Nonetheless, counts for the non-infested control group showed increment but remained very close to normal reference value of sheep. However, the difference in mean lymphocyte count was not statistically significant between *B. ovis* / *M. ovinus* infested and non-infested control group (Table 1).

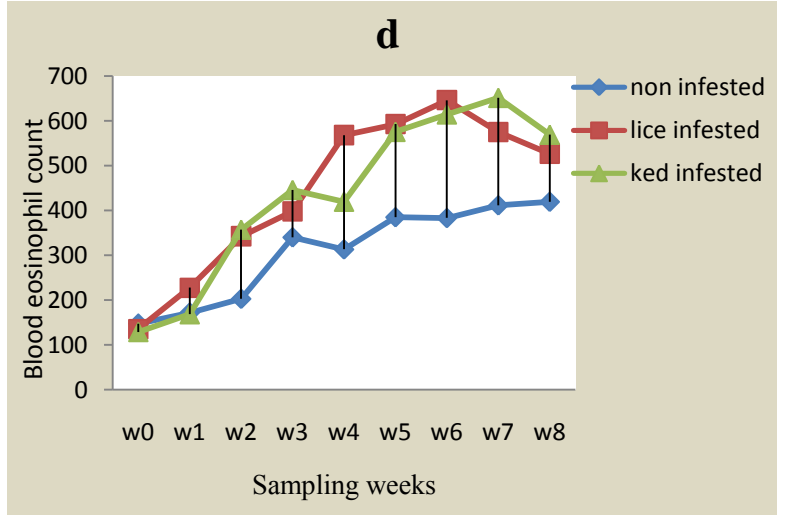
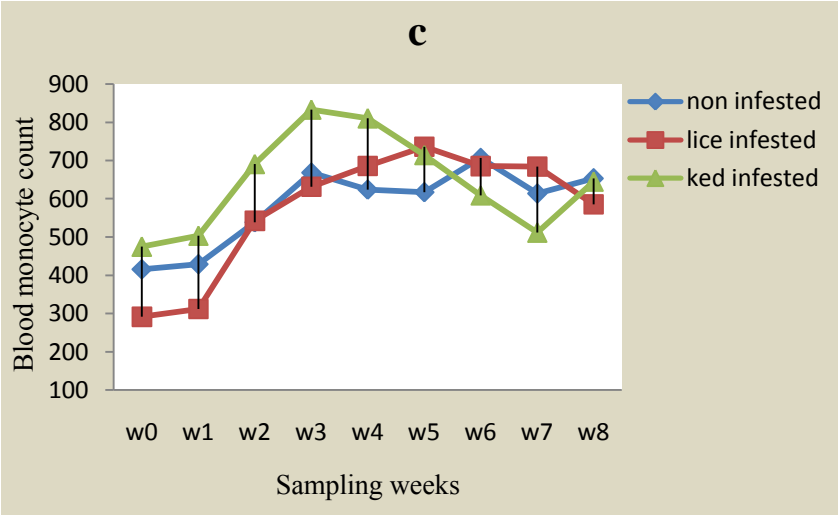
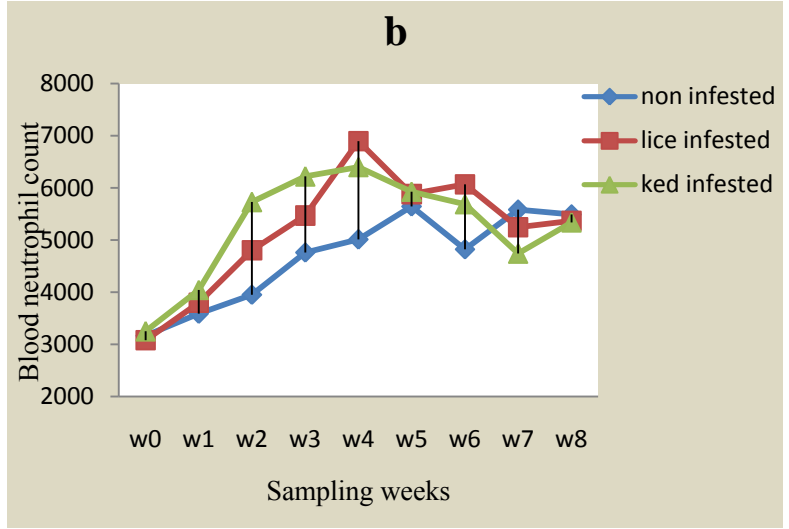
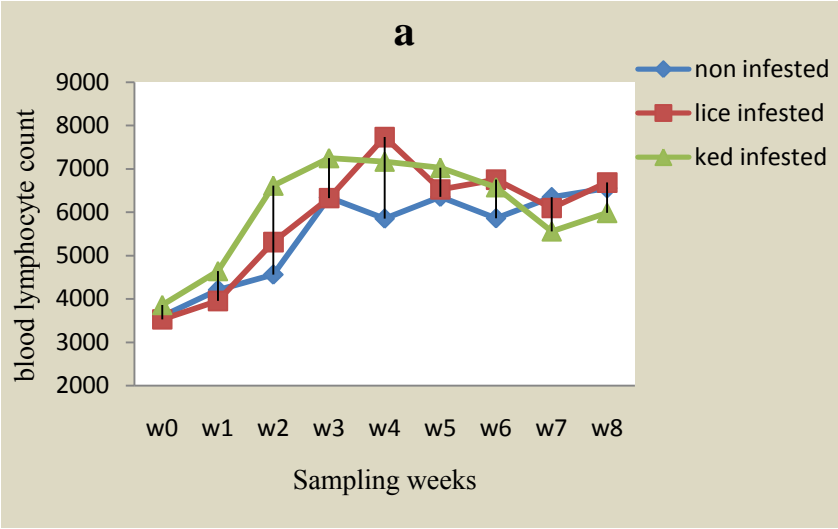
The percentages of neutrophils was fluctuating along the experimental period for non-infested control group whereas a peak of increase was on fourth week for both *B. ovis* and *M. Ovinus* infested groups and it gradually declined but raise up again at week eight (Fig. 6b). Nevertheless, there was no statistically significant variation in the levels of neutrophils between non-infested control and *B. ovis* infested as well as *M. Ovinus* infested group (Table 1). Similarly, blood monocyte proportion fluctuated along the experimental period (Fig. 6c). The difference in monocyte proportion was not significant among the non-infested control, *B. ovis* infested and *M. Ovinus* infested groups (Table 1).

The mean absolute differential eosinophil count revealed significant difference among *B. ovis* infested, *M. ovinus* infested and negative control group. It was higher for *B. ovis* infested group at ( $P = 0.001$ ) and *M. ovinus* infested at ( $P = 0.005$ ) compared to the non-infested control group (Table 1). The mean eosinophil levels were elevated in infested groups up to week six for *B. ovis* infested and week seven for *M. ovinus* infested thereafter it gradually declined (Fig. 6d).

Table 1: Mean and standard deviation of haematological parameters of the study groups

Experimental Groups	Mean $\pm$ standard deviation and p-value of hematological parameters									
	Total WBC count	P-value	Lymphocyte count	P-value	Neutrophil count	P-value	Monocyte count	P-value	Eosinophil count	P-value
Non-infested control	11059.26 $\pm$ 2787.91		5519.35 $\pm$ 1495.01		4670.13 $\pm$ 1327.76		585.15 $\pm$ 189.22		308.43 $\pm$ 149.77	
<i>B. ovis</i> infested	12080.09 $\pm$ 4356.45	0.097	5879.58 $\pm$ 2122.34	0.236	5180.37 $\pm$ 2072.30	0.079	572.49 $\pm$ 292.14	0.763	445.72 $\pm$ 267.06	0.001*
<i>M. Ovinus</i> infested	12478.70 $\pm$ 2873.18	0.046*	6075.78 $\pm$ 1397.46	0.113	5260.74 $\pm$ 1283.82	0.078	643.37 $\pm$ 214.10	0.230	436.69 $\pm$ 240.08	0.005*

\* The mean difference is significant at p-value is below 0.05 level



**Figure 6:** Differential WBC count of experimentally infested and non infested sheep

### 4.3. Pathological Changes

#### 4.3.1. Gross lesions

Most of the gross lesions induced by *M. ovinus* and *B. ovis* were observed to be similar. The non-infested control group did not show gross lesion. In infested groups; the infestation sites and regions where parasite migrated through were erythemic and there were macules of different sizes (Fig.7D). The skin was disfigured with the excreta of parasite. However it was not so intense in *B. ovis* infestation (Fig. 7A & B). It was clearly visible and yellow green in ked infestation (Fig. 7 C).

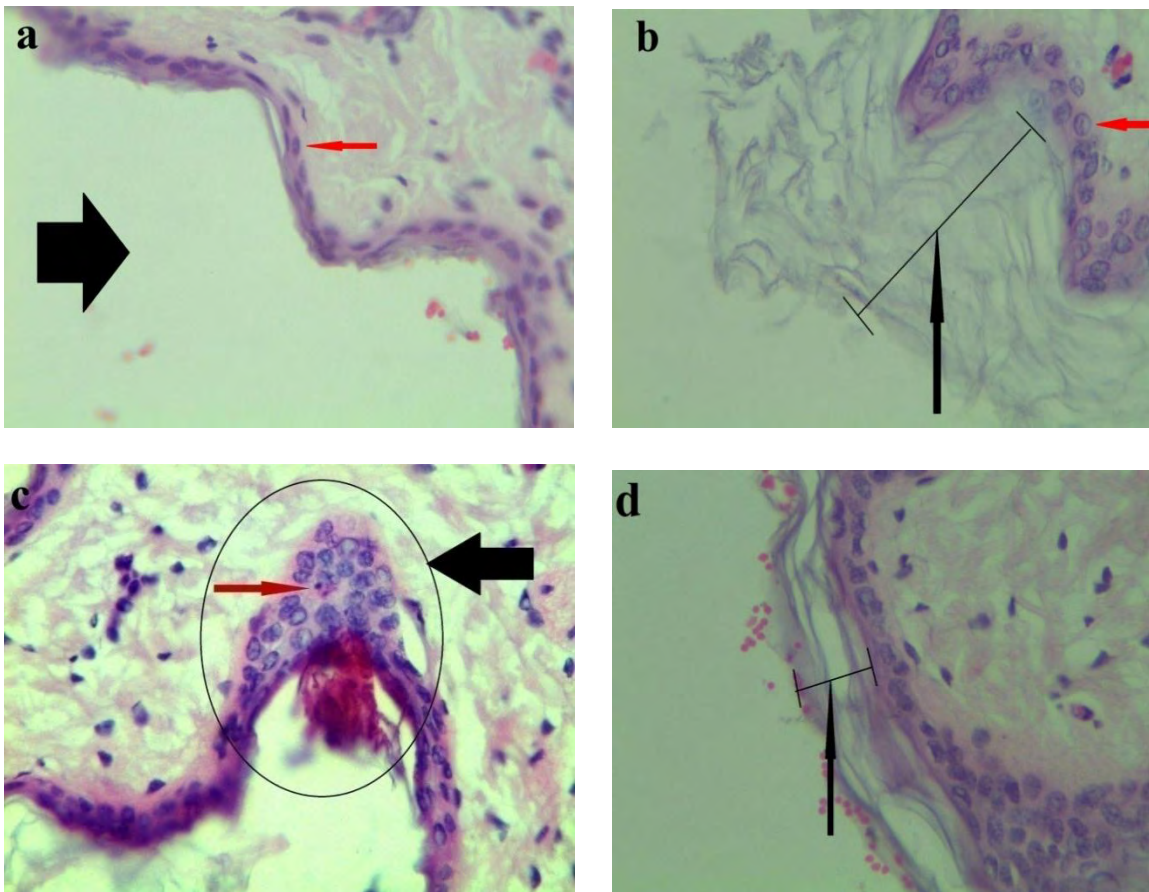


**Figure 7:** Gross lesion of sheep infested by *B. ovis* and *M. ovinus*. A & B, *B. ovis* infested skin lesion with excreta of parasite and skin disfigured (circular insets); C, gross skin lesion taken from *M. ovinus* infested sheep with *M. ovinus* parasite (blue arrow) and

characteristic yellow green discoloration of skin (red arrow) and D, inflamed and erythemic skin taken from *B. ovis* infested sheep.

#### 4.3.2. Microscopic lesions

**Epidermal lesions:** The microscopic lesions induced by *B. ovis* and *M. ovinus* are more or less similar but the severity of individual lesions varies. *B. ovis* infested group elicited moderate hyperkeratosis (Fig.8d) and acanthosis of epidermis. Epidermis of *M. ovinus* infested group demonstrated extensive hyperkeratosis (Fig.8B) and severe acanthosis (Fig. 8c). Unlike the *B. ovis* and *M. ovinus* infested groups, the non infested control group did not show any epidermal lesion (Fig. 8a).



**Figure 8:** Microscopic lesion induced by *M. ovinus* and *B. ovis*: a, skin of the non infested control sheep with normal amount of keratin layers (black arrow) and normal

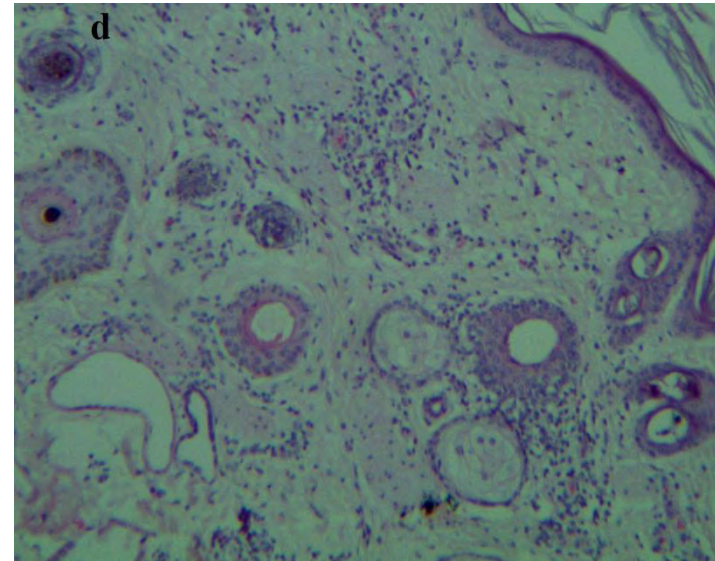
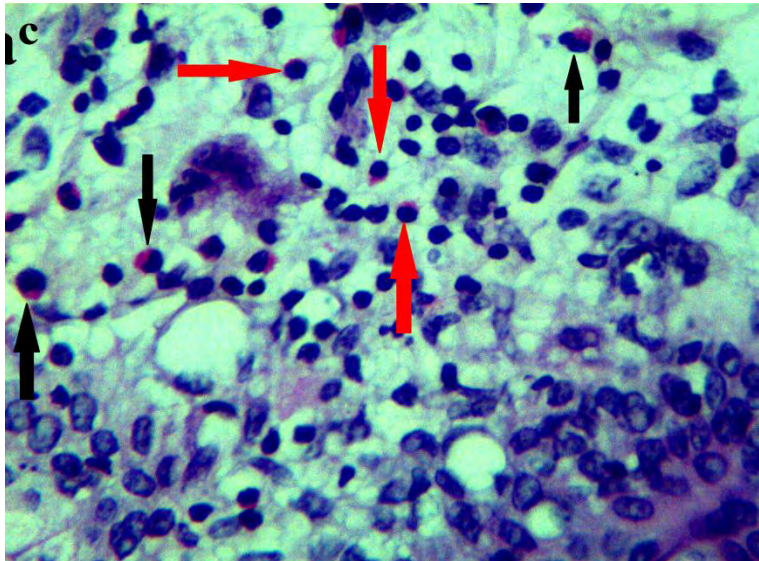
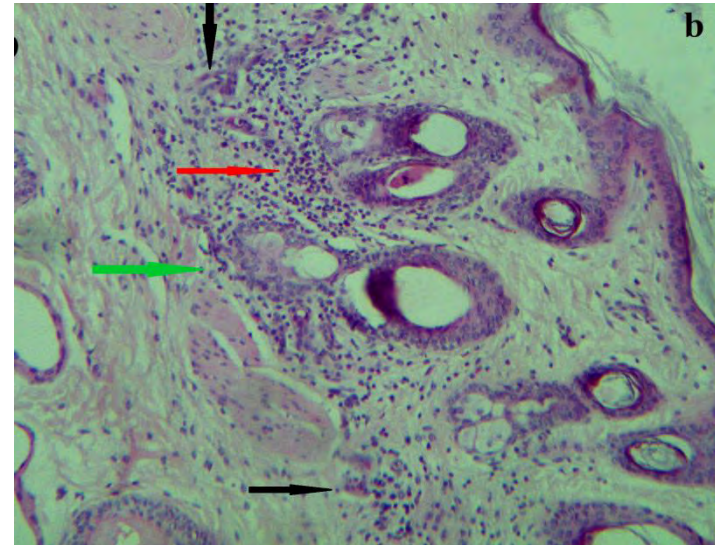
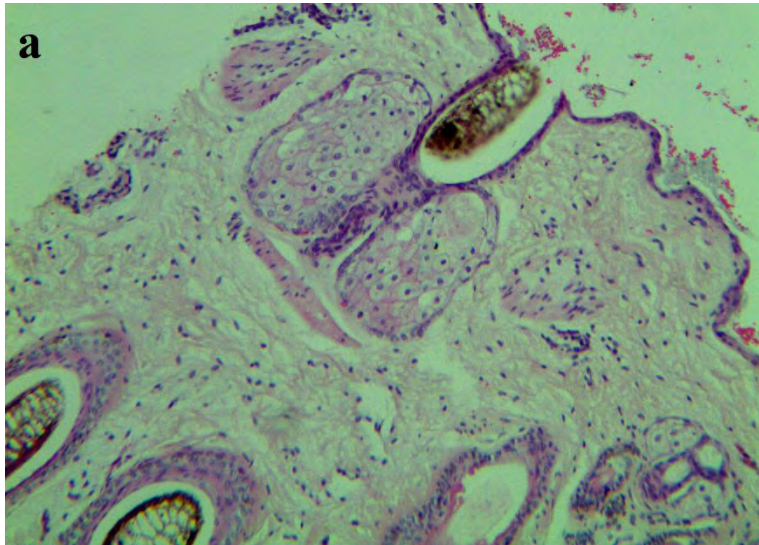
squamous epithelium nucleus (red arrow). b, Severe hyperkeratinized skin of *M. ovinus* infested sheep (black arrow) with activated basal cells (red arrow). c, Acanthosis (black arrow) with mitotic figure which indicates actively proliferative cells (red arrow). d, Moderate hyperkeratosis of sheep skin infested by *B. ovis*.

**Dermal lesions:** *B. ovis* infested skins were characterized by severe dermatitis that was mainly infiltrated by lymphocyte and eosinophils (Fig. 9c), perivasculitis, perifolliculitis, frunculosis and sebaceous adenitis (Fig. 9b).

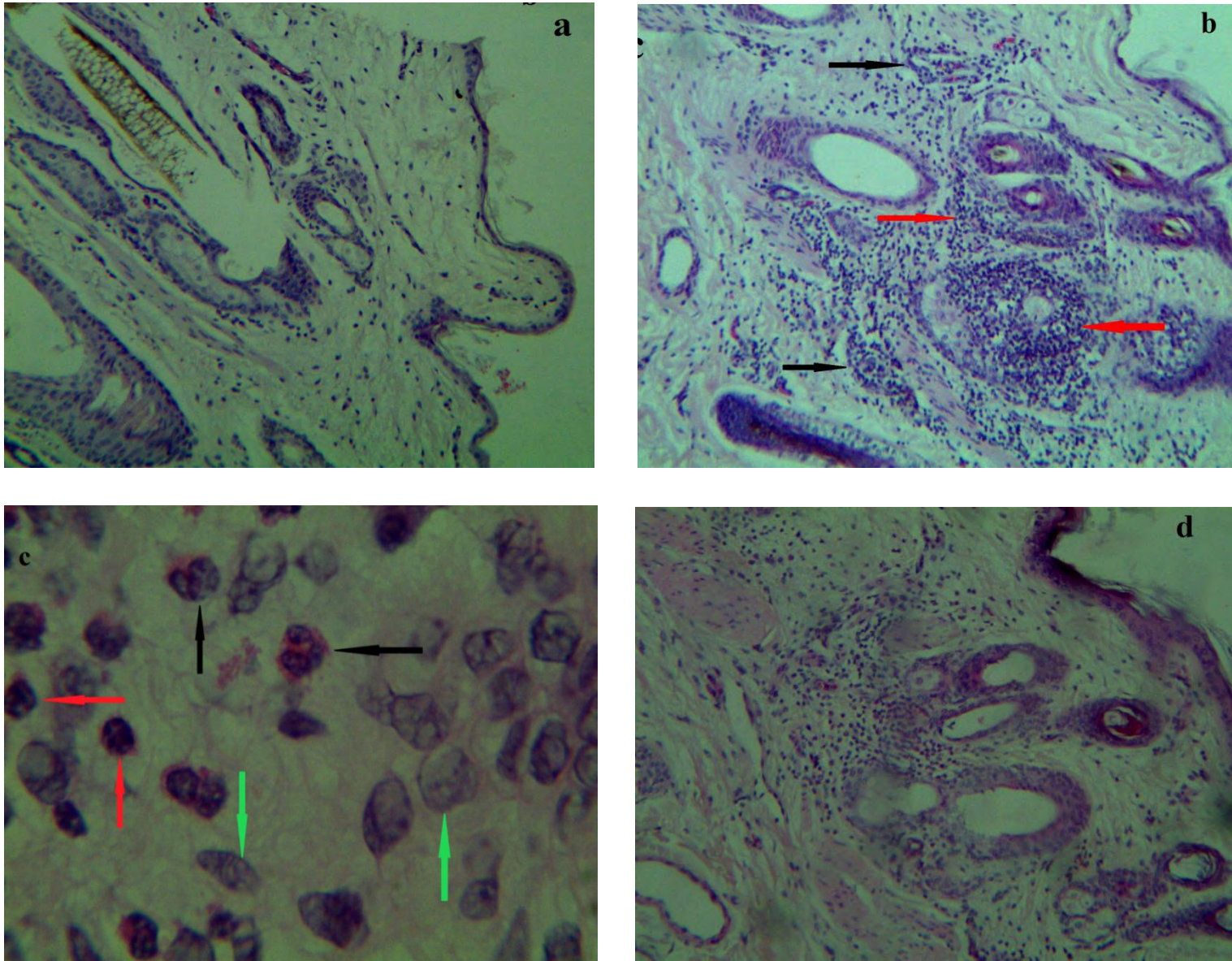
The characteristic microscopic lesions of *M. ovinus* infested sheep skin were diffuse dermatitis mainly infiltrated by lymphocyte and eosinophil (Fig. 10c), pervasculitis, perifolliculitis, frunculosis and many empty capillaries in the deeper part of the dermis.

#### **4.4. Effect of Treatment on Skin Resumption to Normal State**

The most common gross lesions, erythema and macules were disappeared within a week of treatment. Conversely, the skin discoloration by excreta of parasites was persisted for two weeks on most and even more on some animals. Treatment did not significantly improve the microscopic lesion within 30 days. Especially the hyperkeratosis and acanthosis were remained as before treatment until the end of experiment (30 days after treatment). However, treatment will reduce the cellularity of inflammatory cells at site of dermatitis, perivasculitis, perifolliculitis, and sebaceous glands (Fig. 9d and 10d). Specifically, there was high reduction in eosinophil number than other inflammatory cells.



**Figure 9:** Microscopic lesions of *B. ovis* infested dermis: a, Normal skin from non infested group. B, Severe perifolliculitis (red arrow) with aggregation of inflammatory cells, severe perivascularitis (black arrow), and sebaceous adenitis (green arrow). Morphology of inflammatory cells at 40 x objectives (c) and Inflammatory cells extent illustration on the skin tissue taken from treated sheep (d).

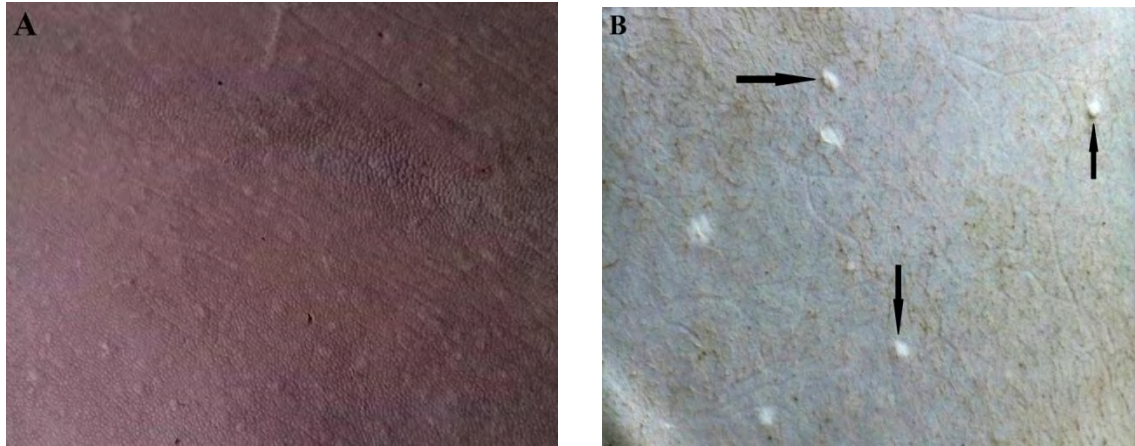


**Figure 10:** Microscopic lesion of *M. ovinus* infested dermis: a, normal skin taken from negative control; b, diffusely infiltrated dermis with inflammatory cells mainly perifolliculitis (red arrow) and prevasculitis (black arrow); (morphology of inflammatory cells at 100x objective c) and inflammatory cells extent illustration on the skin tissue taken from treated sheep (d).

#### **4.5. Cockle formation, Distribution, Extent and Skin quality Grades**

From skin of non-infested control group, 60% of them did not have any visible cockles after processing nevertheless only 40% of processed skins from control group exhibited mild cockles. All skin of *B. ovis* infested untreated sheep showed cockles of different degrees of which 16.7% mild, 33.3% moderate and 50% severe cockles after processed (Table 2). The skin of *M. ovinus* infested untreated sheep were also all demonstrated cockles of which 60% were moderate cockles and 40% severe cockle (Table 2). The cockles caused by *M. ovinus* were large, elevated above processed skin level, easily visible (Figure 11d) although cockles caused by *B. ovis* were small, massive in distribution and cover most parts of the skin (Figure 11c).

Based on cockle related pickled skin grading , 83% of processed skin from non-infested control sheep lied within grades of 1 to 4 (very good to good), 16.7% in grades 5 and 6 (moderate) and no processed skin of the control group were under grades 6B to rejected(poor). However, infestation by *B. ovis* and *M. ovinus* were automatically reversed quality of skin grading, that is no infested untreated processed skins of sheep ( 0.0% ) was graded from 1 to 4 grade (good to very good), 83% were graded as either 5 or 6 grade and 17% were graded as 6B or rejected. Similarly, no processed skin from *M. ovinus* infested untreated sheep were graded as 1 to 4 grade, 50% were graded as 5 or 6 grade and 50% less than or equal to 6B grade.



**Figure 11:** Cockle types and distributions: A, *B. ovis* caused small cockles that widely distributed over large area. B, *M. ovinus* caused severe, large cockles with multifocal distribution (arrow).

#### 4.6. Effect of Treatment on Improving Skin Quality Grade

The difference in cockle distribution and extent between *B. ovis* as well as *M. ovinus* infested untreated group and non-infested control group was statistically significant ( $p < 0.01$ ) but this difference became statistically insignificant after treatment ( $p > 0.05$ ). The details were shown on table 2.

All skins from *B. ovis* infested untreated group demonstrated cockles and none were graded from 1 to 4 grades (very good to good). However, 50% of *B. ovis* infested treated were improved and graded from 1 to 4 grade and only 50% were remain with different extents of cockle and graded as grade 5 or 6 from *B. ovis* infested treated group. The detail information's were revealed on table 2.

Similarly, all skins from *M. ovinus* infested untreated group showed cockles and none were graded from 1 to 4 grades (very good to good). Conversely, from *M. ovinus* infested treated group 33% of skin were improved, had no any visible cockles and were graded as 1 to 4 grades, and 67% of skins showed cockles of different extents and were grouped as

grade 5 or 6. None of skin from *M. ovinus* were graded as grade 6B or rejected after treatment. The fine points were given away on table 2.

Table 2: Summary of effects of cockle on skin grade

Group	No. of skin examined	Extent of cockle on pickled skin				cockle related pickled skin grade		
		No	Mild	Moderate	Severe	G1- 4 (good)	G5-6 (moderate)	G6B-R (poor)
<b>Non-infested control</b>	6	4(60%)	2(40%)	0(0.0%)	0(0.0%)	5(83%)	1(16.7%)	0 (0.0%)
<b><i>B. ovis</i> infested untreated</b>	6	0(0.0%)	1(17%)	2(33%)	3(50%)	0(0.0%)	5(83%)	1 (17%)
<b><i>B. ovis</i> infested treated</b>	6	1(17%)	3(50%)	1(17%)	1(17%)	3(50%)	3(50%)	0 (0.0%)
<b><i>M. ovinus</i> infested treated</b>	6	0(0.0%)	1(17%)	3 (50%)	2(33%)	2(33%)	4(67%)	0 (0.0%)
<b><i>M. ovinus</i> infested untreated</b>	6	0(0.0%)	0(0%)	4(60%)	2(40%)	0(0.0%)	3(50%)	3 (50%)

P < 0.01 for extent of cockle and cockle related skin grade between non-infested control and infested untreated group (*B. ovis*/*M. ovinus*).

## 5. DISCUSSION

In the present study, even though the total mean WBC count increased throughout the experiment in both *B. ovis* and *M. ovinus* infestation there was no statistical significance difference in total mean WBC count between *B. ovis* infested and non-infested control group. Nevertheless, the mean WBC count was significantly higher in *M. ovinus* infested group compared to non-infested control group. This could be due to skin piercing behavior of ked while sucking blood which opens a chance for microbial infection resulting in increments of more leukocytes other than specific ones. This finding agrees with the findings of Bequaert (1942 and 1953) and Losson *et al.* (1988), who in the same way reported higher WBC count in ked infested sheep.

Differential leukocyte count between the *B. ovis*/*M. Ovinus* infested and non-infested control group in this experimental study unequivocally revealed significant eosinophilia which was statistically significant ( $P < 0.05$ ). Both lymphocytes and neutrophils showed increment in *B. ovis* and *M. Ovinus* infested groups but the difference was not statistically significant ( $P > 0.05$ ). This was in agreement with the finding of Aatish (2007) who found significantly increased eosinophil count in sheep infested with mange mite than healthy sheep. This could be due to allergic reactions caused by parasites or their products of inflammatory reactions as well as activation of immune system (Nelson *et al.*, 1977).

Most of the gross and microscopic lesions of *M. ovinus* and *B. ovis* had shown similarities which agreed with the finding of Mersha *et al.* (2010) that described most of ectoparasite lesions overlapping. In the present study, *B. ovis* and *M. Ovinus* infested groups showed erythema and macule/papule on the area where parasite found on the host, discolored skin with the excreta of parasite and crust formation, which is in line with the findings of Small, (2005) and Mersha *et al.*, (2010). Other investigators also described that, the site of attachment of lice on their hosts became red and slightly elevated (Sarkar *et al.*, 2010).

Hyperkeratosis and acanthosis were the common epidermal lesion of *B. ovis* infested group while the common dermal lesions were inflammatory mainly of eosinophil and lymphocyte infiltrated, perifolliculitis, perivasculitis, frunculosis and sebaceous adenitis which is in agreement with the finding of Mersha *et al.*, (2010).

*M. ovinus* caused extensive hyperkeratosis and acanthosis than *B. ovis* and perivasculitis, follicular lesions, diffuse dermatitis and many empty capillaries at the base of epidermis. The findings of Jones *et al.* (1997) have indicated that various types of dermal pathologies varied from follicular lesions to perivascular and/or diffuse dermatitis which is comparable with current findings. Mersha *et al.* (2010) also described that skin lesions caused by sheep ked was the presence of many empty capillaries in the dermis. The findings of Heath *et al.* (1995) have indicated that arteriolar vasoconstriction and fibrinoid degeneration of the tunica media of capillaries and arteries may cause empty capillaries. Heath *et al.* (1995) also described that sheep infested with biting lice only would not have severe skin lesions and scars as do sheep infested with sheep keds alone. This might be due to the fact that *B. ovis* does not suck blood and they only feed on the secretion and debris of the sheep skin.

*B. ovis* and *M. Ovinus* causes a rash called cockle on the skin, which was the principal cause of skin rejection at tanneries in this study. Tefera and Abebe, (2007a); Kassa *et al.* (1998); Asegedech *et al.* (1999) and Tefera and Abebe (2007a & 2007b) reported that cockle or “Ekek” was resulted in connection with the *B. ovis* and *M. ovinus* in sheep. It was found that *B. ovis* caused small cockles that covered larger areas of skin but and *M. Ovinus* caused large and severe cockles. Legg *et al.* (1991) and Heath *et al.* (1996) reported that cockle and hard nodules of *M. Ovinus* was partially due to self scratching.

It was also found that many of *M. ovinus* infested skin lied to low grade quality than *B. ovis* infested sheep skin. Heath *et al.* (1995a), described that sheep infested with lice only would not have severe skin lesions and scars as do sheep infested with sheep keds alone, and the skin of those infested with lice alone heal from the scar faster than the skin of

sheep infested with sheep keds having sucking mouth part that pierce and damage the skin of its host. This study revealed that all skins infested by both *B. ovis* and *M. Ovinus* and processed at tanneries lied in low quality grades, which was in agreement with, Tefera (2004) who reported infestation of sheep with *B. ovis* and *M. ovinus* leads to development of 'Ekek' and is responsible to cause higher proportion of pelts to fall into lower grades. Tadesse and Mebrahitu (2010) reported that the percentage of pickled sheep skins with cockle defect was much higher in lower grades compared with the first three best grades. In agreement with the current findings, Heath *et al.* (1996) had also demonstrated association of *B. ovis* infestation of sheep with downgrading of skins. Similarly, in agreement with this finding, Asp and Tauni (1988) and Ermias (2000) reported increase in intensity of cockle at lower grades.

In this study it was found that treatment removes the common gross lesions caused by both *B. ovis* and *M. Ovinus*. Treatment also reduced the density of inflammatory cells from microscopic lesions. However, treatment was not leads to complete disappearance of skin inflammation within 30 days after treatment. This might be due to time shortage as Heath *et al.* (1995a) mentioned that diazinon with its immediate 'knock down' effect, deals rapidly with lice and in turn cockle disappears relatively quickly. Even though the scars left due to the allergic reaction would take some time to heal depending on the damages done to the skin and the species of the parasite causing the defect. Depending upon wool length, this procedure can take up to 7 weeks as measured by toxicity to biting lice.

It was also observed that only 50% of sheep skin infested by *B. ovis* improved quality grade while the rest 50% still were remained below standard within 30 days after treatment. Of *M. Ovinus* infested treated skins only 33% showed improvement in skin quality grade while 67% remained below quality standard after 30 days of treatment. The most probable reason could be duration after treatment.

## 6. CONCLUSION AND RECOMMENDATIONS

This study revealed *B. ovis* and *M. Ovinus* cause severe lesion to the skin of sheep. Grossly, *B. ovis* and *M. Ovinus* caused more or less similar lesion, of which erythema, disfigured wool and macule were common. However, microscopically *M. Ovinus* caused severe hyperkeratosis and dermatitis with perifolliculitis, perivasculitis than *B. ovis* did. On processed skin *B. ovis* caused small cockles that covered larger areas of skin nevertheless *M. Ovinus* caused large and severe cockles with multifocal distribution. Many of *M. ovinus* infested skin lied to low grade quality than *B. ovis* infested skin. Treatment improved 50% skin quality in *B. ovis* infestation and 33% in *M. Ovinus* infestation after 30 days of treatment. Based on aforementioned conclusions the followings were recommended:

- Awareness should be created for animal owners about the damage of skin due to *B. ovis* and *M. ovinus* as there is economic loss from damaged skin as well as processing of skin to be rejected
- The effect of commonly used anti-ectoparasitic drugs on skin quality improvement should be done
- Strong and extensive work should be done on control, eradications and prevention of diseases causes down grade the quality of skin
- Detailed study should be conducted on the time duration needed for complete resumption of skin lesions after treatment so as to determine the most probable timing between treatments and flaying.

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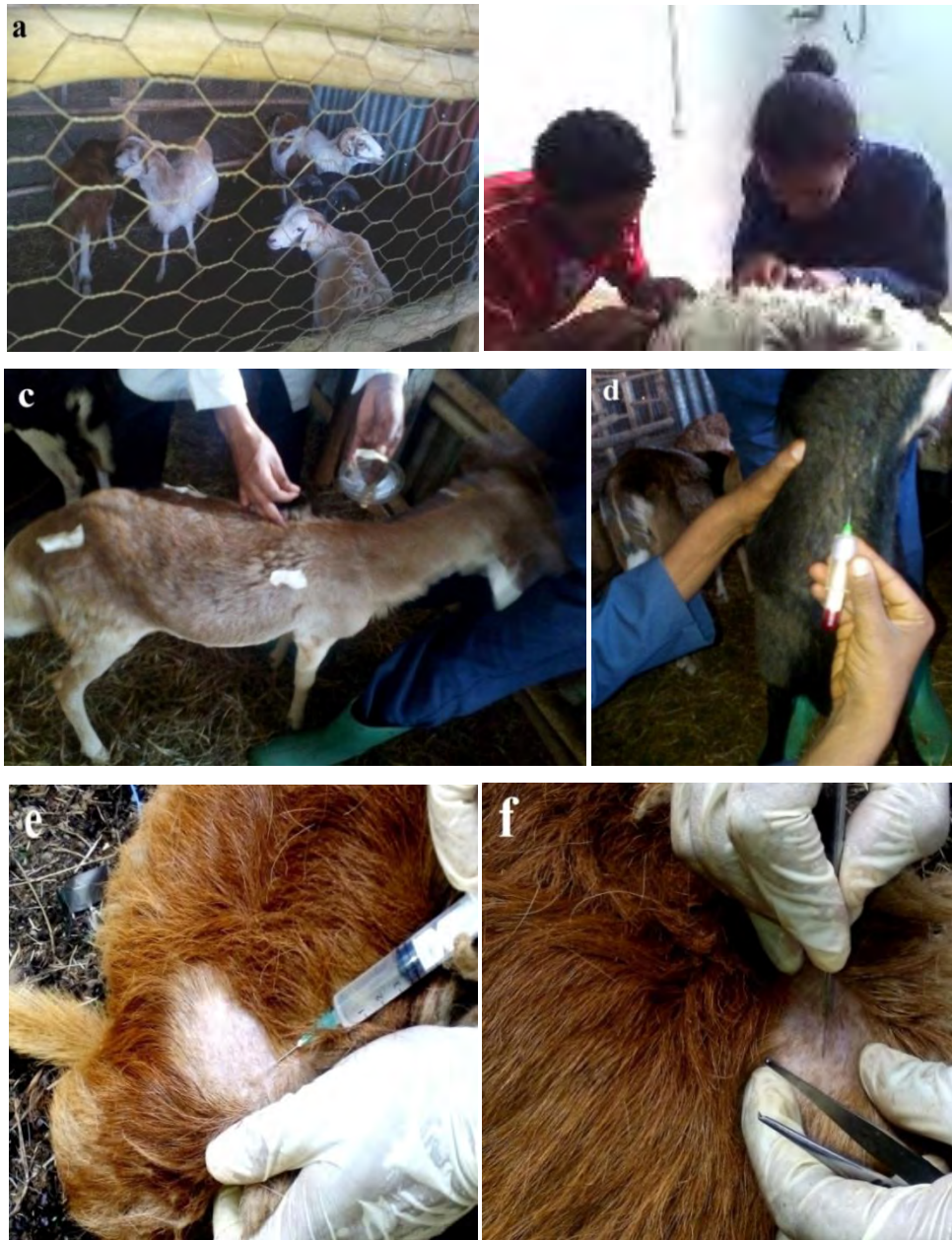
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## 8. ANNEX

### Annex 1: Photo showing on experiment

Experimental group in a separate pen (a), parasite collection from donor animal (b), lice infestation and using scotch tape(c), Blood sample collection from jugular vein (d), administration of local anesthesia to biopsy site (e) and biopsy sample collection (f).



**Annex 2: WBC parameters record format sheet**

Animal group	Id No.	WBC	Differential count				
			Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophil
<b>I</b>							
<b>II</b>							
<b>III</b>							
<b>IV</b>							
<b>V</b>							

**Annex 3: Laboratory materials and procedures for hematology**

1. Total white blood cell (WBC) count

Materials and reagents:

- ✓ Un-coagulated whole blood in EDTA coated vacutainer tubes
- ✓ Micropipette calibrated to dispense 1 to 200 µl with disposable tips
- ✓ Test tube capable of holding 2ml fluid
- ✓ Hemocytometer (improved Neubauer chamber)
- ✓ Special cover glass for the counting chamber

- ✓ A piece of gauze
- ✓ Diluting fluid (1% HCl)
- ✓ Microscope

Procedure:

- About 190  $\mu\text{l}$  of diluting fluid and 10  $\mu\text{l}$  of blood are mixed in a vial, thus diluting the blood to a 1:20 dilution and left for about a minute.
- A cover slip is placed on the hemocytometer, the solution was taken by using a micropipette and holding the micropipette slightly inclined, small volume of the fluid is added under the cover slip that filled the space by capillarity.
- The cells are allowed to settle for a minute.
- Counting of WBC's found at 4 corners (marked in the diagram above as  $\_W^*$ ) is started using low magnification (10 x). WBC's touching the right and lower parts of the lines are not counted to avoid duplicate counting of cells.
- Finally, the total number of WBC counted is multiplied by 50 to get the total WBC's number.

A correction factor of 50 is calculated as:

$$\text{Area of one smallest square} = 1/4 * 1/4 \text{mm} = 1/16 \text{ mm}^2$$

$$\text{Depth of the counting chamber} = 1/10 \text{mm}$$

$$\text{The volume of one smallest square} = 1/10 \text{mm} * 1/16 \text{ mm}^2 = 1/160 \text{mm}^3$$

There are 16 smallest squares in one corner, thus the total squares found in 4 corners =  $16 * 4 = 64$

$$\text{Thus, the total volume} = 1/160 \text{mm}^3 * 64 = 64/160 \text{mm}^3 = 0.4 \text{mm}^3$$

Dilution is 1:20 (i.e., dilution factor is 20), thus it follows that:

$$\text{The total WBC's number} = [(\text{number of WBC counted}) * (\text{dilution factor})]$$

$$/ (\text{the volume in mm}^3)$$

$$= [N * 20] / 0.4 \text{ mm}^3$$

$$= N * 50 \text{ per mm}^3 (\mu\text{l})$$

## 2. Differential white blood cell count

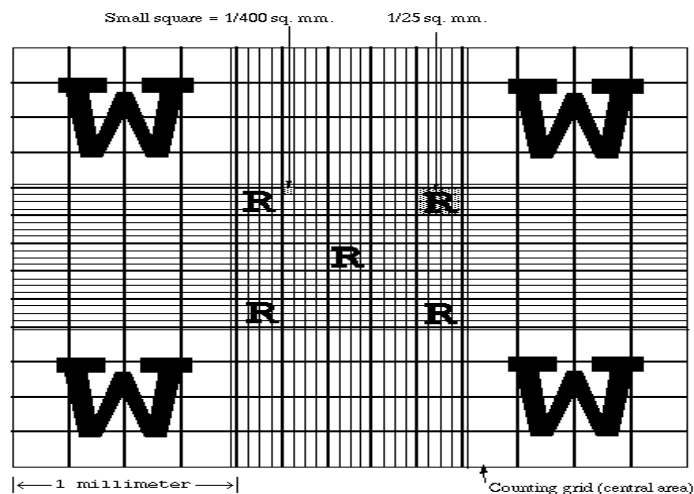
Materials and reagents:

- ❖ Un-coagulated whole blood , Microscope, Glass slides , Immersion oil, Giemsa stain, Xylene , Methanol alcohol , a piece of gauze, Distilled water and Staining rack

Procedure:

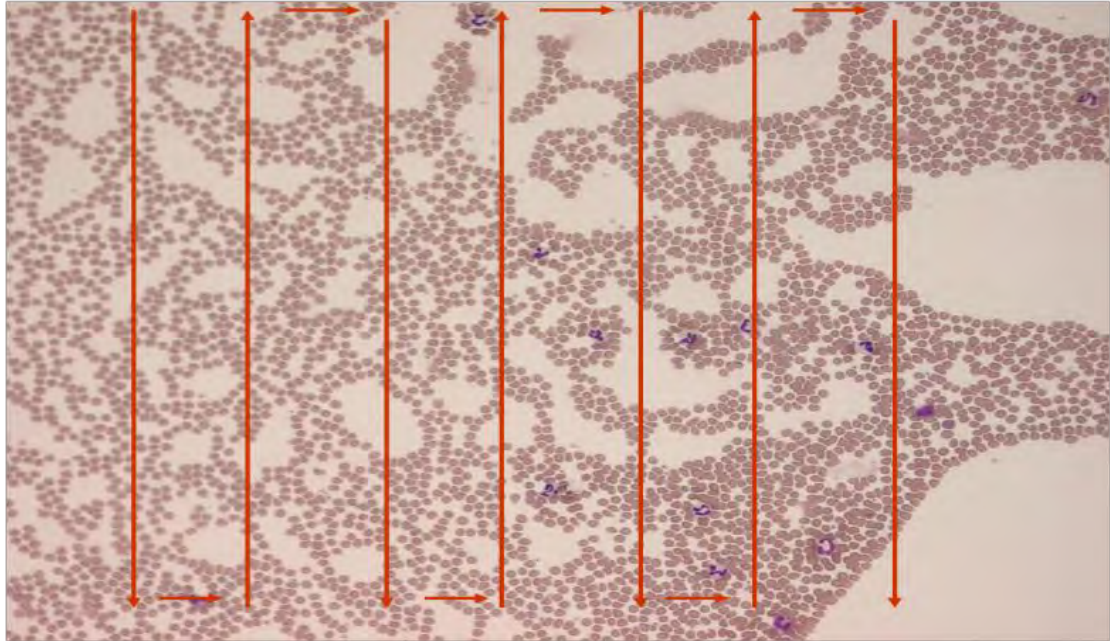
- A drop of blood is placed near to the one end of a clean glass slide.
- A second slid is hold at 45°, attached to the drop of blood, then pushed along the surface of the first in forward direction to form a thin smear. The smear is dried in the air.
- The smear is fixed in methanol for 3-5 minutes.
- The smear is stained with giemsa staining dye and allowed for 30 minutes.
- The slide is rinsed with water and dried in air.
- The stained smear is put on the microscope and one drop of oil is added on the smear. Using oil-immersion objective (100x), 100 WBC's are counted by Battleship method as indicated (annex 3).
- Each type of leukocyte is identified, and results are recorded as % of the total leukocyte count then changed to absolute for more accuracy.

### Annex 4: Ruled area on improved Neubauer hemocytometer.



Total RBC's are counted in the squares labelled with "R". Total WBC's are counted in the (W) area. Source: (Coles, 1986)

**Annex 5: Figure shows differential count method in a stained blood smear (Battleship method)**



**Annex 6: Histopathological Techniques:**

1. Fixation of tissue by 10% neutral buffered formaldehyde
2. Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
3. Tissue specimen processing: fixation of tissue by formalin, Dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax.

Formalin-I 2hr → Formalin-II 2hr → 70% Alcohol 1hr → 95% Alcohol-I 1hr → 100% Alcohol-I 1hr → 100% Alcohol-II 2hrs → 100% Alcohol-III 2hrs → Xylene-I 30hrs → Xylene-II 30hrs → Xylene-III 1:30hrs → Paraffin-I 2hrs → Paraffin-II 3hrs.

4. Embedding processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60c°) is poured and allowed to settle and solidify.
5. Section: sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put an incubator over night.
6. Staining: Hematoxyline eosine stain procedure
  - a. Deparaffinize slides in 2 changes of xylene for 5minutes eac.
  - b. Hydrate slides in 3 changes of 100% alcohol each for 3minutes and 1 changes of 95% alcohol for a minute and 1 change of 70% alcohol for 3minutes
  - c. Rinse in distilled water until repples disappear from slides.
  - d. Place in heamatoxyline (mayer's hematoxline) for 10-15 minutes
  - e. Rinse in tap water until water runs clear
  - f. Decolorize in 1% acid alcohol, 3-6 qiuck dips. Check differentiation microscopically: Nucleic should be distinct; cytoplasm should be uncolored.
  - g. Rinse in tap water until ripples disappear from slides.
  - h. Stain in eosin, 3 dips.
  - i. Rinse in tap water until water runs clear.
  - j. Dehydrate in 95% alcohol of 3dips and 100% alcohol, 3 changes each for 3minutes.
  - k. Clear in 3 changes of xylene for 5 minutes each.
  - l. Mount cover glass with DPX.
7. Examination under the microscope

**Annex7: Photo shows histopathology equipments:**

Tissue processor on processing (a), embedding machine on work (b), microtome on sectioning tissue (c) and tissue ribbon on the bath (d).



### **Annex 8. Procedure for skin processing at tannery**

- 1. Sorting;** On receipt, hides and skins may be sorted into several grades by size, weight, or quality.
- 2. Trimming;** is generally carried out during the sorting process. Some of the edges (legs, tails, face, udders, etc.) of the raw skins can be cut off. Usually this process step is done in the abattoir, but it can also be carried out in tanneries.
- 3. Soaking;** is carried out to allow hides and skins to rehydrate any water which may have been lost after flaying and curing. This is done to clean the hides and skins (removal of dung, blood, dirt, etc), and to remove interfibrillary material. Soaking performed in a processing vessel called a paddle (annex 9b).

4. **Unhairing and liming;** The function of liming and unhairing is to remove hair, interfibrillary components and epidermis and to open up the fibre structure. Unhairing and liming is performed in a processing vessel called a drum (annex 9c).
5. **Fleshing** is a mechanical scraping off of the excessive organic material from the hide (connective tissue, fat, etc.). The pelts are carried through rollers and across rotating spiral blades by the fleshing machine (annex 9d).
6. **Deliming;** The aim of deliming is to remove residual lime from the pelts and to take the pelts to the optimum condition for bating. This involves a gradual lowering of the pH (by means of washing and the addition of deliming chemicals), an increase in temperature and the removal of residual chemicals and degraded skin components. Deliming is performed in a processing vessel called a drum (annex 9c).
7. **Bating** is a partial degradation of non-collagenic protein achieved by enzymes to improve the grain of hide and skin and the subsequent run and stretch of leather. In this process the rest of the unwanted hair roots and scud can be removed.
8. **Degreasing;** Excess grease must be eliminated from fatty skins (sheep, pig) to prevent the formation of insoluble chrome-soaps or to prevent the formation of fat spues at a later stage. Degreasing is most relevant in processing sheepskins, where the natural fat content is about 10 % – 20 % on dry weight. The nature of this fat makes it difficult to remove because of the presence of glycerides and a high melting temperature.
9. **Pickling** is carried out to reduce the pH of the pelt prior to mineral tanning and some organic tannages. The choice of the exact pickling parameters depends on the subsequent tanning step. Very often tanning is carried out in the pickle liquor; however, pickled pelts, e.g. sheepskins, can be traded. Pickled pelt sheepskins must contain fungicides to protect them from mould growth during storage,
10. **Draining, samming and setting;** After tanning, the leathers are drained, rinsed and either horsed up to age, or unloaded in boxes and subsequently sammed to reduce the moisture content prior to further mechanical action, such as splitting and shaving. The setting out operation can be carried out to stretch out the leather. After samming and

setting, hides and skins can be sorted into different grades (annex 9f) after which they are processed further or sold on the market.

**Annex 9. Pictures show materials used for tanning**

Flaying of skin at CVMA abattoir (a); soaking paddle on work (b); skin processor drums (c); fleshing machine (d); processed skin at Draining, samming and setting room (e) and skin on selection and grading (f).





### **Annex 10. Quality grades characteristics**

**Grade 1:** skin with good grain and full of substances with 2 or 3 minor defects outside the butt and shoulder areas which do not depreciate the quality of the skin.

**Grade 2:** skin with good grain appearance with closed minor defects possibly along belly and back and 2 or 3 similar defects randomly spread on the butt and shoulder area, this skin have a cutting value of about 90%.

**Grade 3:** skin with good grain appearance with closed minor defects possibly along belly and back and 2 or 3 similar defects randomly spread on the butt and shoulder area, this skin have a cutting value of about 80%.

**Grade 4:** skin with cutting value of at least 70%, major defects visible on neck region with minor defects on butt and shoulder.

**Grade 5:** skins having at least 60% cutting value with random and minor skin blemishes all over the skin.

**Grade 6:** skin having similar defects as grade 5 and having a cutting value of about 50%.

**Grade 7:** skin having the same grain characteristics as grade 6, but slightly poorer in substances and having cutting value of not less than 25%.

**Grade 8:** skins with empty substances with major deformation in shape and highly irregular in grain character with 10-25% cutting value.

**Rejected skins:** reject the total product