COMPARATIVE STUDY ON PATHOLOGICAL CHANGES IN SHEEP AND GOATS EXPERIMENTALLY INFECTED WITH *HAEMONCHUS CONTORTUS*

MSc THESIS

BY

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BISHOFTU, ETHIOPIA

COMPARATIVE STUDY ON PATHOLOGICAL CHANGES IN THE ABOMASUMS OF SHEEP AND GOATS EXPERIMENTALLY INFECTED WITH *HAEMONCHUS CONTORTUS*

MSc THESIS

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 Veterinary Science in Tropical Veterinary Pathology

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DEDICATION

This thesis is dedicated to all my family especially my father Tarekge Gabremikael and my uncle Ashenafi Teashale and my aunt Nigist Teashale for nursing me with affection and love and for their exhaustive support in the success of my life.

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LISTS OF ABRVATIONS

ECL	Entrochromaffin-Like
EDTA	Ethylenediaminetetraacetic acid
FAMACHA	FAfa Malan CHArt
GI	Gastro-Intestinal
GIN	Gastrointestinal nematodes
Hgb	Haemoglobin
PCV	Packed Cell Volume
SPSS	Statistical Package for the Social Science
TEC	Total erythrocyte count
TSP	Total serum protein

ABSTRACT

The experimental study was done between October, 2019 and June, 2020 in the fly-proof experimental animal facility located in the premise of the college of veterinary medicine and Agriculture at Bishoftu. A total of 14 male goat (G1 and G2) and 14 male sheep (G3 and G4) were allotted in to four equal groups. Single dose of 10,000 of infective larvae of *Haemonchus contortus* (L3) was orally administered to each animal in G1 and G3. Parameters such as body weight, PCV, haemoglobin, worm count, serum total protein, serum albumin, alkaline phosphatase and aspartate aminotransferase were measured. Moreover, histopathological sections were stained and examined for general changes as well as for changes in specific cells such as tissue eosinophilia and parietal cell population. The findings show that 1) All infected sheep and goats developed the infection with higher mean worm burden in goats (5590) than sheep (2887) and the difference was significant ($P<0.05$); 2) All infected sheep and goats exhibited a progressive anaemia; the level being more severe in goats than in sheep ($P< 0.05$) with mean PCVs of 13% and 18.6% respectively; 3) While body weight gain was minimal in sheep, goats have lost significant weight compared to pre-infection levels, to control animals or in relation to infected sheep ($P< 0.05$); 4) analysis of biochemical changes also revealed marked reduction in serum total protein and albumin but again it was much more significant in goats than in sheep ($P< 0.05$); 5) infected sheep and goats' abomasa have shown thickening, nodule development, eosinophilic infiltration and damage to parietal cells. Tissue eosinophilia was more prominent in sheep while parietal cell loss was severe in goats. In conclusion, goats under experimental infection and similar management condition with sheep develop much more severe infection and associated pathology compared to sheep and hence deserve special attention.

Keywords: Anaemia, Biochemical changes, *H. contortus* Histopathology, Goat, Sheep.

1. INTRODUCTION

Gastrointestinal parasitism is an important problem of livestock in many places of the globe (Vercruysse and Claerebout, 2001). Its economic impact due to production losses, mortality and costs of treatment can be huge in susceptible populations (Hawkins, 1993; Selemon, 2018). Among the most pathogenic and highly prolific gastrointestinal tract (GIT) helminthes is the blood feeding abomasal parasite, *Haemonchus species* affecting ruminants and camels (Urkuhart *et al*., 1996). Abomasum is in fact one of the most important sites for nematodes belonging to *Trichostrongylidae* family in small ruminants. It is the site for four species of GI nematodes; *Haemonchus spp.*, *Teladorsagia spp.*, *Ostertagia spp.* and *Trichostrongylus spp* (Sultan *et al*., 2010; Vlassoff and McKenna, 2010). Although *Haemonchus* infections are reported in climates ranging from tropical to temperate, it has a more significant impact in tropical and subtropical regions of the world (Besier *et al*., 2016; Selemon, 2018). High humidity, at least in microclimate of the faeces and the herbage is essential for larval development and survival. Surveys in countries around the world have shown that amongst domestic animals, sheep and Goats suffer more frequently from haemonchosis (Maqsood *et al.,* 1996; Nwosu *et al.,* (2007); Tariq *et al.,* (2008). Similar reports have also confirmed that *Haemonchus contortus* is the most important gastrointestinal parasite in sheep (Fleming *et al.,* 2006; Sharma and Ganguly, 2016). *H. contortus* sucks about 0.05 mL of blood per day by ingestion (Qamar and Maqbool, 2012) and an additional blood is also lost into the gastric lumen because of mucosal irritation and oozing out from when the parasite is detached from the feeding site altogether leading to mild to severe anemia.

The pathological consequences of helminth parasitism and the ability of the host to develop immunity and express resistance against them depends on a number of factors such as host traits (species, breed, age, physiological status, feeding behavior, etc) and worm characteristics (burden, feeding behavior, lifecycle, etc) (Hoste *et al.,* 2008; Rowe *et al.,* 2008). For example, Amarante *et al.,* (2007) found an inverse relationship between inflammatory cells and worm burden in sheep infected with *Trichostrongylus colubriformis* and suggested that this condition possibly impaired establishment,

development and survival of parasite. Similarly, Terefe *et al* (2007a) has demonstrated that resistant breeds of sheep had higher number of circulating eosinophils. In addition, Terefe *et al* (2007b) has also demonstrated that in vitro pre-exposure of *Haemonchus contortus* L3 to blood eosinophils reduces their establishment potential in sheep. The cytokines secreted by Th2 cells promote mast cell hyperplasia, eosinophilia and the production of antibodies. However, such allergic/Th2 type responses are often accompanied by pathological consequences that can be aggravated by the direct impacts of the parasites in the tissue or lumen of the digestive system (Else and Finkelman, 1999; Grencis, 2001; Gause *et al*., 2003).

Most of the available pathological studies due to *H. contortus* have been carried out on experimentally infected goats). Pérez *et al. (*2001), while studying the pathology of the abomasum and abomasal lymph nodes in experimentally infected goats with *H. contortus,* have demonstrated a marked increase in the secretion of mucus and huge infiltration of eosinophils, mast cells, T- lymphocytes, B- cells, plasma cells and globule leukocytes in the abomasal mucosa., Bambou *et al.,* (2013) studied the effect of *H. contortus* on local cellular responses in experimentally infected resistant and susceptible young goats and reported an increase infiltration of eosinophils and mononuclear cells in both resistant and susceptible breeds.

The major cause of pathogenesis within the host is due to direct morphological changes to the abomasal mucosa by the larval stages and subsequent infiltration of the site by inflammatory cells (Fox, 1997; Huntley *et al*., 2004). After ingestion, exsheathed L3 enter the gastric glands of the abomasum and remain for about two weeks. Their presence within the gastric mucosa induces morphological changes within the infected glands, resulting in the replacement of the specialized mucous, zomogenic and parietal cells with an undifferentiated, mucous secreting columnar epithelial cell type (Soulsby, 1982). As L4 emerges from infected glands 7-14 days later, an undifferentiated cellular hyperplasia occurs among the surrounding gastric glands, resulting in the inability to secrete HCl by non-functional parietal cells and an increase in abomasal pH. As pH increases within the abomasum, pepsinogen is unable to convert to pepsin which causes a disruption in

digestion. Additionally, a breakdown of cellular junctions causes serum hypoproteinemia as serum proteins begin to leak into the abomasum (Murray *et al*., 1970; Soulsby, 1982).

Reports from many studies show controversial findings with regards to GIT helminth profiles between sheep and goat. Some research outputs have shown that the prevalence haemonchosis is higher in sheep than in goats in the same geographical area (Mushonga *et al.,* 2018). However, whether this difference between host species is due to the inherent factors of the hosts or due to difference in their exposure to the parasite owing to their feeding habit is not clear. On the contrary, Kumsa *et al*., (2011) has reported an almost equivalent infection rates between sheep and goats in central Oromia of Ethiopia while Tony (2007) described that goats appeared to be more susceptible to helminthes than sheep. Such variations may be attributed to differences in host breed, animal feeding and management and/or parasite factors (strain and host-adaptation, ect). Obviously, such inconsistency in the host-parasite association could be accompanied by less defined local and systemic pathophysiological changes. As an explanation to such variations and to aid the understanding of the role of host species on the development of gastrointestinal parasitism, it is essential to study the degree and type of pathological consequences occurring in sheep and goats infected with specific parasite, *H. contortus*. We hypothesize that sheep and goats show similar pathophysiological responses if exposed to similar risk of helminth parasite infection under similar management condition.

Therefore, the objectives of this thesis were:

- To demonstrate pathophysiological changes and compare these pathophysiological changes between the two species of hosts using selected indicators.

2. LITERATURE REVIEW

2.1. Abomasal helminth parasites of sheep and goats

The majority of gastrointestinal strongyles of ruminants belong to the family Trichostrongylidae. A complex of related genera and species of Trichostrongylide nematodes are known to develop in the abomasum in different species of ruminants. These include: *Ostertagia ostertagi, O. lyrata, Haemonchus placei,* and *Trichostrongylus axei* of cattle, *Teladorsagia (Ostertagia) circumcincta, Teladorsagia trifurcate, Haemonchus contortus, and T. axei* of sheep and goats, and *Haemonchus longistipes* and *T. axei* of camels (Jubb *et al.,* 1993). Some cross-infections by these species occur between sheep, cattle and camels, but are of minor significance (Achi *et al*., 2003).

2.2. Haemonchosis in small ruminants

2.2.1. Morphology of H. contortus

The morphological characteristics of *H. contortus* are a mouth capsule with a single dorsal lancet and two prominent cervical papillae in the oesophageal area. The male parasite is characterised by its copulatory bursa formed of two large lateral lobes and a small asymmetrically positioned dorsal lobe. The females have a reddish digestive tube filled with ingested blood, spirally surrounded by two white genital cords (ovaries). They have a sharply pointed slender tail and a vulva with or without anterior vulval flap. The eggs are of strongyle type with a diameter between 70 and 85 µm (Urquhart *et al*., 1996).

2.2.2. Life cycle

Eggs passed in feces develop to infective stage (L3) in the environment. Ruminants acquire infection by ingestion of infective L_3. The L3, after removing its L2 cuticle (exsheathment) in the rumen, passes to the abomasums where it enters the gastric glands by penetrating the mucosa. The L3 then develops into L4 by moulting and then return to

the lumen where they molt to L5 and finally adult stages. The L4 remains in the mucous membrane for about 7-11 days before emerging as late L4 into the lumen (Rahman and Collins, 1990). Adult male and female *Haemonchus* copulate and the female starts laying thousands of eggs a day. The prepatent period is 2-3 weeks, but the L4 may go into the stage of arrested development (hypobiosis), which significantly alters the prepatent period (Urquhart *et al*., 1996).

2.2.3. Clinical signs

Clinical signs of disease may become more frequent following these peaks owing to higher worm burdens. They include severe anemia, seen as pale mucous membranes periorbital and submandibular edema resulting from hypoproteinemia caused by the *Haemonchus* blood sucking activity, lethargy, emaciation, weakness, wool loss and even death (Dunn, 1978). The adult worms feed on host blood and move from one feeding site to another, leaving behind wounds that continue to hemorrhage and resulting in anemia, which is the most common clinical sign. The volume of blood that is lost and consumed with a heavy worm burden may result in the host death (Soulsby, 1982). The degree of anemia depends on the abomasal worm numbers. Clinically, haemonchosis can be categorized into three types; hyperacute, acute and chronic. The hyperacute form occurs in young stage and unhealthy lambs exposed over a short period of time to heavy infection (ingested a massive number of L3s > 10,000) and is rare and results in lamb death (Mehlhorn, 2016).

Acute cases usually occur in young lambs that get heavily infected with or without diarrhea, but the host mounts an erythropoietic response resulting in the partially compensation for the blood loss. The parasite burden is mild, 1,000-10,000 individuals and all ages of sheep are affected, regardless of present health status. Furthermore, anemia is accompanied by hypoproteinemia and edema, which may contribute to death. A common remark in these cases is sub-mandibular edema designated “bottle-jaw” (Taylor *et al.*, 1990).

2.3. Pathophysiology of haemonchosis in sheep and goats

2.3.1. Anaemia

Most of the pathophysiological changes in haemonchosis are caused by the L4 and adult stages and depends on factors including: intensity of the infection; hosts' species, age, breed and physiological status as well as environment and management factors (Kassai, 1999; Taylor *et al.,* 2007). The L4 and L5 larvae and adult worms are robust blood-sucking stages of the parasite. Movement of the worm causes wounds and secretion of anti-coagulants resulting in a continuous haemorrhage from the abomasal wall (Tehrani *et al.,* 2012).

Anaemia is the key pathogenic process leading to *haemonchosis* and blood loss is often correlated with *H. contortus* burden (Le Jambre, 1995) in the abomasums. Critical values associated with terminal haemonchosis are evident from field and pen observations: a fall in haematocrit (packed cell volume) to <15% for an individual animal is generally fatal, unless immediate treatment is given (Albers *et al.,* 1989) and despite treatment recovery is unlikely at values of <10-12%. Using the more consistent index of haemoglobin concentration (Roberts and Swan, 1982) considered values of <8.5 g/100 mL to be indicative of heavy *H. contortus* burdens.

2.3.2. Gross pathological changes in the abomasums

Gross lesions, such as petechial haemorrhages on account of the *Haemonchus* parasite attachment and feeding behavior and severe congestion in the abomasal mucosa are common observations (McKenna, 1998). The abomasal mucosa becomes oedematous and congested and showed petechial haemorrhages in all *Haemonchus contortus* infected goats. These changes are more severe in fundic than in cardiac and pyloric areas. Gastric lymph nodes are also oedematous and markedly enlarged in all infected goats (Pereza *et al.,* 2001). The abomasal, the content was watery, seldom with foul smelling. In these abomasal, the mucosa had different patterns as multifocal to diffuse thickening and

corrugation, focal to multifocal thickening and presence of scattered pale to whitish small nodules in the mucosa.

According to Biswajit *et al. (*2017), following *H.contortus* infection, all the visceral organs were pale and the abomasal contents were fluidal and sometimes mixed with free blood with large number of adult *Haemonchus contortus* parasites. In few cases ulcerative haemorrhagic spots were seen on the abomasal mucosa where the parasites were found adhered with hyperaemia of the abomasal folds. The large burdens (many thousands) of worms considered typical of acute haemonchosis leaves little doubt about the diagnosis. The mucosa is oedematous and appears covered with worms, with petechiae and often frank blood-seepage evident.

2.3.3. Histopathological changes in the abomasums

Studies of field parasitism and experimental larval infections identified the morphological and physiological effects of abomasal nematodes including nodule development, mucous cell hyperplasia and superficial epithelial damage with accompanying changes in secretions (Simpson, 2000). Glands with developing larvae are often lined by a flat epithelium containing few secretary cells and reduced number of parietal and chief cells. Fundic mucosal tissues are thicker due to mucous cell hyperplasia and marked accumulation of inflammatory cells such as lymphocytes and eosinophils (Scott *et al*., 2000), which sometimes result in nodule development. *Haemonchus* species larvae emerge earlier from the gastric glands and are therefore, ambulatory over the abomasal surface. Hyperplasia is therefore, usually generalized in haemonchosis and is thought to occur in the more superficial layers of the epithelium, so that parietal and chief cell numbers are not necessarily affected (Hunter and Mackenzie, 1982). After parasites emerge into the lumen, there is more widespread mucosal hyperplasia and parietal cell loss. Reduction in the number of chief cells may result from the failure of immature mucus neck cells to mature to chief cells in the absence of the correct signal from parietal cells (Simpson, 2000). Along with the rapid decrease in abomasal acid secretion, the parietal cells develop dilated canaliculi and/or degenerative changes typical of necrosis (Scott *et al*., 2000).

Biochemical changes in abomasal parasitism

H. contortus or *T. circumcincta (Ostertagia circumcincta)* infected sheep have increased gastric pH, elevated serum concentrations of pepsinogen, gastrin and diminished acid secretion (Simcock *et al*., 1999). The abomasal pH of uninfected sheep is approximately 2.8 but this may quickly increase to about 4 or above during infection (Lawton *et al.*, 1996) probably due to reduced production of HCl following damage to parietal cells. Hypergastrinaemia begin at the same time as abomasal pH became increased, however stayed high and continued to augment when pH had reached a maximum and had returned towards normal. Serum gastrin is also depressed when abomasal pH exceeds 5.5 in *T. circumcincta* (*O. circumcincta)*-infected sheep (Lawton *et al*., 1996).

Hyperpepsinogenaemia is frequently employed in ruminants as an indicator of abomasal parasitism (Lawton *et al*., 1996; Simcock, 1999). Serum pepsinogen usually elevates at the time of parasite emergence from the glands or a little earlier and remains increased for up to 30-60 days after a single infection while the parasites are still present in the abomasum in *T. circumcincta* (*O. circumcincta)* and *H. contortus*-infected sheep (Lawton *et al*., 1996; Scott *et al*., 2000). After transfer of adult *H. contortus* (Simpson *et al*., 1997), *Ostertagia ostertagi* (McKellar *et al.,* 1986) or *T. circumcincta* (Lawton *et al*., 1996; Simpson *et al*., 1999; Scott *et al.,* 2000), abomasal pH, serum gastrin and pepsinogen concentrations increase within the first day. These effects on abomasal secretion are proposed to be initiated by abomasal parasite chemicals (Anderson *et al*., 1985; Lawton *et al*., 1996; Scott *et al.,* 2000).

Nematodes also secret proteolytic enzymes which for blood-sucking worms like *Haemonchus* have potent anticoagulant activity (Suchitra and Joshi, 2005). It is also believed that the parasites may initiate the pathophysiology through their ES products either by acting directly on parietal cells or indirectly through Entrochromaffin-Like (ECL) cells thereby provoking inflammation and disruption of the protective mucosal defense system (Simpson, 2000).

3. MATERIALS AND METHODS

3.1. Study Area

The experimental study was done between December, 2019 and April, 2020 in the fly-proof experimental animal facility located in the premise of the college of veterinary medicine and Agriculture at Bishoftu. Bishoftu is located at a distance of about 47km South East of the capital city of Ethiopia, Addis Ababa. The area is located at an altitude of 1850 meters above sea level. It experiences a bimodal pattern of rain fall with a long rainy season from June to October and a short rainy season from March to May and has an average annual rainfall of 800mm. The area has an average maximum and minimum temperature of 27.7 ^{o}C and 12.3 ^{o}C, respectively (CACC, 2003). The animal house was equipped with different pens containing feeding and watering troughs.

3.2. Experimental Animals and grouping

All sheep and goats to be used for the experimental study were purchased from Assela open market. Before, forming experimental groups, the animals were acclimatized for one month during which they were monitored for helminth eggs by treated with broad spectrum anthelmintics and sprayed with acaricides, body weight changes and general health. All animals were housed in four separate boxes with raised concrete based units and a solid partition separated by adjacent pens. Care was taken to avoid contamination of pens with nematode larvae from outside by wearing boots before enter to each pens of the animals. Two groups of sheep (infected and negative control) and two groups of goats (infected and control) each with seven animals were formed. Animals were allowed to feed dried hay with sufficient quantities of concentrate feed and water throughout the adaptation and experimental period. Animals were handled and managed according to guiding principles of ethical use and management of experimental animals.

3.3. *H. contortus* isolation and preparation

The adult female *Haemonchus contortus,* were isolated from abomasums of freshly slaughtered goats originating from Borena area. The worms were pooled and homogenized to release the eggs. A fecal material was collected from apparently helminth free animals, tested for absence of eggs and the prepared eggs seeded in it. The mixture was then kept at room temperature for 14 days with frequent mosturization and aeration. Larvae was recovered by the modified Baermann (annex 1) technique and stored for three weeks at +4 degree centigrade until donor animals were infected for further propagation. Two donor sheep were infected with 10,000 L3 of the prepared larvae. After three weeks, following appearance of a good number of fecal eggs, large volumes of fecal materials were collected daily to culture and recover large quantity of L3 for infecting experimental groups of sheep and goats.

3.4. Experimental design and animal infection

At the end of the adaptation period animals were weighed and ear tagged for easy identification. A randomized block design was used where goats and sheep were allocated into four groups based on their body weights. Group 1 (G1) was infected goat, Group 2 (G2) non-infected goat, Group 3 (G3) infected sheep and Group 4 (G4) non-infected sheep (Table 1). Each group consisted of seven (7) animals. Group 1 and 3 (G1 and G3) were infected by oral route with 10, 000 *H. contortus* L3/animal (all at once) as described in Terefe *et al.* (2007a). All animals were managed following standard ethical principles for use of laboratory animals with permission from the Animal Research Ethics Review Committee of the college of Veterinary Medicine and Agriculture (Certificate Ref. No: VM/ERC/29/01/12/2020).

Table 1. Experimental groups and sampling schedule

Group	Infection status	Sampling days								
G1	Infected goats (Day0)	D0	D7	D14	D21	D28	D35	D42	D49	D56
G2	Non- Infected goats									
G3	Infected sheep (Day0)									
G4	Non- Infected sheep									

3.5. Data collection

3.5.1. Clinical observations

The experimental animals were thoroughly observed daily for clinical changes with special attention to appetite, general body condition, color of visible mucous membranes and consistency of the feaces.

3.5.2. Body weight measurement

The animals were weighed weekly for the whole duration of the experiment using spring balance. The body weight of each animal was recorded starting from day0 and all the way through the end of the experiment (D56).

3.5.3. Confirmation of infection

Animals in all groups were monitored for fecal egg by using fecal floatation technique (Urquhart *et al*., 1996). Once, eggs were detected, animals were periodically tested to confirm that the infection persisted throughout the experiment.

3.5.4. Measurement of anaemia

Blood samples were taken weekly from Day0 to Day56 from each individual sheep and goat in order to determine packed cell volume (PCV) and haemoglobin (Hgb) concentration. About 4ml of blood was collected into EDTA coated vacutainer tube by puncturing the jugular vein. The packed cell volume was determined using microhaematocrit technique (Jain, 1986). For PCV determination fresh blood samples were drawn in capillary tubes and centrifuged in a microhaematocrit centrifuge (Hawksley and sons Ltd. England) at 12000 rpm for five minutes. The PCV percent was read by using a microhaematocrit reader. Haemoglobin concentration was measured by Sahli's method (acid haematin method) as described by Jain (1986). The method depends

on the conversion of haemoglobin to acid haematin by adding a small amount of diluted hydrochloric acid. The resulting brownish-yellow color was matched with the standard color of the apparatus. The reading was converted to g/dl.

3.5.5. Serum protein

Blood sample for the evaluation of biochemical parameters was drawn from the jugular vein and transferred to disposable blood collecting tubes (BD vacutainer stubes) and allowed to coagulate at 37°c for 30 min. The serum was separated by centrifugation at 3000 rpm for 10 min and transported to the laboratory (Arsho Laboratories PLC-Addis Ababa) in a thermacool box Total serum protein (TSP) and Serum albumin were anlaysed.

Total serum protein and albumin serum determination

The system reagent for the quantitative determination of total protein and albumin in serum were using Beckman Coulter AU analyzers (Walker *et al*., 1990). For determination of total serum protein concentrations using the modification of Weichselbaum method were employed. Cupric ions (Cu2+) in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce a blue violet colored complex. For determining albumin concentrations in serum were using the modified the Doumas and Rodkey methods. The albumin reacts with pH 4.2 neutral buffered solution of bromocresol green to form an intense green complex. The results were given the analyzer machine by automatically printed out for each sample in g/dL.

3.5.6. Gross examination of the abomasum

At the end of the experiment, 56 days post infection (PI), all sheep and goats were killed humanely. The entire abomasum from each control and infected animal was examined visually for gross lesion such as presences of hemorrhages, nodules, ulcerations, presence of adult worms, etc. The contents of infected abomasum were recovered and preserved in

70% alcohol. The volume of the material was adjusted to 1 litre and worms were counted in 10% aliquot as described previously by Terefe *et al*. (2005).

3.5.7. ***Histopathology***

Representative samples were taken by including parasite specific predilection sites such as the fundic and pyloric regions. About 1 cm^2 tissue samples from both infected and control animals were collected and preserved in 10% buffered formalin for observing eosinophil and description of tissue damages. After routine histological processing, 5 µm-tick paraffin-embedded tissue sections were prepared. These were then be deparaffinised and stained with haematoxylin and then counterstained with eosin according to the methods described by Winsor, (1994). The slides were examined at x400 magnification in 10 microscopic fields.

3.6. Statistical analysis

Data were recorded in Excel spreadsheet and summarized with descriptive statistics (means, standard error and percentages) were calculated. Means were compared among groups through analysis of variance (ANOVA) and analyzed using STATA version 12.0 (Stata Corporation, 2009).

4. RESULTS

4.1. Parasite establishment

Fecal eggs were first detected on day 21 post infection (PI) and persisted throughout the experimental period in all infected animals. No worm eggs were demonstrated in the non-infected sheep and goat at all times. Further confirmation was done at postmortem by the presence of numerous adult worms on the surface of the abomasal mucosa in all animals of G1 (infected goats) and G3 (infected sheep). The mean numbers of *H. contortus* recovered at necropsy (Table 2) from abomasum of G1 and G3 animals were respectively (5590 ±1927.546) and (2887.143±2746.232), the difference between both groups being significant (P<0.05).

Table 2. Mean worm burden, goats and sheep experimentally infected with *H. contortus*

Worm burden	**Group**	**Mean+SD**	**95%CI for Mean**	**P -value**
Adult parasite	G1	5590 +_1927.546	3807.317-7372.683	<0.05
	G3	2887.143+_2746.232	347.3018-5426.984	

4.2. Clinical observations

No clinical signs were observed in uninfected control group during the experimental period. On the other hand, all infected groups showed depression, variable degree of inappetance, pale mucous membrane and reduced of body condition (Figure 1).

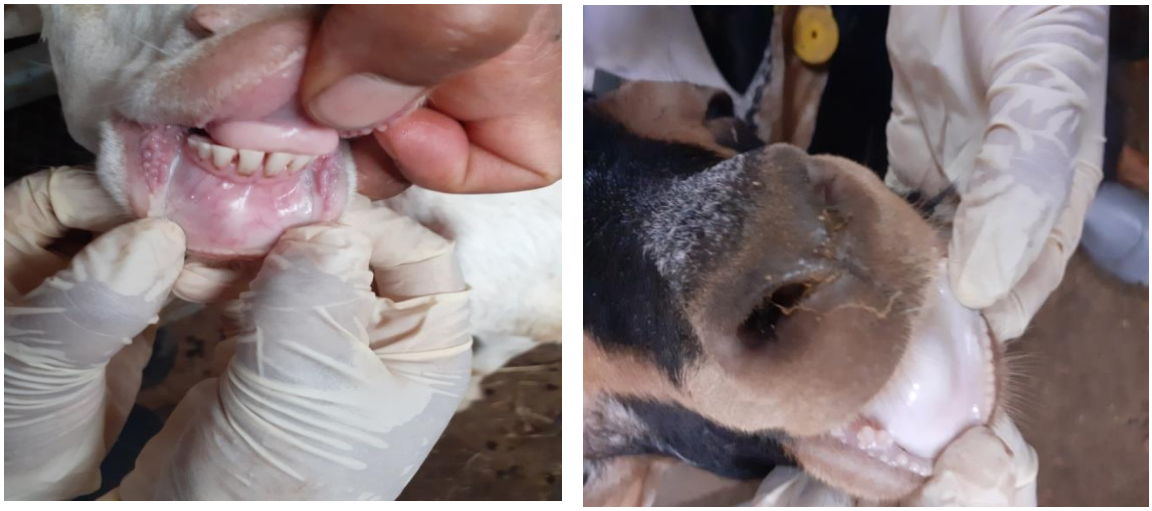

Figure 1: Development of anemia as expressed by change in the color of the mucous membrane: Goat with pinkish mucous membrane (left: non-infected), and pale mucous membrane (right; infected)

4.3. Body weight changes

There was no significant variation in the body weights of all the four groups of animals at the beginning of the experiment since they were grouped by blocking body weight. The weight of animals in the negative control groups (G2 and G4) had gradually increased or remained relatively unchanged throughout the experimental period. In response to *H. controtus* infection, goats (G1) and sheep (G3) have responded very differently with regards to their body weight changes (Figure 2). Infected goats have shown progressive decline while G3 animals displayed mean body weights that more or less maintained their pre-infection values. At the end of the experiment (D56 PI), G1 had significantly lower mean body weight than animals in G2 with mean values of 19.7±1.3kg and 24.9±4.9kg respectively and the difference was significant ($P<0.05$). On the same measurement point, no significant difference in body weight was observed between G3 and G4 sheep ($P>0.05$). On the other hand, although there was no difference at the start of the experiment, infected goats have lost significantly higher body weight (19.6% loss) than

infected sheep (4.75% gain) at the end of the experiment **(**P <0.05) compared to day0 values.

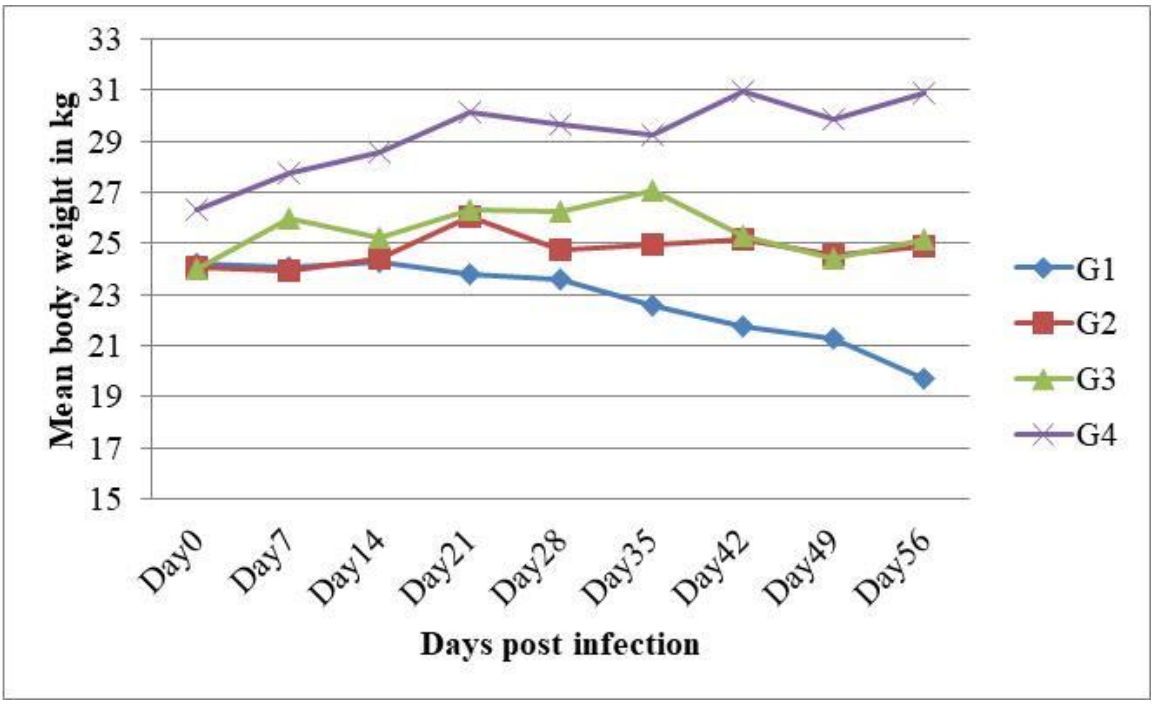

Figure 2: Mean body weight of infected G1 and G3) and non-infected (G2 and G4) groups

4.4. Hematological finding

4.4.1. Changes in packed cell volumes (PCV)

At the beginning of the experiment, all four groups had comparable PCV values. While these values remain almost at pre-infection level for the non-infected control groups, PCV progressively declined until the end of the experiment in the infected groups (Figure 3). Hence, for most part of the experimental period, PCV of infected animals was significantly lower than that of their control counterparts (P<0.05); attaining values of 13±1.41% and 29.86±2.67% in infected and control goats respectively and 18.57±5.35% and 29.17±2.48% in infected and control sheep respectively at the end of the experiment.

When this measurement was compared between infected groups (G1 and G3), it was significantly lower for G1 compared to that of G3 ($P < 0.05$) as of D28 PI.

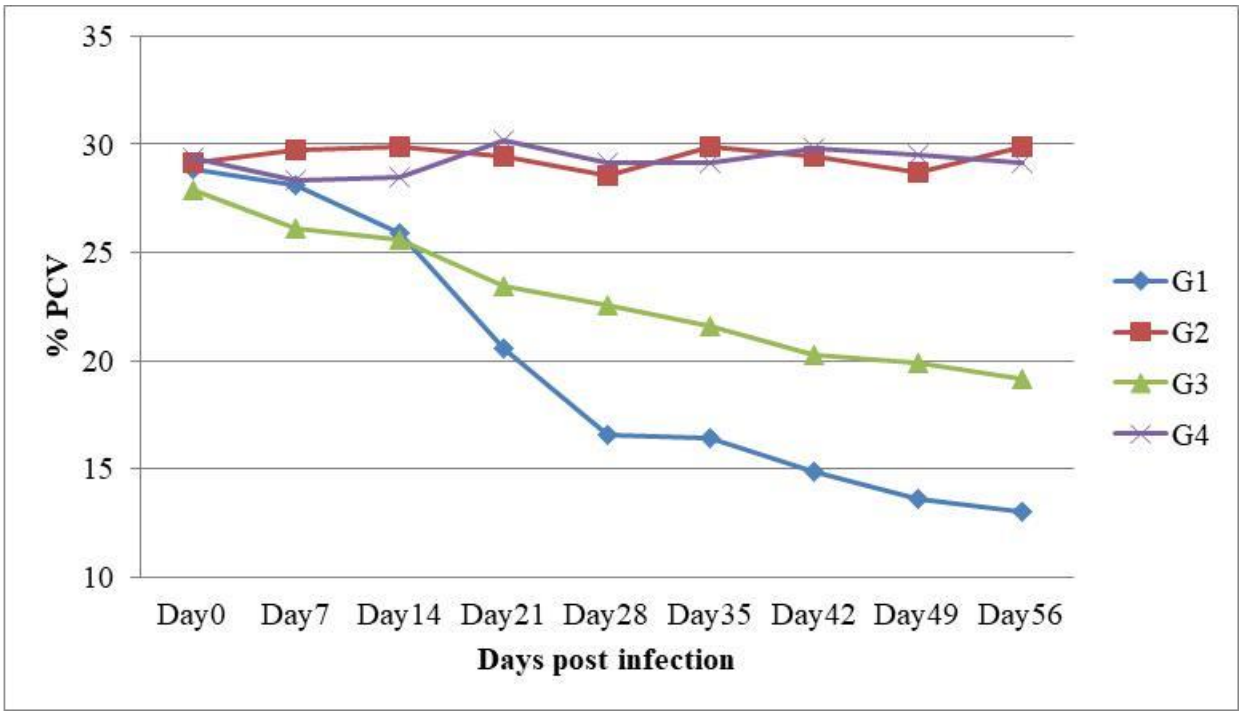

Figure 3: Comparison of mean PCV values in the four experimental groups

4.4.2. Haemoglobin (Hgb) concentration

A significant reduction from pre-infection level ($P< 0.05$) of Hgb concentration was observed as of Day14 and Day28 PI in all infected goats and sheep respectively (Figure 4). This was also true between infected and control groups. At the end of the experiment, G1 had mean Hgb concentration of 7.23g/dl while group 3 had mean Hgb of 8.56g/dl but with no significant difference ($P>0.05$). The change in blood hemoglobin concentration was directly proportional to the decline in PCV ($R= 0.6373$, $P< 0.05$).

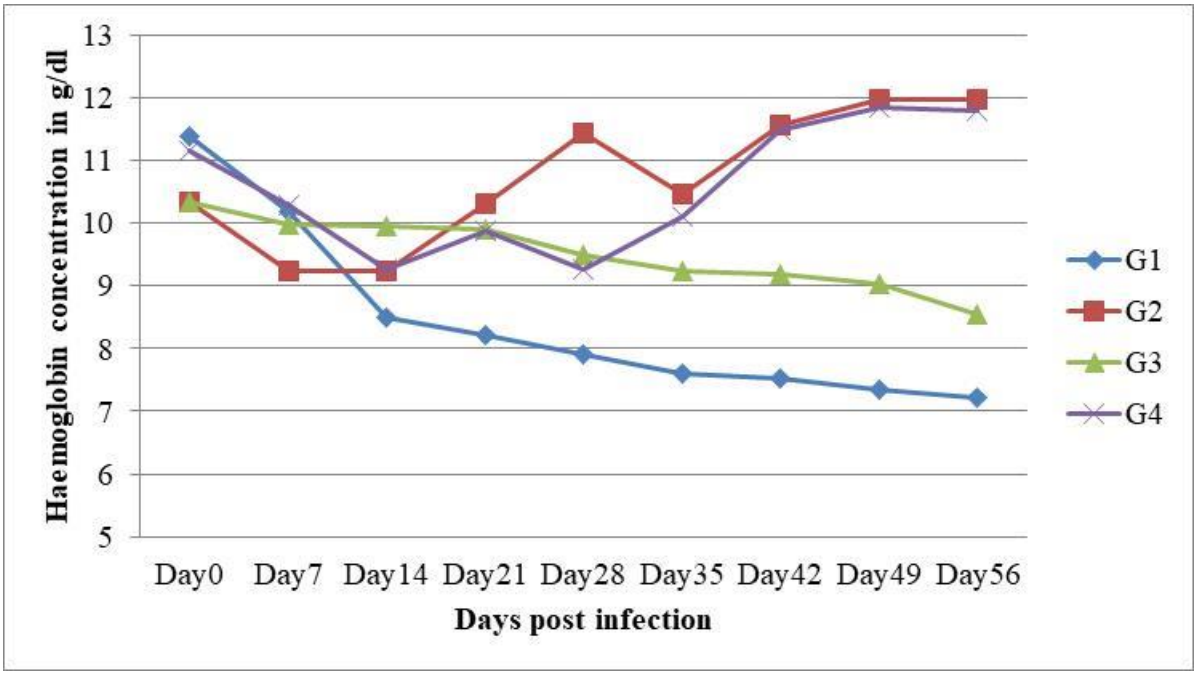

Figure 4: Mean haemoglobin concentration in infected and control goat and sheep

4.5. Serum biochemical changes

4.5.1. Serum total protein and albumin levels

At the beginning of the experiment (D0), the concentration of total serum protein was similar between the four groups (Figure 5). Significant fall from the initial values was observed in G1 on days 28 and 56 PI ($P<0.05$). Similarly, when infected (G1) and non-infected (G2) goats are compared, TSP was much lower in G1 than in G2 ($P<0.05$). Comparison of TSP between infected sheep and goats also revealed TSP was significantly lower in G1 than in G3 ($P<0.05$) on day 28 PI. Because there were notable variation between groups at day 0 (before infection), albumin concentration in g/dl was transformed into percentage changes in concentration with values at day0 taken as 100%. With a similar pattern to the observation for TSP, albumin concentration in group G1 has sharply declined on day 28 PI compared to its initial value before infection ($P<0.05$). Infected goats have also shown significantly reduced serum albumin level compared to the non-infected control group ($P<0.05$) 28 and 56 days PI and compared to infected

sheep (P<0.05) at day 28 PI. Such difference was not detected in sheep when compared to initial readings and between G3 and G4 (Figure 6). The changes in serum albumin concentration was directly proportional to the decline in total serum protein (R= 0.8683, P< 0.05).

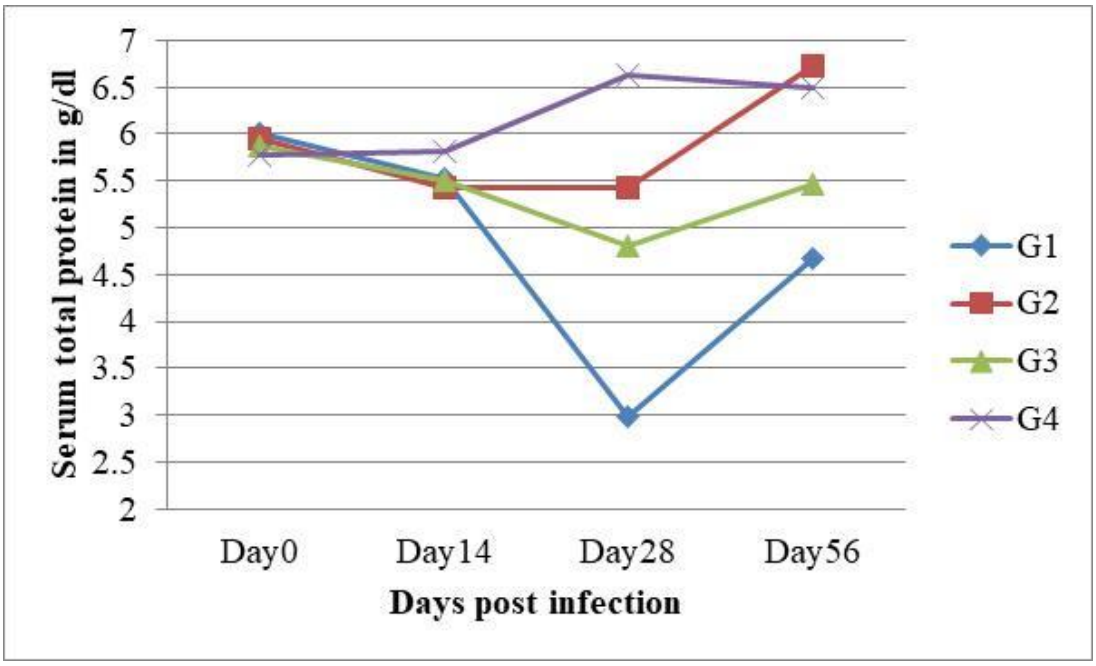

Figure 5: Mean serum total protein concentration in infected (G1, G3) and non-infected (G2, G4) goat and sheep

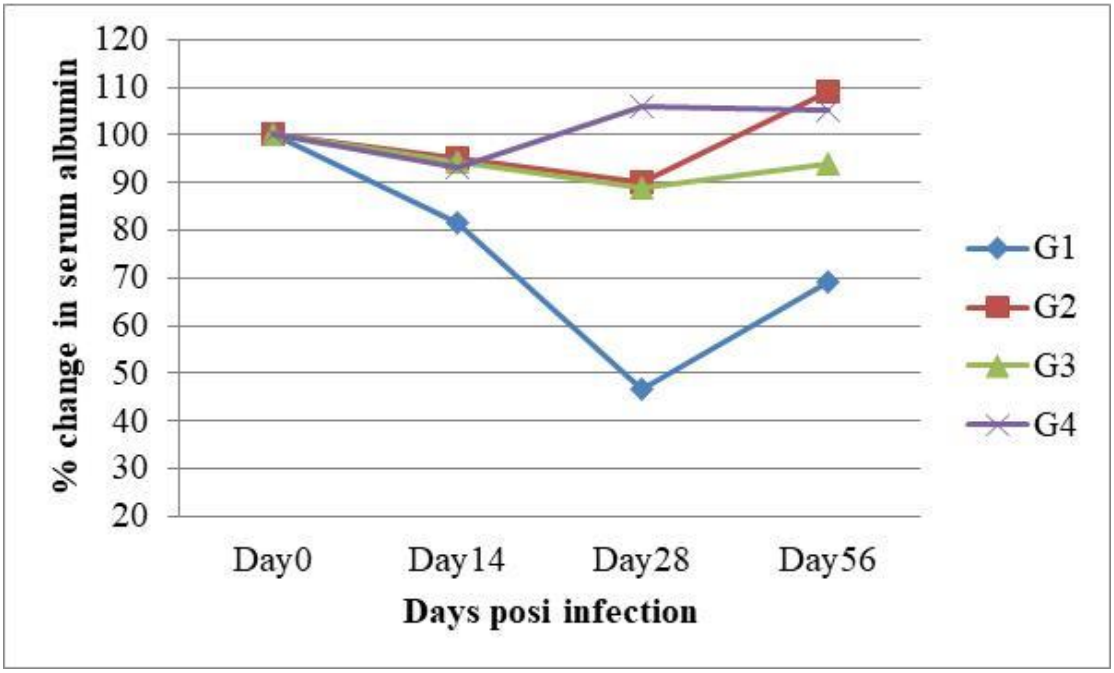

Figure 6: Percentage changes in mean serum Albumin concentration in infected (G1, G3) and non-infected (G2, G4) goat and sheep.

4.6. Gross and histopathological findings

4.6.1. Macroscopic findings

Harboring variable number of worms, the abomasum was endowed with gross lesions such as thickening of mucosal folds, petechial haemorrhages and nodule development which are more visible on the fundic region in both sheep and goats (Figure 7). Such lesions were more prominent in goats than in sheep.

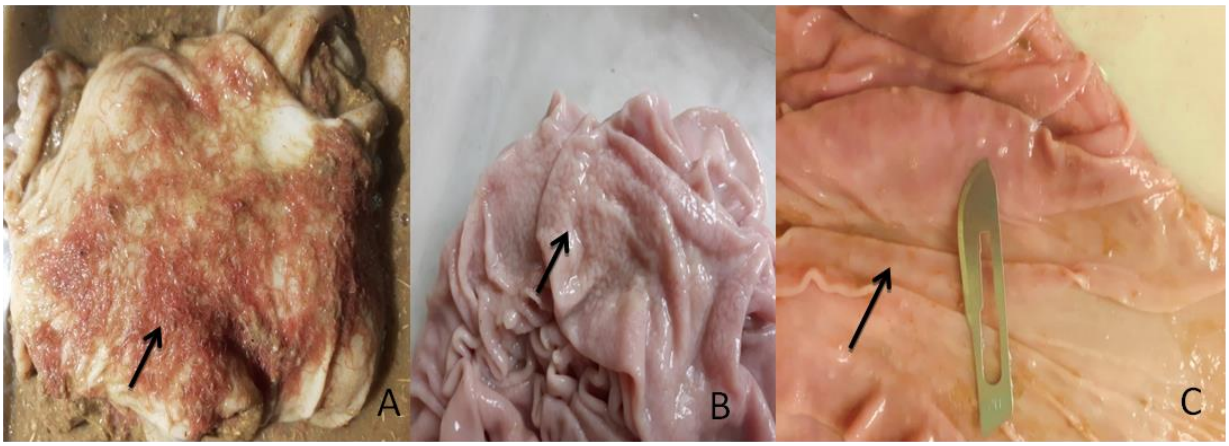

Figure 7: Abomasum of goat infected with 10,000 L3 of H. controtus: (A) large number of adult worms; (B) thickened and rough mucosal folds (arrow), (C) nodular lesion (blade) and petechial haemorrhages (arrow) in the fundic region

4.6.2. Microscopic findings

Histological examination of the fundic region of the non-infected abomasums revealed intact surface epithelium with goblet cells and glandular structure with few leukocyte populations in the lamina propria and submucosa (Figure 8). On the other hand, denuded surface epithelium, damaged gastric pits, hemorrhagic submucosal layer as well as a cross-section of tissue doweling immature worm can be observed at low magnification in

infected sheep and goats (Figure 9). At higher magnification, degenerating chief and parietal cells, as well as very high cellular infiltration dominated by eosinophils is a common characteristics of infected abomasal. Tissue eosinophilia was more extensive in sheep both in terms of distribution along the different layers of the mucosa and the number of cells under a single field of vision (Figure 10).

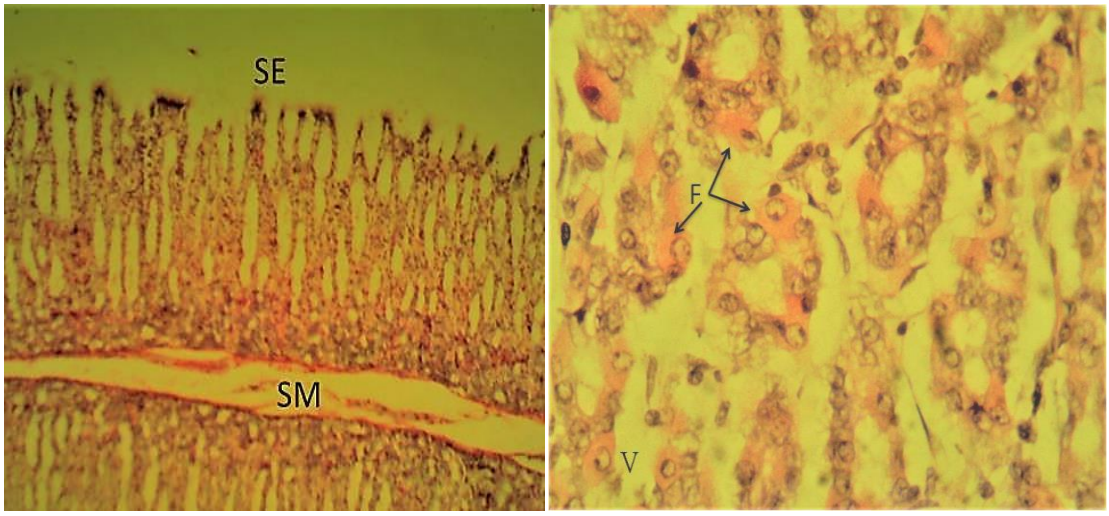

Figure 8: Histological section of non-infected abomasum of sheep showing intact surface epithelium with goblet cells (SE), the sumbucosa (SM), pink staining normal parietal cells (F) with numberous chief cells nearby. (HE staining, 10x and 40x magnification)

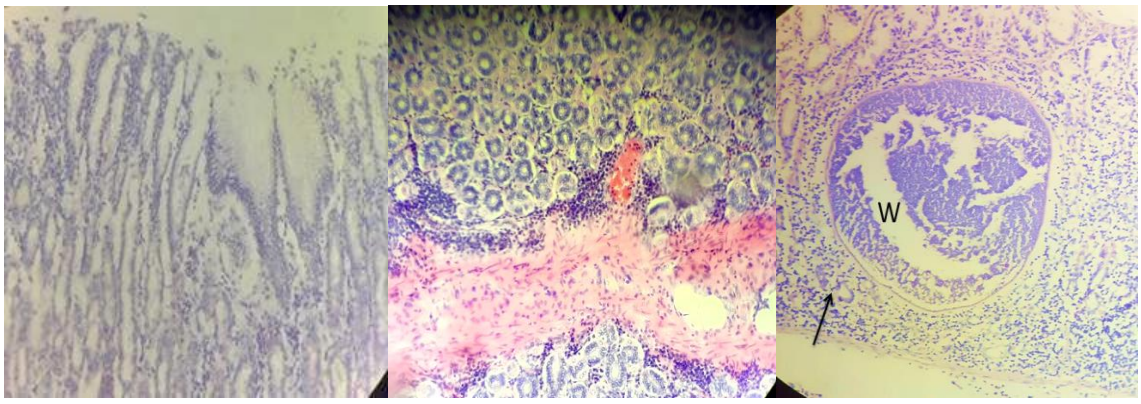

Figure 9: Histopathological changes in the abomasal fundus of *H. contortus* infected goat under low magnification (10x): (A) denuded surface epithelium with damaged gastric pits, (B) high cellular infiltration and hemorrhagic submucosa, (C) tissue doweling worm section (W) surrounded by cellular infiltration (arrow); (HE staining)

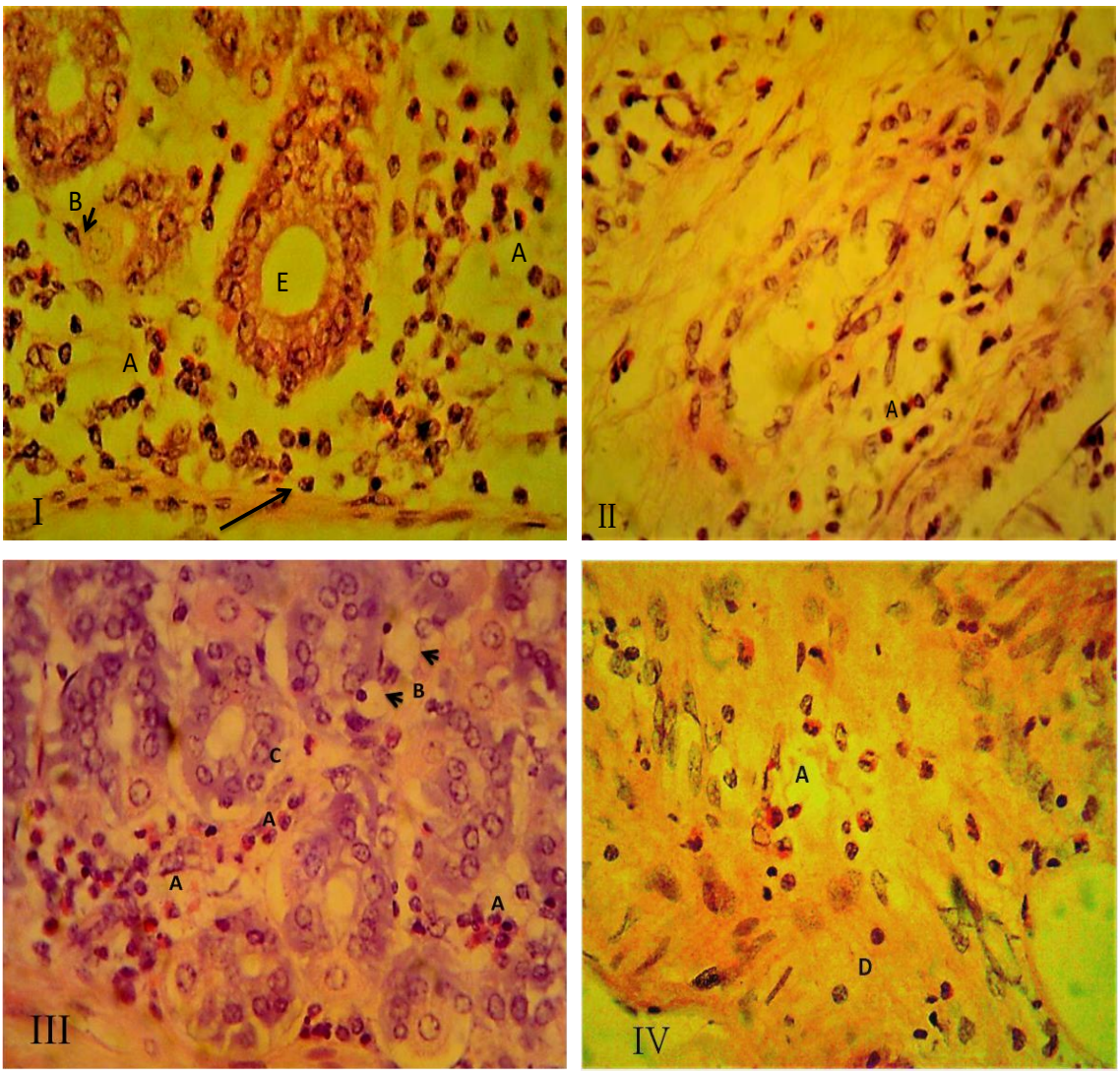

Figure 10: Histopathological changes in the abomasal fundus of representative goat (I and II) and sheep (III and IV) infected with *H. contortus*: (A) numerous pink staining eosinophils in the lamina propria and the submucosa, (B) damaged/degenerating parietal cells, (C) intact chief cells, (D) neutrophil and lymphocyte (also arrow in I) in the submucosa, (E) body of abomasal gland; HE staining, 40x magnification

5. DISCUSSION

5.1. Both sheep and goats have developed pathological changes due to *H. controtus*

The pathology caused by parasites and the host reaction in response to parasitic infection depends on the parasite attachment organs, depth of penetration and site of location and worm burdens (Esmaeilnejad *et al*., 2012; de Oliveira *et al*., 2013). In this study, both infected sheep and goats have demonstrated one or more signs of systemic and gastrointestinal disturbances such as depression, reduced appetite and mild to intense pale mucus membrane especially in goats, loss of body condition and body weight during the first few weeks. Similar findings have been reported in other studies (Fox, 1997; Saminathan *et al*., 2015). Such changes in health condition may be related to local pathological changes in the abomasum and/or systemic changes resulting from anemia and gastric secretions such as the hormone gastrin, release of enzymes such as pepsin and the acid HCl (Fox, 1997; Lawton., 1996; Pérez *et al*., 2001).

In agreement with the above findings, both infected sheep and goats had reduced PCV and hemoglobin concentration the level of which varied with the number of adult worms (Clark *et al*., 1962). This supports the report of Rouatbi *et al* (2016) in a study on the effect of *H. contortus* on the haematological values of rams in South Africa. Body weight reduction was more marked in goats and can be related to the reduced appetite, loss of serum protein and digestion problems linked to disturbances in gastric secretion (Fox, 1997). Similar reduction in body weights have been documented by previous studies in sheep and/goat (Kelkele *et al*., 2012; Starling *et al*, 2019).

On the other hand, Rouatbi *et al* (2016) reported that live weight was not different between infected and non-infected rams despite infection with 30,000 L3 of *H. contortus*. This difference could be attributed to the level of previous exposure to the infection or genetic resistance of the animals. In this regards, infected goats have lost significant amount of serum protein during the infection. This agrees with the reports of Fausto *et al.* (2014) and Rouatbi *et al* (2016). As serum albumin was reduced proportional to the

reduction in total serum protein, it is expected that serum globulin concentration, which is a marker of the level of immunoglobulin production (Bisher, 1990), also follows the same pattern of reduction.

Gross and histological examinations have revealed that both infected sheep and goats have shown changes of various degrees. Such changes have also been reported by previous studies (Fox, 1997; Tehrani *et al*., 2012). These lesions could be responsible for some of the changes in blood protein and systemic signs of reduced appetite and reduction in body weight gain compared to the non-infected control groups.

5.2. Goats have suffered more than sheep from *H. controtus* infection

Haemonchus controtus worm burden was much higher in infected goats than in sheep suggesting that the former was unable to control the establishment and persistence of the parasite in the abomasum. In this regards, several previous researches have reported conflicting findings. In support of the current finding, Tony (2007) described that goats appeared to be more susceptible to helminthes than sheep. This might be due to the fact that sheep are more resistant to the parasitic infection as they are able to elicit a strong immune response **(**Watson and Hosking 1989). It might also be possible that, goats as browsing animals are less adapted to the feed (grass hay) provided to them throughout the experimental period which might have consequently reduced their resistance. On the contrary, helminth prevalence was more significant in sheep for Mushonga *et al.* (2018) and equally important in both species for Kumsa *et al*., (2011). Animal genetic and physiological traits, animal management, parasite strain and method of study could be possible factors for such variation.

Infected goats had significantly lower body weight, hematocrit and hemoglobin values compared to sheep infected with equivalent number of worms and managed under similar in house condition. The large nematode burden in goats may be responsible not only for these changes but also for the lower serum protein values. The larger the number of *H. contortus* parasites, the more blood it consumes and the more anemic the animal would

be (Clark *et al*., 1962; Hoste *et al*. 2008). Such large population of the abomasal parasite in goats could also be responsible for greater damage to the abomasal tissue subsequently leading to destruction of parietal cells and hence reduced acid secretion and reduced protein digestion with losses through the GIT and hence the animal becomes unable to gain weight (Fox, 1997). Decline in the total serum proteins might also be attributed to haemodilution, which is a compensatory mechanism for the abomasal haemorrhage caused by the parasite leading to the loss of large quantities of serum protein into the gut Kelkele *et al*. (2012). Furthermore, due to abomasal haemorrhage haemodilution occurs which can cause relative hypoproteinemia and hypoalbuminemia (Angulo-Cubillán *et al.,* 2007; Sathis *et al*., 2017). Weight loss during GIT parasitism could also be ascribed to the reduction in appetite as a result of the heavy infection (Fox, 1997). Loss of protein also means that, the animal will be deficient in major ingredient to mount adequate level of protective antibody responses (Bisher, 1990).

In agreement with previous reports, (Mannan *et al*. 2017), marked thickenings of the abomsal mucosa were observed in both infected sheep and goats suggesting that the organs have undergone marked inflammatory processes. Eosinophils are said to be one of a Th2 type effector cells responsible to defend the host against helminth parasitism (Balic *et al*., 2006; Terefe *et al*., 2007a). It has been demonstrated that activated eosinophils in the presence of antibodies and/or complement proteins can effectively kill *H. contortus* larvae in vitro (Rainbird *et al*., 1998; Terefe *et al*., 2007a). The lower resistance of infected goats in this study can also be explained by the less population of tissue eosinophil observed compared to the situation in infected sheep where the submucosa and the laminapropria as well as intergladular spaces were highly infiltrated. A report by (Terefe *et al*., 2009) has clearly shown that an apparently nematode resistant breed of sheep, the Barbados black belly, had huge blood and tissue eopisophilia as compared to the susceptible INRA 401 breed suggesting that these cells have a potential to limit helminth development in the host.

Marked increase in the secretion of mucus by mucous cells together with an abundant infiltration of eosinophils, mast cells and globule leukocytes were recorded in the

abomasal mucosa especially in the early stages of infection with *H. contortus* (Pérez *et al*., 2001) suggesting that this cell type may have been involved in rejection of adult nematodes in resistant species as compared to the more susceptible ones. Similar to the findings of our study, other studies have also reported development of numerous nodular lesions with thickening of the fundic mucosa, reduction in the population of parietal cells followed by mucous cell hyperplasia in the fundic mucosae of sheep infected with adult *Ostertagia circumcinta* (Scott, *et al*., 1998).

6. CONCLUSION AND RECOMMENDATIONS

This study was executed to demonstrate development of pathological features in sheep and goats and compare the degree of such changes between the two hosts. The study confirmed that both hosts have developed the infection and expressed various clinical, gross and histopathological as well was serum biochemical changes. It was clearly demonstrated that under similar feeding and management conditions and with experimental challenge infection of 10,000L3 per animal, goats were more susceptible to the infection where significantly larger number of parasites was recovered at the end of the experiment. Concomitant with the difference in worm burden, goats have shown marked anemia, reduced total serum protein, serum albumin, and tissue eosinophilia; all suggesting goats have developed more pathology than sheep. Moreover, in addition to the loss of appetite, the reductions in the number of functional parietal cells seem to have contributed in the reduction in protein utilization and consequently reduction in body weight.

So depending on the above conclusions the following recommendations were forwarded:

- Under situations of comparable grazing management where goats may be exposed to inadequate access to brows species of plants, they should be closely monitored for helminth parasitism to minimize losses
- This study should be repeated under field conditions where goats and sheep have free access to natural feeds to demonstrate whether goats are genetically more susceptible or it is simply due to the effect of changes in nutrition and feeding habit
- As hypoproteinemia was one of the most important problems observed in this study, helminth infected animals should be supplemented with protein rich feeds such as legumes

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8. LIST OF ANNEXES

Annex 1: Procedure of Baerman technique for larval collection

1. Take 10 gram of cultured faeces.
2. Wrap the faeces using gauze and place into the Baerman apparatus to which a sieve is placed.
3. Cover the faeces using lukewarm water (40-45°c).
4. Leave to stand overnight.
5. The clipper on one end of the rubber tube is the released and the liquid collected in a test tube.
6. Centrifuge the collected material for 2 minutes at 2000rpm.
7. Discard the supernatant and examine the sediment for moving larvae.
8. Count the number of larvae by using equally dividing Petri dish under microscope and multiply by a factor to arrive at the total larvae in 10ml the collected material.

Annex 2: Procedures for recovery of nematodes from the abomasums

1. Separate the abomasums from the intestine for wash.
2. Open the abomasums in bowl and collect the contents.
3. Wash the abomasums wall via under slow flow of tap water and to avoid rub of the mucous membrane with fingers carefully remove any adhering to it.
4. Pour the contents of the bowl a little to a wire mesh with hole of 0.15 mm and then wash with slow flow a stream of tap water until remove coloured matter or feed particle.
5. Flush the content on the sieve with a fast flow of water from the tap.
6. Fill the content of the graduated cylinder to 1 litter.
7. Agitate the whole content vigorously and taken an aliquot of 50 ml by using a beaker.
8. Place content in glass Petri dish and examine under a stereomicroscope.
9. Count the number of each species and multiply by a factor to arrive at the total parasite burden.

Annex 3: Process of packed cell volume (Jain, 1986)

1. Blood was collected EDTA tube.
2. The capillary haematocrit tube was filled up to 3/4 of tube.
3. One end of tube was sealed with crystal sealant or soap.
4. The tube was placed in the microhematocrit centrifuge with sealant at the outer end.
5. The blood in capillary tube was centrifuged at 12,000 rpm for 5 minutes.
6. The tubes were taken from the hematocrit centrifuge and placed on the hematocrit reader to determine the PCV rule of haematocrit reader.

   A). Align the top of sealant or bottom of erthyrocyte to the zero line.

   B). Align the top of the plasma to 100 or top of line.

   C). Take the measurement just on the top of erythrocyte.

7. Express the result in terms of percentage.

Annex 4: Procedure for Hemogilobin concentration (Hgb) determination

SAHILI'S ACID HEMATIN METHOD

1. Fill the graduated tube of the hemoglobin meter to 2 marks with 0.1N HCl.
2. The 4 ml blood was collected from jugular vein in heparinized (EDTA) tube and take anticoagulant blood with sahli's to the level of 20 marks.
3. Wipe the outer part of the pipette with a piece of cotton to remove blood.
4. Expel the blood into the graduated tube of the hemoglobin meter and rinse the inside part of tube several times.

5. Leave to settle for 1 minute to complete the reaction.
6. Adding of water drop by drop to the graduated tube and mix with glass stirring rod.
7. Continue the above process until the colour of the solution in the graduated tube much to the colour of the standard on the hemoglobin meter.
8. Take the measurement on the upper meniscus of the solution.
9. Express the results in grams per deciliter (gm/dl).

Annex 5: Histopathological procedures (Takulder, 2007)

1. Fixation of tissue by 10% neutral buffered formaldehyde.

2. Trimming part of the tissue in a way that the lesion 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-I2hr ➡ Formalin-II 2hr ➡ 70% Alcohol 1hr ➡ 95% Alcohol ➡ 100% Alcohol-I 1hr ➡ 100% Alcohol-II 2hrs ➡ 100% Alcohol-III 2hrs ➡ Xylene-I 1:30hrs ➡ Xylene-II 1:30hrs ➡ Xylene-III 1:30hrs ➡ Paraffin-I 2hrs ➡ Paraffin-II 3hrs.

4. Embedding of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60°c) is poured and allowed to settle and solidify.

5. Sectioning: 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.

6. Later label and put an incubator over night.

7. Staining with heamatoxyline and eosin stain.

Heamatoxyline and eosin stain procedure

1. Deparaffinize slides in 2 changes of xylene for 5 minutes.

2. Hydrate slides in 3 changes of 100% alcohol each for 3 minutes and 1 changes of 95% alcohol for a minute and 1 change of 70% alcohol for 3minutes.
3. Rinse in distilled water until ripples disappear from slides.
4. Place in heamatoxyline for 10-15 minutes.
5. Rinse in tap water until water runs clear.
6. Decolorize in 1% acid alcohol 3-6 quick dips.
7. Check differentiation microscopically: Nucleic should be distinct; cytoplasm should be uncolored.
8. Rinse in tap water until ripples disappear from slides.
9. Stain in eosin 3 dips.
10. Rinse in tap water until water runs clear.
11. Dehydrate in 95% alcohol of 3 dips and 100% alcohol, 3 changes each for 3 minutes.
12. Clear in 3 changes of xylene for 5 minutes each.
13. Mount cover glass with DPX (mounting medium).
14. Examination of the prepared slides under the microscope.

Annex 6: The picture during experimental animals handling, recovery of worms and trimming part of the tissue and tissue processing