Clinical And Hematological Analysis In Experimentally Infected Sheep With *Haemonchus Contortus*

Eyachew Ayana

Bahirdar Animal Health Investigation And Diagnostic Laboratory, P.O. Box 70, Bahirdar, Ethiopia
eyuelayana83@gmail.com

ABSTRACT: An experimental study was conducted to evaluate the clinical response and haematological effects on sheep experimentally infected with *Haemonchus contortus.* The study was carried out at the experimental animal house in the College of Veterinary Medicine and Animal Sciences of University of Gondar. A total of eight sheep were used in this experiment and assigned randomly into two groups of four animals (Group I- infected and Group II- non-infected). All sheep in Group I were experimentally infected with 5000 infective larvae (L3) of *H. contortus* whereas Group II animals received normal saline water at the same time. The experimental animals were examined daily for clinical observations and weekly interval for body weight measurement, faecal examination and haematological analysis (packed cell volume (PCV), haemoglobin (Hgb) concentration, total red blood cell (TRBC), total white blood cell (TWBC) and differential leukocyte counts) for 10 consecutive weeks. Postmortem examination was done in all animals at the end of the experiment to determine the worm burden and appreciate abomasal pathologies. The clinical observations on the infected sheep were depression, weakness, weight loss, reduced feed intake and pallor of visible mucus membranes. Faecal egg counts showed patent infections in the infected group which was confirmed by the presence of worm during postmortem examination. The pathological alterations caused by *H. contortus* in infected sheep were mainly confined to the abomasum which showed pallor of abomasal mucosa and adult worms. The result of haematological analysis showed that *H. contortus* infected sheep significantly decreased (p<0.05) in Hgb concentration, PCV, TRBC and neutrophil counts, and body weight gain compared with the non-infected. Furthermore, there was an evidence of a significant increase (p<0.05) in the number of eosinophil of infected group while the TWBC, lymphocytes, basophiles and monocyte counts of infected group were not significantly different (p>0.05) compared with the non-infected. In conclusion the finding of the present experiment revealed that *H. contortus* was established in experimentally infected sheep and the parasite had induced detectable clinical signs and there was a significant effect on the haematology of infected sheep with resultant anaemia.
[Eyachew Ayana. **Clinical And Hematological Analysis In Experimentally Infected Sheep With *Haemonchus Contortus.*** *Life Sci J* 2024;21(11):21-40]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com. 03. doi:10.7537/marslsj211124.03

Key words: Haemonchus contortus; clinical observations; haematological analysis; sheep; experimental infection.

1. INTRODUCTION

Ethiopia has 25.49 million sheep and 24.06 million goats [76]. These livestock are almost entirely managed by the resource-poor, small-holder farmers and pastoralists. However, they make a critical contribution to food self sufficiency for rural households by providing milk, meat, skin, manure and traction, as well as generating direct cash income. In addition, livestock are a source of risk mitigation against crop failures. Small ruminants (sheep and goats) are particularly important resources for their owners, because they require smaller investments, have shorter production cycles, faster growth rates and greater environmental adaptability than cattle. Therefore, they form an important economic and ecological niche in all agricultural systems throughout the country [70].

Although, small ruminants represent a great resource for the nation, the productivity per animal is low. Small ruminant disease particularly the gastrointestinal helminthes are among the major causes of reduced productivity in Ethiopia [70]. Gastrointestinal parasitism is arguably the most serious constraint affecting sheep production worldwide. Economic losses are caused by decreased production, costs of prophylaxis and treatment and death of the infected animals. The blood-feeding nematode, *H. contortus,* is among the most important gastrointestinal (GI) parasites of sheep and it is recognized globally as a major constraint to both small and large-scale small ruminant production systems in developing countries, leading to significant economic losses [71].

Haemonchus contortus, better known as barber pole worm or red worm, is a pathogenic nematode that uses

sheep as a host and causes haemonchosis, an infection characterized by anemia and digestive disturbances. *H. contortus* is active mainly in warm, humid climates in the summer months. Adult worms colonize the abomasal mucosa of the sheep and feed on their blood. The eggs they produce are excreted in the feces, hatch and are ingested by the sheep through the consumption of grasses especially those that are short and/or covered in dew [64]. *H. contortus*, as the highest egg producer of all sheep worms, is one of the more devastating internal parasites [77]. Haemonchosis, if untreated, can lead to protein deficiency, anemia, bottle jaw or the swelling of the lower jaw [72]. It is the most prevalent and pathogenic parasite and also economically important of small ruminants [12].
Haemonchus contortus is a species most commonly found in sheep and goats but *Haemonchus placei* is the usual species in cattle and there may be cross infection occur when small ruminants and cattle graze together but its severity is usually less [9]. The infection causes anaemia and occasional death of the animals and is a major animal welfare problem. The main problem for the farmer is production losses due to decrease in weight and growth of the host animal that in turn leads to economic losses. *H. contortus* is frequently found in tropical and subtropical regions, where the conditions for the survival of this parasite are optimal. However, the parasite has also become a growing problem in temperate regions [14].

Diagnosis is made on the basis of clinical signs, grazing history, season, demonstrating eggs of fecal examination is confirmative but specific identification is difficult and required specialized laboratories and observation of adult parasite during post mortem examination [61].

Consequently, there is an urgent and ever-present need to control infections caused by *H. contortus* in small ruminants in the tropics. Control is generally achieved by the use of synthetic anthelmintics in combination with grazing management. Ideally, this may entail an integrated approach, including biological control, reduced frequency of anthelmintic treatments, parasite vaccines, livestock breeds that are resistant to parasites and the use of plants with anti-parasitic properties as well as the use of traditional herbal remedies or ethnoveterinary medicine [63].

The understanding of the effect of this parasite on haemoglobin (Hgb) concentration, packed cell volume (PCV), total red blood cell (TRBC) count, total white blood cell (TWBC) count and differential leukocyte count (DLC) is essential to reduce the losses caused by this infection in sheep. However, no attempt was made to evaluate the effect of *H. contortus* infection on Hgb, PCV, TRBC, TWBC and DLC in the current study area.

Therefore, the objectives of this research paper are:
- To determine the susceptibility of sheep to the experimental infection with infective larvae (L3) of *H. contortus*.
- To determine the effect of *H. contortus* on haematological values.
- To determine changes in parasitological parameters associated with *H. contortus* infection.

2. LITERATURE REVIEW

Gastrointestinal nematodes of the order *Stronglida* are the most common causes of clinical helminthosis. These parasites infest the wall or the lumen of abomasum, small intestine and large intestine. The most common gastrointestinal nematodes of small ruminants belong to the following genera: *Haemonchus, Trichostrongylus, Ostertagia, Oesophagostomum, Cooperia, Nematodirus, Marshalagia, Strongyloides and Trichuris.* Mixed infection of several genera is common in most of the natural infections [34].

2.1. *Haemonchus contortus*

Haemonchus contortus is a voracious blood sucking abomasal nematode and is responsible for extensive losses in sheep, goat and cattle especially in the tropics. Haemonchosis is for the most part a primary parasitosis, predisposing causes for infestation including overcrowding, lush pasture and hot humid climatic conditions. However, the development of clinical illness is favored by a fall in plane of nutrition particularly in young animals [44].

Haemonchus contortus known as barber pole worm or red worm is a pathogenic nematode that uses sheep as a host and causes haemonchosis, an infection characterized by anemia and digestive disturbances. *Haemonchus contortus* is active mainly in warm, humid climates in the summer months. Adult worms colonize the abomasal mucosa of the sheep and feed on their blood. The eggs they produce are excreted in the feces, hatch and are ingested by the sheep through the consumption of grasses especially those that are short and/or covered in dew [64]. *Haemonchus contortus*, as the highest egg producer of all sheep

worms, is one of the more devastating internal parasites [77].

Lambs and kids are the most affected members of the flock and older sheep and goats under stress also may have total anaemia [73]. The haematocrit reading become less than 15% and progressive weight loss. But diarrhea is not a common feature of haemonchosis; the lesions are those associated with anemia. The abomasa become edematous and in the chronic phase the pH increase causing gastric dysfunction [12].

At peak infection, naturally acquired populations of *H. contortus* can remove one fifth of the circulating erythrocyte volume per day from lambs and may average one tenth of the circulating erythrocyte volume per day over the course of nonfatal infections lasting two months. Thus the anaemia of haemonchosis is generally considered to be moderately macrocytic normochromic in nature [20].

Observation of a phenomenon called self-cure is found to be the characteristic feature of haemonchosis in endemic areas in which the major part of the adult worm burden is expelled resulting in sharp drop in egg per gram to near zero after the advent of a period of heavy rain [12].

2.1.1. Morphology

The members of the genus *Haemonchus* are large worms. They are known as the large stomach worms of ruminants because they are the largest worms of the abomasum [27]. An adult *H. contortus* measures about 15 to 30 mm long, the male being shorter than the female. The morphological characteristics of *H. contortus* (Figure 1) 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. Together with the two chitinous spicules, which are inserted in the female genital opening during copulation, this part of the worm is important for identification. The females have a reddish digestive tube filled with ingested blood, spirally surrounded by two white genital cords (ovaries) giving the appearance of a barber pole. They have a sharply pointed slender tail and a vulva with or without anterior vulval flap [35]. The adult worm is yellowish and usually red in color when engorged with blood [50]. The eggs are of strongyle type with a diameter between 70 and 85 µm [49].

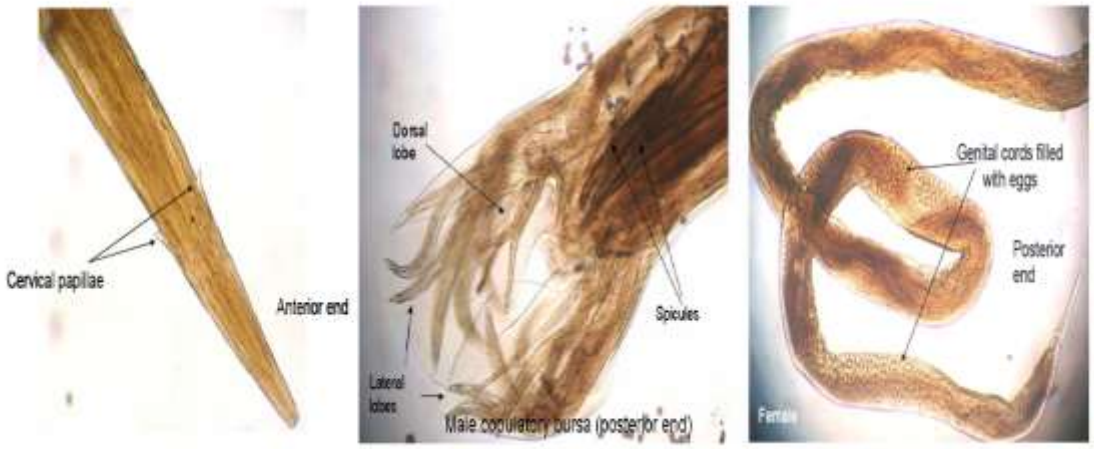

Figure 1: Morphology of male and female *H. contortus* [68].

2.1.2. Life cycle

The life cycle of *H. contortus* is typical to its superfamily *Trichostrongylidae* which have a direct life cycle [27]. An understanding of the life cycle of *Haemonchus* is important for effective control programs. Adult *Haemonchus* worms live in the abomasum and lay eggs that are passed in the faeces (Figure 2). Each adult female parasite has a tremendous egg laying potential (5000 -10000 eggs per day). The eggs that are excreted together with faeces hatch and pass

through three larval stages, the third stage (L3) being infective to the host. The period required for hatching of the egg and development of the larvae ranges from 5 days to several months depending on the weather conditions. Ingestion of the L3 together with grass while grazing leads to infection of the host. L3 then penetrates the mucus membrane of the abomasum and molt to L4 within the next few days. L4 remain in the mucus membrane for 10-14 days after which they emerge and molt into adult stage and females start egg production within 14-21 days post infection [62].

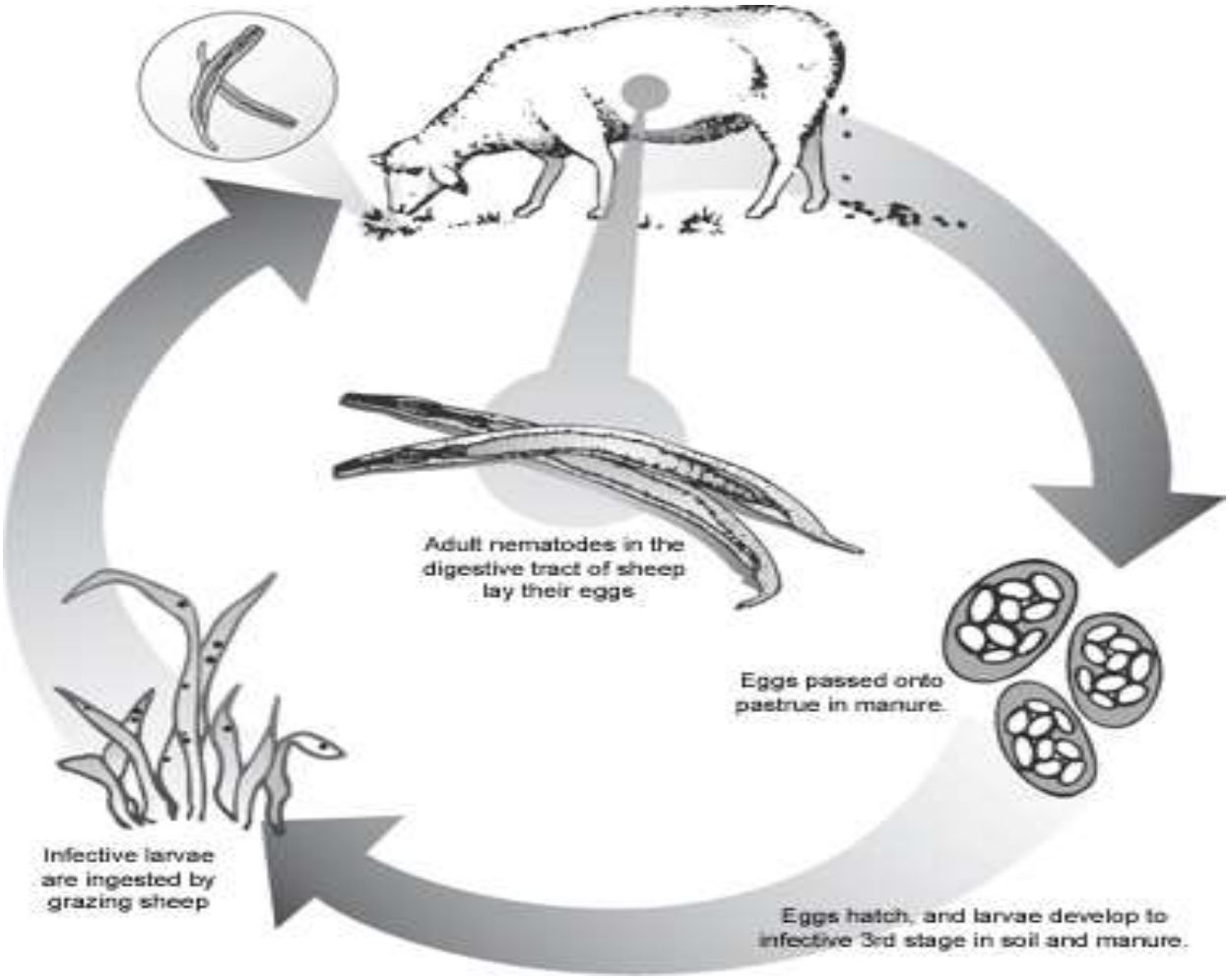

Figure 2: Life cycle of nematode parasite *H. contortus* [62].

2.1.3. Epidemiology

Haemonchus contortus has a worldwide distribution with concentrations in the tropics and subtropics where there are high temperatures and a lot of rainfall. The parasite can also be found in more temperate areas, such as the United States. In the southeast United state, *H. contortus* predominates over other gastrointestinal nematodes, such as *Teladorsagia* and *Trichostrongylus* species and can be found in large numbers because of the warm and humid environment. The optimum environmental temperatures for *H. contortus* are 31°C- 34°C (O'Connor *et al*., 2006), but it can be found in temperatures as low as 10°C and as high as 36°C [67]. High humidity (more than 85%) is important for the parasite because a lot of moisture in the air helps to protect it from desiccation at high temperatures [66]. *Haemonchus contortus* does not endure the cold very well [67] and eggs do not hatch below 9°C [66].

2.1.4. Pathogenesis

Haemonchosis is characterized by hemorrhagic anemia attributable to blood loss via the blood-sucking

activities of worms in the abomasum. The mechanism of blood sucking involves the worm attaching to the mucosa and extruding its oral lancet to slit capillaries in the abomasal mucosa. Fourth stage larvae (L4) as well as adults ingest blood flowing from these slit capillaries [5]. They also secrete anticoagulant into the bleeding lesion ensuring the continual bleeding after the worm has moved away, thus causing hemorrhagic anemia. Each worm removes about 0.05 ml of blood per day through ingestion and seepage from lesions so that a sheep with 5000 parasites may lose about 250 ml of blood per day [13]. The pathogenic effect of *H. contortus* results from the inability of the host to compensate for blood loss. The spectacular depression of hemoglobin level accompanied by weakness and death are the classical features of haemonchosis [20].

2.1.5. Clinical manifestation and diagnostic methods

Haemonchosis in sheep may be classified as hyperacute, acute, or chronic. In the hyperacute form, death may occur within one week of heavy infection without significant signs. This form of the disease is very rare and appears only in highly susceptible lambs. The acute form is characterised by severe anaemia accompanied by edema ("bottle jaw"). In acute haemonchosis, grazing sheep develop a sudden onset of anemia. In the absence of treatment, the situation of the animals will progressively worsen. At first, the packed cell volume (PCV) drops gradually, followed by a rapid drop signaling exhaustion of the erythropoietic system. Death is the usual outcome if not treated. At necropsy, mucus membranes of these animals become pale and edematous due to loss of blood and plasma protein respectively. 2,000-20,000 worms may be found in the abomasum. Abomasal content will be brownish due to the presence of blood. Hemorrhagic lesions are also detected on the abomasal mucosa [5].

Anaemia is also characteristic of the chronic infection, often of low worm burdens and is accompanied by progressive weight loss [12]. The chronic form is the most commonly observed during natural infections. The lesions are associated to anaemia resulting from blood loss. With the exception of the L3, all other stages of development feed on blood. *Haemonchus contortus* is known to produce calcium and a clotting factor binding substance known as calreticulin [65], enabling the parasite to feed easily on host blood and in so doing cause haemorrhagic lesions. At post mortem, the abomasum appears edematous with petecheal haemorrhages, occasional nodular developments and a rise in pH [10].

The case history provides useful information especially the data about the season and climate, managemental system, animal age and nutritional and physiological status of the animal [12].The clinical signs, mainly anaemia, edema and loss of weight in association with reduced haematocrit values might be characteristic of haemonchosis in sheep. The clinical signs may be tentatively used for the diagnosis of haemonchosis in areas and seasons in which the disease is predominant. South African researchers have also developed a visual colorimetric assessment (FAMACHA) of the level of anaemia caused by parasitic infections in sheep [60].

However, all these signs can be shared by a number of parasitic and non-parasitic diseases and hence must be supported by other diagnostic methods. In this regard, demonstration of parasite eggs in faecal material can prove the presence of infection and is the most commonly used diagnostic method. Nevertheless, this method does not always reveal the presence of the parasite during low level of parasitic burden and prepatent periods [54], requiring repeated examinations. Host resistance to GI helminths also delays egg laying [19] and a change in female worm size affects its fecundity [51]. Hence, egg counts do not necessarily reflect the number of worms present. Other methods like measurement of parasite specific antibodies can be used as supplementary diagnostic tools [3]. In general, a more accurate diagnosis lies on the utilization of all available information regarding the epidemiology, clinical manifestations and laboratory diagnostic methods [68].

2.1.6. Abomasal pathophysiology

In the parasitized abomasum, gastric dysfunction and superficial epithelial damages caused by the presence of larvae in the gastric glands (stretching the glands) and the movement of adult worms that feed on the mucosal surfaces presumably compromise the protective barrier to diffusion and allow parasite and luminal chemicals access to host tissues. The marked changes in gastrointestinal secretions that accompany abomasal nematode infections in ruminants are well established and include a reduction in gastric acid secretion and an increase in circulating pepsinogen and gastrin levels. The abomasal hypoacidity may reduce pepsinogen activation and appears to be responsible for increased gastrin secretion in the initial phase of infection. Increased serum pepsinogen concentration is attributed mainly to the increased

back-diffusion of luminal pepsinogen through the more permeable mucosa [68].

Abomasal secretion begins to change around the time of parasite emergence from the glands. Because of the timing, dysfunction has been attributed to tissue damage during emergence [41]. Parasites may inhibit the parietal cells inadvertently by provoking inflammation or disrupting the protective mucosal defense system or, alternatively, by targeting these cells through excretory/secretory chemicals. Therefore, inhibition of acid secretion and loss of parietal cells appears to be a key event responsible for both the secretory dysfunction and the altered cellular composition of the gastric glands [68].

The contributions of the host and the parasite to the pathophysiology of abomasal parasitism may have quite different costs and benefits to each of them. The ability to inhibit acid secretion may allow colonization of a hostile acid environment, which also contains proteolytic activity capable of digesting an unprotected parasite [56]. Raising the pH may also enhance egg laying; e.g. for *H. contortus* it is maximal between pH 4 and 4.5 [25]. A curious feature of the inflammation caused by parasites is that the parasites may themselves actively recruit granulocytes through secreted chemotaxins. Eosinophil chemotactic factors have been found in a wide range of parasites including *Ostertagia ostertagi* and *H. contortus* [15].

The cellular response to abomasal nematodes involves the accumulation of inflammatory cells such as mast cells, globule leucocytes, eosinophils and lymphocytes [6]. Cell pattern and time course of this cellular infiltration may vary according to host factors such as age, immune status, genetic predisposition, reproductive status and plane of nutrition [4].

Lymphocytes, eosinophils etc. began accumulating 1-2 days after adult parasite transfer and were present in large numbers after 8 days [11]. Most studies of the cellular changes in the GI mucosa of sheep infected with nematode larvae rely on post mortem sampling of groups of animals at specified time during the infection. Sequential abomasal or intestinal biopsy also offers the advantages of more frequent sampling than is practical with killing groups of sheep and enables to study mucosal inflammatory responses throughout the course of the infection [4].

2.1.7. Treatment

Various anthelmintic drugs are used for the treatment of animals infected with *H. contortus*. The most important are the nematocides group such as thiabendazole, benzimidazole, levamisole, morantel and naphthalphos. Also many trematodocides such as closantel, clioxanide, rafoxanide and nitroxynil had significant high efficiency against *H. contortus* [42]. When an acute outbreak has occurred the sheep should be treated with one of the benzimidazoles, levamisole, an avermectinimilbemycin or salicylanilide and immediately moved to pasture not recently grazed by sheep. When the original pasture is grazed again, prophylactic measures should be undertaken, as enough larvae may have survived to institute a fresh cycle of infection. Chronic haemonchosis is dealt with in a similar fashion [13].

However, recent studies indicated that many strains of *H. contortus* showed resistance to various anthelmintics. The anthelmintic resistance is measured by the inability of the drug to reduce the faecal egg count of infected animals. Less than 85% reduction in faecal egg count suggests that the parasite strain may be resistant to the drug used [43].

2.1.8. Control methods of haemonchosis

The aim of most parasite control strategies is not to totally eliminate the parasites in livestock, but to keep the population under a threshold, above which it would otherwise inflict harmful effects on the host population [55]. The relative success or failure of any control strategy can be judged in terms of immediate and/or long-term objectives, the ultimate goals being increased production, minimizing risks of drug resistance and addressing consumer and environment associated problems. Generally, nematode control strategies can be directed against the parasite in the host and/or in the environment [68].

Commercial anthelmintics have been used for some decades throughout the world to minimize the losses caused by helminth infections [47]. However, the threats of anthelmintic resistance, risk of residue, availability and high cost especially to farmers of low income in developing countries have led to the notion that sustainable helminth control cannot be achieved with commercial anthelmintics alone. Therefore, today the strategy of helminth control has shifted to integrated control scheme involving grazing management, utilization of natural immunity together with anthelmintics for sustainable control of helminth parasites [45]. Other options like, biological control,

vaccine and traditional medicinal plants are being examined in different parts of the world [63].

3. MATERIALS AND METHODS

3.1. Study Area

The current experimental study was carried out in the premises of Tewodros campus at experimental animal house of the College of Veterinary Medicine and Animal Sciences, University of Gondar, Northwest Ethiopia from December 2016 to March 2017. Gondar town is found at latitude of 12.3-13.8°N, at a longitude of 35.3-35.7°E and at 2200m.a.s.l. The annual mean minimum and maximum temperature of the area vary between 12.3-17.7°c and 22-30°c, respectively with an annual average temperature of 19.7°c. The average annual precipitation being about 1000mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June to September. The livestock population of North Gondar is estimated to be 1,936,514 cattle (exotic, cross and local), 524,083sheep, 682,264 goats, 36,828 horses, 12, 473 mules, 223,116 donkeys and 3,165,068 poultry [74].

3.2. Parasitological Techniques

3.2.1. Collection of abomasal samples

A total of 101 abomasa were collected from naturally infected sheep slaughtered from restaurants and hotels in Gondar town. Each abomasum was ligated from both ends and immediately separated from the rest of the digestive tract. Then the abomasum was opened along their greater curvature and their content was washed. Then the parasites were identified by close visualization for the presence of adult *Haemonchus* [43].

3.2.2. Adult worm recovery and harvesting of infective larvae

Mature gravid *H. contortus* females were collected directly from the abomasum of naturally infected sheep which were slaughtered from restaurants and hotels. The female worms were individually picked up from the abomasal contents and collected in to petridishes containing normal saline. The eggs were released from the gravid uteri by crushing the collected worms using a pestle and mortal. The faecal culture was prepared according to Urquhart *et al.* (1996). Sterilized egg-free horse faeces were crushed into small particles. A small amount of water was added to the faecal mass to form a moist crumbly material. The pure eggs were then added to the faeces and well mixed on the petridishes and plastic bottles. Then the faecal cultures were kept for a minimum of 2 weeks at room temperature. During this period the larvae hatched from the eggs and developed into L3 and infective dose was harvested. To collect third-stage larvae the cultures were filled with water and put upside down in a petri dish containing water. After a period of 24 hours larvae had migrated towards the clear water and assembled in the reservoir of the Petridish. The collection of harvested *H. contortus* infective larvae was done by application of Modified Baermann Technique (baermannization) described by Urquhart *et al.* (1996).

3.2.3. Faecal egg count

Faecal egg count was performed by the use of McMaster technique [12]. Three grams of faeces were thoroughly homogenized and mixed with 42ml of tap water in a wide mouth glass stoppered bottle. The faecal suspension was well mixed and then filtered through a sieve and the filtrate was further centrifuged for 3 minutes at 1500 rpm. The supernatant was then discarded and the packed sediment was emulsified with saturated sodium chloride (NaCl) solution up to the previous volume. Two McMaster chambers were filled with the tube content using a clean Pasteur pipette and examined under microscope. *Haemonchus contortus* eggs were then counted within the entire marked areas. The number of eggs per gram of faeces was calculated by multiplying the number of eggs counted in both chambers by 50 and or one chamber by 100.

3.2.4. Postmortem examination and worm count

Postmortem examination was performed in all sheep at the end of the experiment. The carcass and all organs were thoroughly examined for gross lesions with special attention to the gastrointestinal tract. In addition, the abomasum was ligated, removed and carefully examined for the presence of worms and related lesions and all mature worms were recovered and counted. This was done according to the method described by Urquhart *et al.* (1996).

3.3. Experimental Design

3.3.1. Experimental animals and their management

Eight sheep were purchased from local market in Gondar town. They were allowed to acclimatized for 1 month during which, they were become dewormed. Experimental sheep were screened and confirmed negative for *H. contortus.* Pre-infection parameters were taken from the sheep before the commencement of the experiment. The sheep were all subjected to thorough clinical examination and blood and faecal samples were further examined to ensure their sound health and absence of internal parasites. They were then ear-tagged, divided into infected and non-infected (control) groups and housed with free access to feed and water, in general they were well managed till the end of experiment. The handling of animals during the experiment was based on international guiding principles for biomedical research involving animals, as proposed by the Council for International Organizations of Medical Sciences [31]. The research was authorized by the Animal Research Ethics Review Committee of the College of Veterinary Medicine and animal science of University of Gondar.

3.3.2. Experimental groupings

Based on the experimental protocol 8 sheep were divided in to 2 groups (each group containing 4 animals) randomly. Group I was infected with L3 of *H. contortus* while Group II was not infected and serves as a negative control.

3.3.3. Parasite challenge

All individual sheep in Group I were challenged with 5000 larvae per 100ml of saline orally while each sheep in Group II were received 100ml of saline water. At the end of the experiment all sheep from the infected and non-infected groups were humanely slaughtered for abomasal pathology, adult worm recovery and count.

3.3.4. Clinical observations

The experimental sheep were thoroughly observed daily for clinical changes with special attention to general appetite, visible mucous membranes, body condition and for development of submandibular edema. The body weight of each experimental animal was measured at weekly intervals throughout the experimental period.

3.3.5. Faecal samples

Faecal samples were collected from the rectum of individual animals from both infected and control groups once a week starting from second week of *H. contortus* infection until the end of the experiment for immediate examination and to measure egg excretion.

3.4. Haematological Analysis

Blood samples from all animals were collected by jugular vein puncture at weekly interval from the beginning up to the end of the experiment. Whole blood samples were collected from each animal in to ethylene diamine tetra acetic acid (EDTA) coated vacutainer glass tubes.

3.4.1. Haemoglobin (Hgb) concentration

Haemoglobin concentration was measured by Sahli's method (acid hematin method) described by Jain (1988). The method depends on the conversion of haemoglobin to acid hematin by adding a small amount of diluted hydrochloric acid (HCl). The resulting brownish-yellow color is matched with the standard color of the apparatus. The result reading was made in g/dl.

3.4.2. Packed cell volume (PCV)

The packed cell volume was determined by the use of the microhaematocrit technique [38]. Fresh blood samples were drawn in capillary tubes and centrifuged in a microhaematocrit centrifuge for five minutes. The PCV was read by PCV reader. The reading was expressed as percentage of packed red cells to the total volume of the whole blood.

3.4.3. Total red blood cell (TRBC) and total white blood cell (TWBC) count

Total red blood cell (TRBC) and total white blood cell (TWBC) count were determined by haemocytometric method [28] by using isotonic salt and 0.1N HCl solutions respectively as a dilution fluid.

3.4.4. Differential leukocyte count (DLC)

Thin blood samples were prepared for the purpose of carrying out differential leukocyte count. Thin smears were first air dried and fixed with methanol for 3-5 minutes and stained with Giemsa stain solution, washed with distilled water and dried on the air. Thin smears were microscopically examined under oil immersion magnification (X100) and counting and classifying of 100 leukocytes were made using battlement method and finally values were expressed in percentage and then converted into numbers using

the TWBC counted for that particular study period [48].

3.5. Data Management and Analysis

All the data collected during the study period was checked, coded and entered in to Microsoft Excel spreadsheet and analyzed using SPSS software version 20. The variables were analyzed statistically using Student's independent-samples T-test. Student's independent-samples T-test was used for comparison of mean values for Body weight, Hgb, PCV, TRBC, TWBC and DLC between the two groups (infected and non-infected) where as descriptive statistics was used for FEC and Worm count. $p<0.05$ was considered as statistically significant.

4. RESULTS

4.1. Clinical Observations

The results of the current experimental study showed that the first symptoms of the disease was observed at second week post infection in infected sheep (Group I) with detectable clinical signs of weakness, depression, inappetance, pale mucous membrane and loss of body condition. The development of submandibular edema was not observed throughout the experiment. The animals in non-infected group (Group II) showed no signs of illness during the experiment period.

4.2. Body Weight

The results of body weight measurement in *H. contortus* infected (Group I) and non-infected group (Group II) is illustrated in Figure 3. There was a variation in body weight gain between the two groups. Infected group showed a significant reduction (p=0.000) in their mean body weight while non-infected group showed a gradual increase in their body weight throughout the observation period.

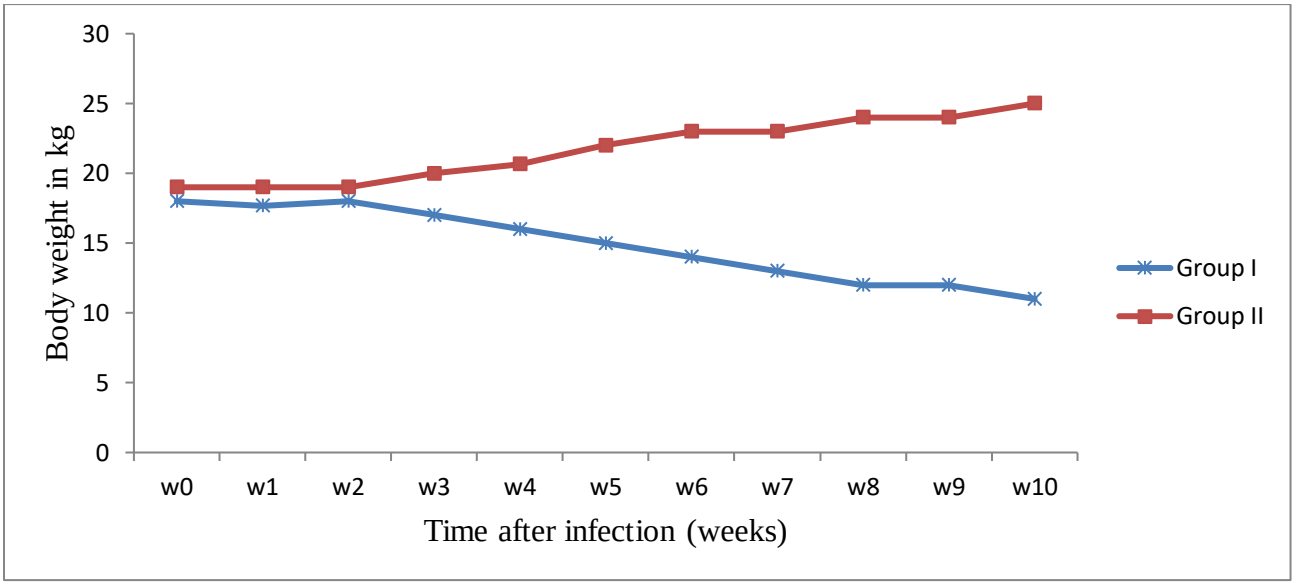

Figure 3: Mean values of body weight gain in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

4.3. Faecal Egg Count (FEC)

No egg excretion was observed in non-infected group throughout the experimental period. Eggs of *H. contortus* in infected group were recorded for the first time during the 3rd week after larval administration (Figure 4). The maximum egg count, in terms of eggs per gram (EPG) was recorded on week 8 post infection. From week 8 post infection onwards, the mean FEC value fell gradually. The prepatent period was found to be 21 days.

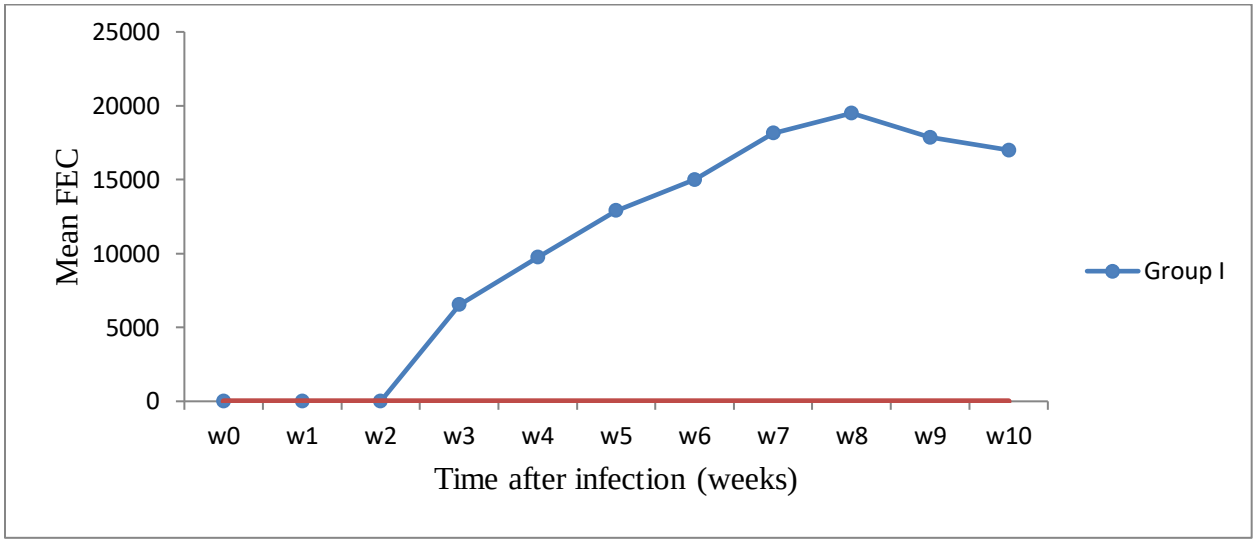

Figure 4: Mean values of faecal egg count (FEC) in experimentally infected sheep (Group I) with *H. contortus.*

4.4. Haematological Findings

Haematological findings of infected and non-infected sheep are shown in Table 1. Haematological values recorded for the non-infected were found to be fluctuating within the normal range throughout the experiment. While that of *H. contortus* infection caused significant reduction in mean values of Hgb concentration, PCV and TRBC count of infected group. The TWBC count was found to be within the physiological range in both infected (Group I) and non-infected group (Group II). Moreover, the infection of sheep with the parasite also resulted in significant increase in the mean values of eosinophils and a decrease in neutrophils where as lymphocytes and monocytes in both groups fluctuate within the normal range throughout the experimental period. Observations on each haematological parameters in all groups were as follows:

Table 1: Hematological parameters in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

Parameters	Group	Mean±SD	Mean difference	*p*-value	95% confidence interval of the difference	
					Lower	Upper
PCV	I	18.58±6.305	-11.273	0.000	-13.523	-9.022
	II	29.85±1.460	-11.273		-13.559	-8.987
Hgb	I	5.91±3.156	-5.212	0.000	-6.382	-4.042
	II	11.12±1.166	-5.212		-6.395	-4.029
TRBC($\times 10^6$/mm^3)	I	6.58±2.136	-3.303	0.000	-4.113	-2.493
	II	9.88±0.927	-3.303		-4.120	-2.486
TWBC($\times 10^3$/mm^3)	I	7.42±0.830	-0.515	0.466	-0.923	-0.108
	II	7.94±0.827	-0.515		-0.923	-0.108
Neutrophil	I	19.79±9.120	-17.727	0.016	-21.542	-13.913
	II	37.52±6.094	-17.727		-21.552	-13.902
Eosinophil	I	14.55±4.452	8.909	0.003	7.190	10.628
	II	5.64±2.148	8.909		7.177	10.641
Lymphocyte	I	63.39±5.841	8.727	0.938	5.788	11.666
	II	54.67±6.107	8.727		5.788	11.666
Monocyte	I	2.24±1.146	0.000	0.684	-0.577	0.577
	II	2.24±1.200	0.000		-0.577	0.577

4.4.1. Packed Cell Volume (PCV)

The results of mean PCV values in *H. contortus* infected and non-infected groups are illustrated in Figure 5. There were PCV differences between the two groups. A significant decrease (p=0.000) in PCV was observed in the infected group (Group I). The lowest mean PCV value was recorded on week ten in infected group. On the other hand, no significant alterations in the mean values of PCV were observed in non-infected group (Group II).

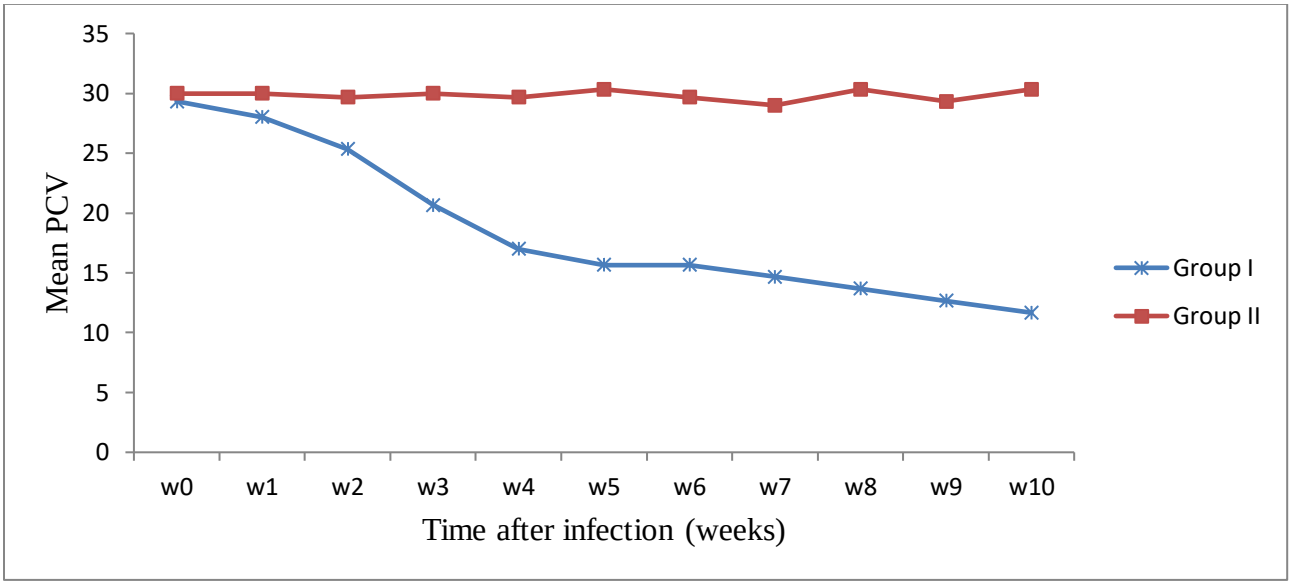

Figure 5: Mean values of Packed Cell Volume (PCV) in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

4.4.2. Haemoglobin (Hgb) concentration

The results of Hgb concentration in *H. contortus* infected and non-infected groups are illustrated in Figure 6. A significant reduction (P=0.000) of Hgb concentration was observed in all infected group starting from the second week of infection. The lowest values were recorded on week nine and ten. On the other hand, no significant changes in Hgb concentration were observed in non infected control group (Group II). It was found to be within the physiological range.

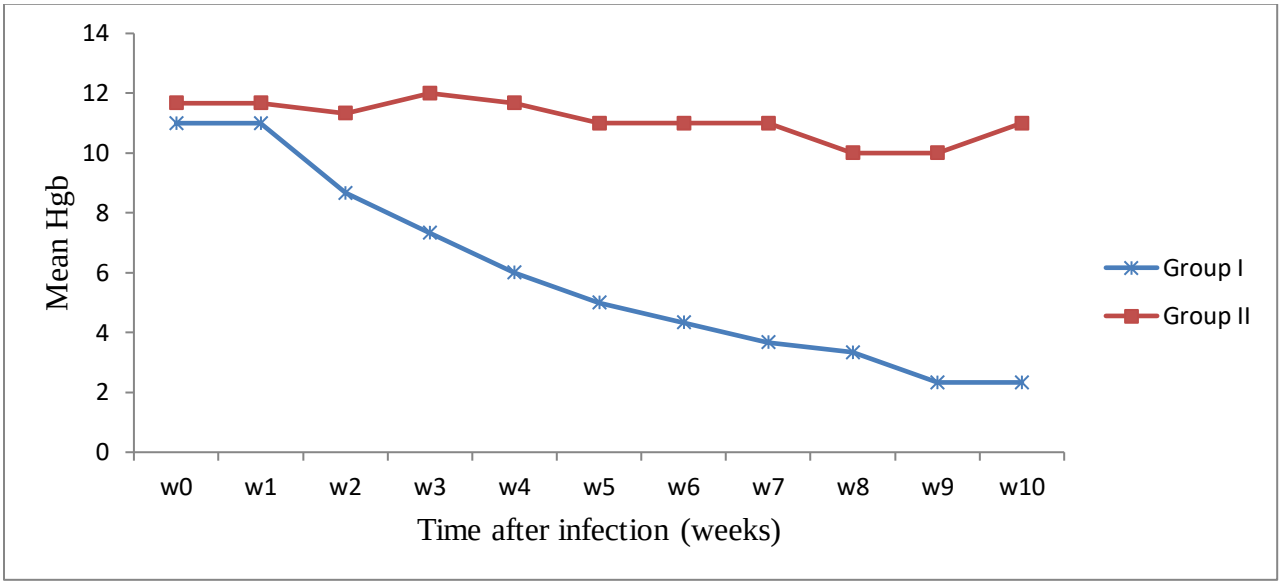

Figure 6: Mean values of Haemoglobin (Hgb) in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

4.4.3. Total red blood cell (TRBC) count

The results of TRBC count in *H. contortus* infected and non-infected groups are illustrated in Figure 7. A significant reduction (p=0.000) of TRBC count was observed in all infected sheep in the infected group starting from the second week of infection and gradually continues up to week ten. The lowest values were recorded on week ten. On the other hand, there were no significant changes in the mean values of TRBC count observed in non-infected group (Group II). It was found within the physiological range.

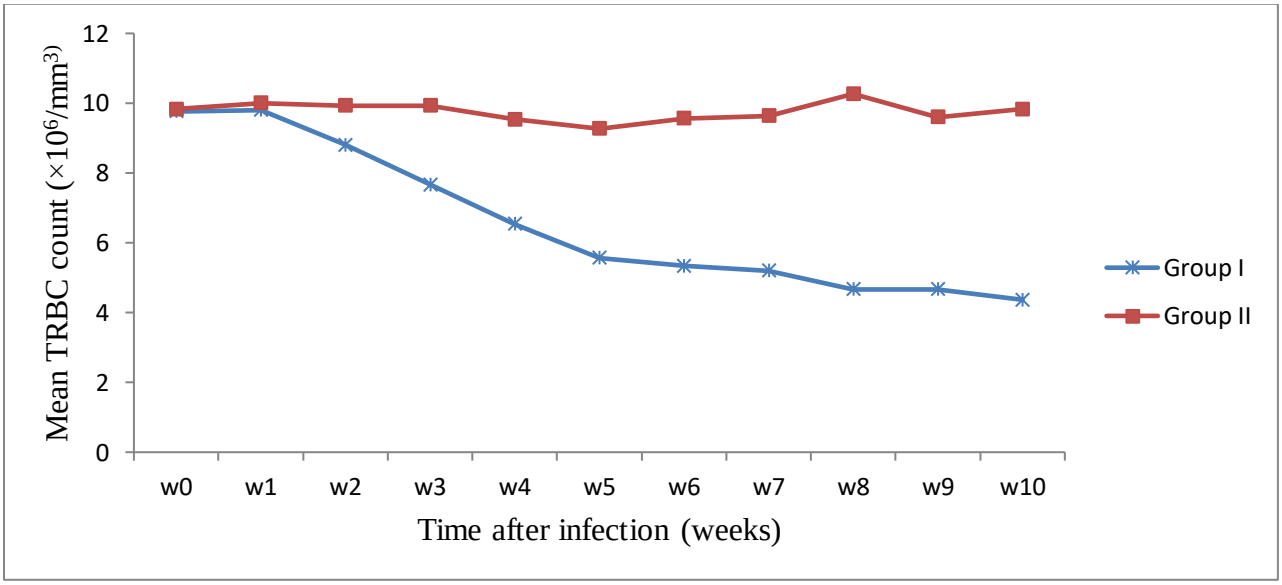

Figure 7: Mean values of Total Red Blood Cell (TRBC) count in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

4.4.4. Total white blood cell (TWBC) count

The results of TWBC count in *H. contortus* infected and non-infected groups are illustrated in Figure 8. There was no significant (p=0.943) change of TWBC count in both infected and non-infected groups throughout the experiment. It was found to be within the physiological range.

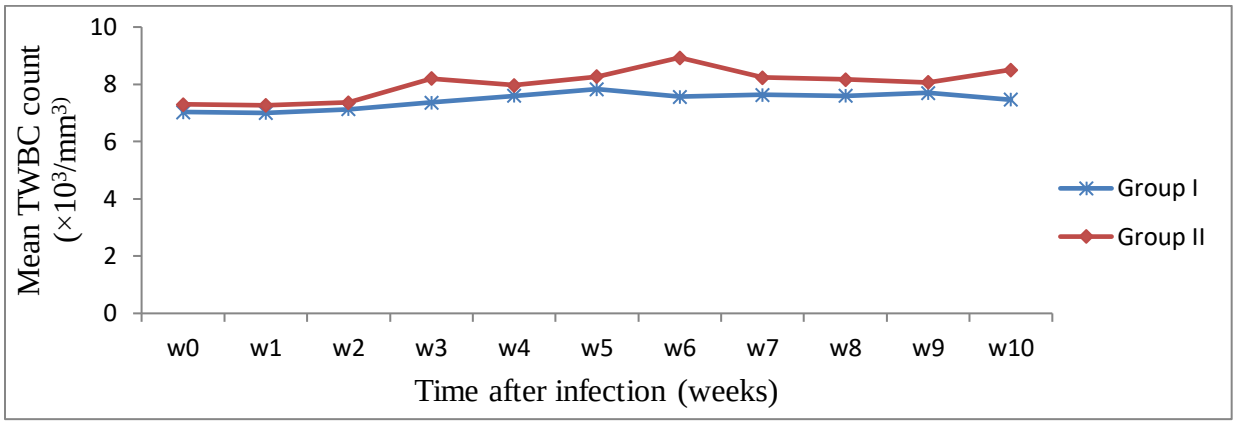

Figure 8: Mean values of Total White Blood Cell (TWBC) count in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

4.4.5. Differential leukocyte count (DLC)

The results of DLC count in *H. contortus* infected and non-infected groups are illustrated in Table 1. The number of blood eosinophils in the non-infected group was at a physiological level throughout the experiment. However, the eosinophil counts in infected group was significantly increased (p=0.003) compared to the non infected group as it is shown in Figure 9. Blood eosinophilia was very prominent phenomenon in sheep infected with *H. contortus.* There was also a difference in the mean values of neutrophil of the two groups. Moreover, the infection of sheep with the *H. contortus* resulted in significant decrease in neutrophils (p=0.016) of infected group (Figure 10).Whereas lymphocytes (p=0.938) and monocytes (p=0.684) were insignificant in both groups and fluctuates within the normal range.

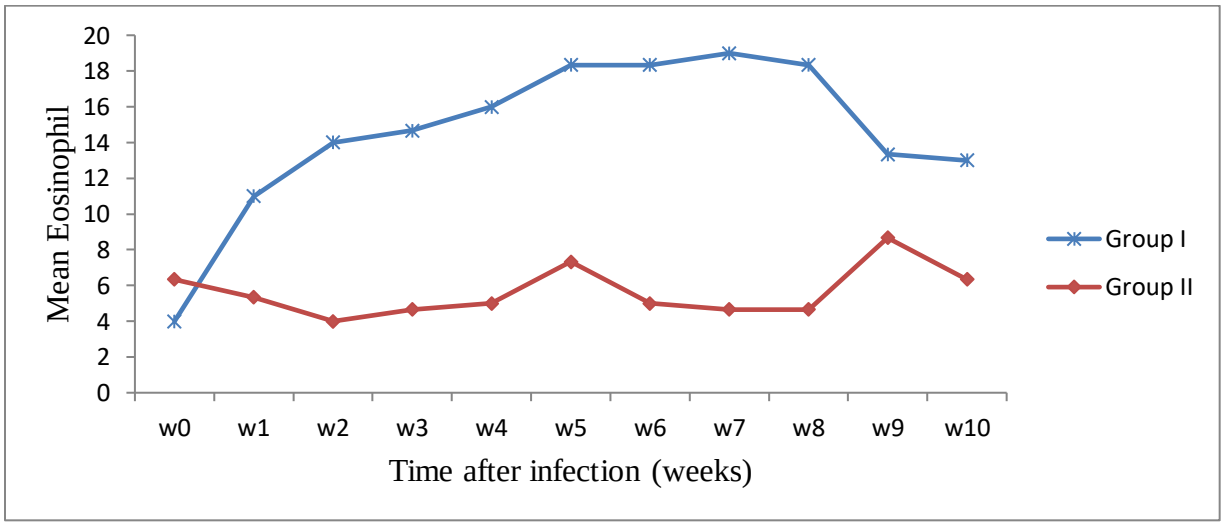

Figure 9: Mean values of eosinophil in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

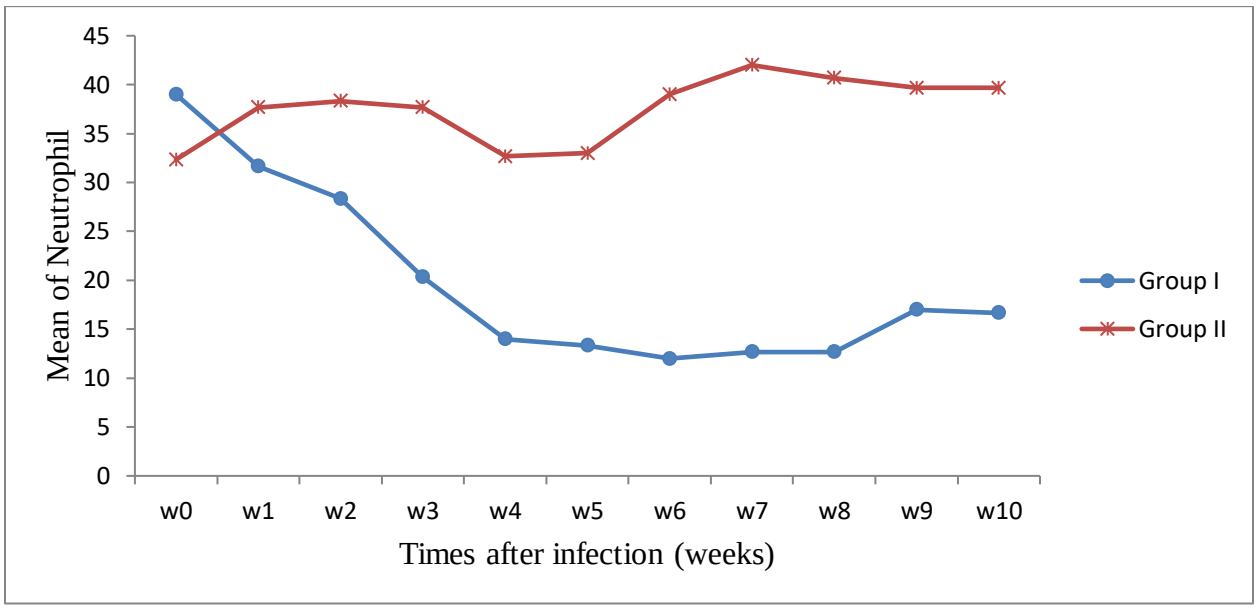

Figure 10: Mean values of neutrophil in experimentally infected sheep (Group I) with *H. contortus* and non-infected (Group II).

4.5. Postmortem Finding and Worm Count

At postmortem examination emaciated carcass; adult worms and pallor abomasal mucosa were evident in infected sheep as shown in Figure 11. Variable numbers of adult worms were found within the abomasal content or closely adherent to the abomasal mucosa. The infected group of sheep had a mean worm burden of 1519, representing a mean establishment of 30.38%. It was calculated in such a way that as 5000 worm have 100% establishment then what will be for 1519 worm and or (1519×100/5000). However, sheep in the non-infected group did not show any worm in the abomasum at post mortem examination.

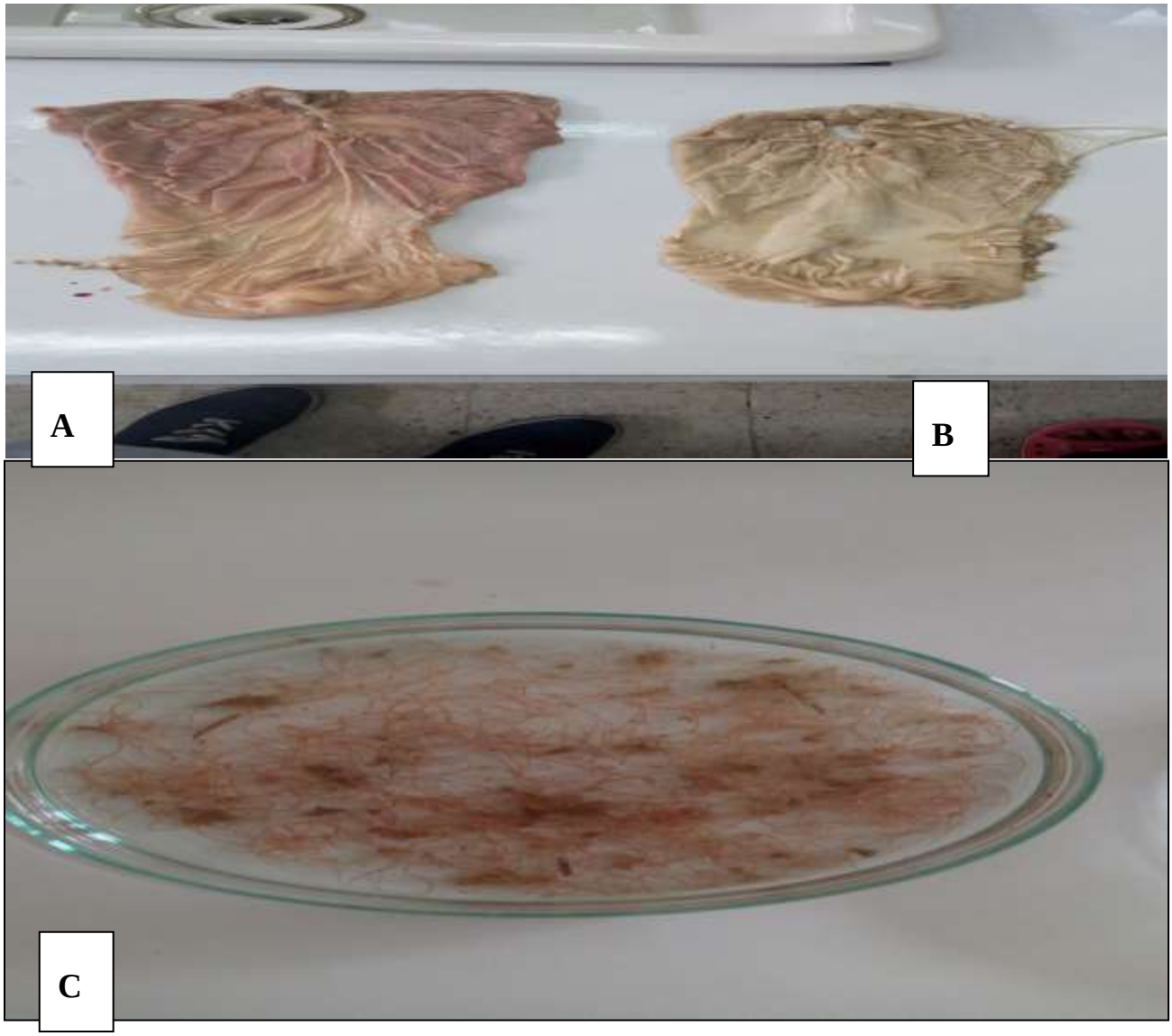

Figure 11: Postmortem findings; abomasum from non-infected (A), infected (B) and adult worms (C).

5. DISCUSSION

The present study was designed to investigate the haematological effect and clinical response of sheep to experimental infection with *H. contortus*. The prepatent period in *H. contortus* infected sheep in the present experimental work was found to be 21 days (third week) post infection as judged by faecal egg counts. This finding was in agreement with the previous results of AlLatif *et al*. (1980); Hunter and Mackenzie (1982); Abbott *et al*. (1986); Ahmed and Ansari (1989); Omar (1999); Abakar *et al*. (2000); Getachew *et al*. (2005) and it was found to be within the established range.

The pattern of egg shedding in *H. contortus* infected sheep was similar to that reported by Abakar *et al*. (2000). High faecal egg count in infected sheep was recorded on day 56 (week 8) post infection indicating higher worm burden in the abomasum and thereby producing significant effect on haematological parameters. However, the time of the maximum egg shedding was longer than that reported by Rahman and Collinis (1990). This might be probably due to the fact that the current *H. contortus* used for experimental infection was had less adaptability to infect sheep due to seasonal factors since the experiment was conducted during dry season.

The clinical signs demonstrated by the infected sheep in this experiment were weakness, depression, inappetance, pallor visible mucous membranes and loss of body condition and this finding is in line with those reported by Dargie and Allonby (1975); Hunter and Mackenzie (1982); Al-Quaisy *et al.*(1987); Mottelib *et al*. (1992); Kelkele *et al*. (2012). However, the submandibular edema (bottle jaw) reported by some of the previously mentioned authors was not observed in this experiment. The absence of submandibular edema is mainly due to the fact that this clinical feature requires much protracted course of the disease with high worm burden resulting in chronic severe hypoproteinemia and this finding was consistent with the report of Ahmed (2002).

Mean live body weight of the infected sheep showed that haemonchosis had a significant effect and a decrease in body weight was observed in *H. contortus* infected sheep in the present study. This finding was in agreement with previous findings of Idris (1980); Barger and Cox (1984); Jubb *et al*. (1985); Al-Quaisy *et al*. (1987); Rahman and Collins (1990); Omar (1999); Abakar *et al*. (2000); Kelkele *et al*. (2012). The decrease of body weight in *H. contortus* infected sheep may be due to anorexia which results in decrease of food intake and/or decrease of the digestibility of nutrients due to alteration in abomasal pH [7].

The current experimental work reveals that *H. contortus* infected sheep had developed anaemia as indicated by a significant decrease (P<0.05) in PCV, Hgb concentration and TRBC count values. This might be related to the blood loss resulting from invasion of the abomasal mucosa by fourth stage larvae and adult worms. This finding is in line with those of Soulsby (1976); Abbott *et al.* (1986); Rahman and Collins (1990); Dorchies *et al*. (1997); Sharma *et al*. (2000); Getachew *et al*. (2005).

Infection with *H. contortus* did not lead to significant changes in TWBC counts (P>0.05) in both infected and non-infected groups and it fluctuates within the normal range throughout the experimental period and this finding is in line with that of Rahman and Collins (1990).

In the current experimental study, eosinophilia was evident in blood of infected sheep. Eosinophils are considered to be important elements in the response against helminth infections and are frequently associated with the expression of resistance to the parasites [37; 8; 53]. The increase in the number of eosinophils is a common feature observed during infection with *Haemonchus*. Consequently, our finding is in agreement with Salaman and Duncan (1984); Abakar *et al*. (2000); Getachew *et al*. (2005); Kelkele *et al*. (2012).

In this study, the number of lymphocytes in both infected and control group was found to be within the physiological range. However, increases in lymphocyte numbers were reported by Abakar *et al*. (2000). Even if the mean values of neutrophil of both infected and control groups fluctuates within the normal range, there was a difference between the two groups and this difference was statistically significant (P<0.05). Similar results were reported after experimental *H. contortus* infection by El Hassan (2002).

The pathological findings in *H. contortus* infected groups were pallor of visible mucus membranes, emaciated carcass, pale abomasal mucosa and adult worms were found in the abomasum. This finding is consistent with the previous report of Idris (1980); Hunter and Makenzie (1982); Rahman and Collinis (1991); Omar (1999); Abakar *et al*. (2000).

Therefore, the findings of the present experiment revealed that the stomach worm *H. contortus* was established in experimental sheep. The parasite had induced detectable clinical signs and there was significant decrease in Hgb, PCV and TRBC count value with resultant anaemia which was clinically manifested by pallor visible mucus membranes.

6. CONCLUSION AND RECOMMENDATIONS

Among the parasites of sheep and goats that restraint the survival and productivity, *H. contortus* being of overwhelming significance and is one of the most pathogenic helminths belonging to gastrointestinal parasites. This experimental work conducted to evaluate the clinical response and haematological effect of experimental *H. contortus* infections in sheep showed the establishment of infection with a prepatent period of 21 days post infection. The major clinical signs that were appreciated in infected sheep during the experimental period include weakness, lack of appetite and pale mucous membranes. These clinical signs start to occur at the second week post infection. *Haemonchus contortus* infection negatively affects the body weight gain of infected sheep that leads to a significant loss of bodyweight. Changes and differences in sheep infected with *H. contortus* were detected in circulating eosinophils with a significant

increase in infected group rapidly after infection indicating the possible role of these cells during parasitism. In addition, there was a significant reduction in the number of neutrophils of infected sheep. Infection with *H. contortus* also resulted in significant reduction of TRBC count, PCV and Hgb concentrations. The pathological alterations caused by *H. contortus* in infected sheep were mainly confined to the abomasum which showed pallor of abomasal mucosa and adult worms.

Based on the above conclusive remarks the following recommendations are forwarded:

- This experimental study should be rationalized and conducted by using *H. contortus* derived from different agroecological zones.
- Further studies should be conducted by using different infective doses of *H. contortus* to assess the effect on haematological, serum-biochemical changes and parasitological parameters.
- Immunological investigation should further be conducted to understand the mechanism and the role of host immune or inflammatory response.

Corresponding Author:
Dr. Eyachew Ayana
Bahirdar Animal Health Investigation And Diagnostic Laboratory, P.O.Box 70, Bahirdar, Ethiopia
Telephone: (+251)918575003
E-mail: *eyuelayana83@gmail.com*

7. REFERENCES

1. Al-Quaisy, H. H. K., Al-Zubaidy, A.J., Altaif, K.I. and Makkawi, T.A., 1987. The pathogenicity of haemonchosis in sheep and goats in Iraq; Clinical, parasitological and haematological findings. *Veterinary Parasitology*, **24**: 221–228.
2. Getachew, T., Hailu, Y., Grisez, C., Prevot F., Dumas, E., Bergeaud J. P., Dorchies Ph., Hoste H. and Jacquiet, J., 2005. *Haemonchus contortus* egg excretion and female length reduction in sheep previously infected with *Oestrus ovis* (Diptera: *Oestridae*) larvae. *Veterinary Parasitology*, **128**: 271-283.
3. Gomez-muñoz, M.T., Cuquerella, M., Gomez-Iglesias, L.A., Méndez, S., Fernàndez-Pérez, F.J., de la Fuente, C. and Alunda, J.M., 1999. Serum antibody response of Castellana sheep to *Haemonchus contortus* infection and challenge: relationship to abomasal worm burdens. *Veterinary Parasitology*, **81**: 281-293.
4. Huntley, J.F., Jackson, F., Coop, R.L., Macaldowie, C., Houdijk, J.G.M., Familton, A.S., Xieh, H.L., Stankiewicz, M. and Sykes, A.R., 2004. The sequential analysis of local inflammatory cells during abomasal nematode infection in periparturient sheep. *Veterinary Immunology and Immunopathology*, **97**: 163-176.
5. Johnstone, C., Guerrero, J., Chou, S., Melissa, D., Howe-Smith, R. and Eisenberg, A., 1998. The Nematodes, Haemonchus pathogenesis and Parasitic Diseases of Domestic Animals an online book of text and images. Available at: http://www. In: cal.vet.upenn.edu/merial/Trichos/trich_5b.htm (accessed on 6 June 2017).
6. Lacroux, C., Nguyen, T. H. C., Andreoletti, O., Prévot, F., Grisez, C., Bergeaud, J. P., Gruner, L., Brunel, J. C., Francois, D., Dorchies, P. and Jacquiet P., 2006. *Haemonchus contortus* (Nematoda: Trichostrongylidae) infection in lambs elicits an unequivocal Th2 immune response. *Veterinary Research*, **37**: 607-622.
7. Nicholls, C.D.; Lee, D.L.; Adrian, T.E.; Bloom, S.R. and Care, A.D., 1988. Hypergasterinaemia of sheep infected with *H. contortus*. *Research in Veterinary Science*, **45**: 124-126.
8. Pfeffer, A., Douch, P. G. C., Shaw, R. J., Gatehouse, T. K., Rabel, B., Green, R. S., Shirer, C.L., Jonas, W. E. and Bisst, S., 1996. Sequential cellular and humoral responces in the abomasal mucosa and blood of Romney sheep dosed with *Trichostrongylus axei*. *International Journal for Parasitol*ogy, **26**(7): 765-773.
9. Radostits, O. M., Gay, C. C., Blood, D. C. and Hnchcliff, K. W., 2000. Veterinary Medicine. A text book of the disease of cattle sheep, pigs, goats and horse, 9th ed .W.B sounders. London, pp.1347-1350.
10. Scott, I., Dick, A., Irvine, J., Stear, M. J. and McKellar, Q. A., 1999. The distribution of pepsinogen within the abomasa of cattle and sheep infected with *Haemonchus contortus*. *Veterinary Parasitology*, **82**: 145-159.
11. Scott, I., Khalaf, S., Simcock, D.C., Knight, C.G., Reynolds, G.W., Pomroy, W.E. and Simpson, H.V., 2000. A sequential study of the pathology associated with the infection of sheep with adult and larval *Ostertagia circumcincta*. *Veterinary Parasitology*, **89**: 79–94.
12. Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. and Jennings F. W., 1996. Veterinary Parasitology, 2nd ed. Blackwell Science, pp. 19-16.
13. Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. and Jennings, F. W., 2007. Veterinary Parasitology, 2nd ed. Blackwell Science, pp. 19-16.

14. Waller, P. J., Rudby-Martin, L., Ljungstrom, B. L. and Rydzyk, A., 2004. The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to overwinter survival strategies. *Veterinary Parasitology*, **122**: 207-220.
15. Wildblood, L. A., Kerr, K., Clark, D. A. S., Cameron, A., Turner, D. G. and Jones, D. G., 2005. Production of eosinophil chemoatractant activity by ovine gastrointestinal nematodes. *Veterinary Immunology and Immunopathology*, **107**: 57-65.
16. Mottelib, A. A.; Haroun, E. M.; Magzoub, M. and El-Basheer, E., 1992. The effect of gastrointestinal parasites on blood picture in sheep and goats at Al-Gassim. *Assiut Veterinary Journal*, **55**: 215-223.
17. Tibbo, M., Mukasa-Mugera E., Woldemeskel M. and Rege J. E. O., 2003. Risk factors for mortality associated with respiratory disease among Menz and Horro sheep in Ethiopia. *The Veterinary Journal*, **165**: 276-287.
18. Githiori, J. B., Höglund, J., Waller, P. J. and Baker, R. L., 2004. Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infections in sheep. *Parasitology*, in press.
19. Silverman, P.H. and Patterson, J. E., 1960. Histotrophic (parasitic) stages of *Haemonchus contortus*. *Nature* **185**: 54-55.
20. Allonby, E. W. and Urquhart, M., 1975. The epidemiology and Pathogenic Significance of Haemonchosis in Merino Flock in Eastern Africa. *Veterinary Parasitology*, **1**: 129-143.
21. Dargie, J. D., and Allonby, E. W., 1975. Pathophysiology of single and challenge infections of *Haemonchus contortus* in merino sheep: studies on red cell kinetics and ''self cure'' phenomenon. *International Journal for Parasitology*, **5**:147–157.
22. Soulsby, E. J. L., 1976. Pathophysiology of parasitic infection. Academic Press, New York.
23. Altaif, K. I.; Al-Abbassy, S. N. and Abboud, H. B., 1980. The response of Awassi sheep to re-infection with *H. costortus* larvae. *Parasitology*, **80**: 233-240.
24. Idris, U.A., 1980. Studies on the chemotherapy of experimental haemonchosis in goats. M.V.Sc. thesis, University of Khartoum.
25. Honde, C. and Bueno, L., 1982. *Haemonchus contortus*: egg laying influenced by abomasal pH in lambs. *Experimental Parasitology*, **54**: 371-378.
26. Hunter, A. R. and Mackenzie, G., 1982. The pathogenesis of a single challenge dose of *H. contortus* in lambs under six months of age. *Journal of Helminthology*, **56**: 135-144.
27. Soulsby, E. J. L., 1982. Helminthes, Arthropods and Protozoa of Domesticated Animals. Bailliere Tindall, London. Pp. 809.
28. Coles, E. H., 1980. Veterinary clinical pathology, 3rd ed. W B soundars company London.
29. Barger, I. A. and Cox, H.W., 1984. Wool production of sheep chronically infected with *Haemonchus contortus*. *Veterinary Parasitology*, **15**: 169-175.
30. Salman, S. K. and Duncan, J. L., 1984. The abomasal histology of worm free sheep given primary and challenge infections of *Haemonchus contortus*. *Veterinary Parasitology*, **16**: 43-54.
31. CIOMS, 1985. Council for International Organizations of Medical Sciences; International Guiding Principles for Biomedical Research Involving Animals.
32. Jubb, K. V. F.; Kennedy, P. C. and Palmer, N., 1985. Pathology of domestic animals. Academic Press, California.
33. Abbott, E. M., Parkins, J. J. and Holmes, P. H., 1986. The effect of dietary protein on the pathophysiology of acute ovine haemonchosis. *Veterinary Parasitology*, **20**:291-306.
34. Soulsby, E. J., 1986. Helminths, arthropods and protozoa of domesticated animals Balliere, Tindall, London, pp. 809.
35. Morales, G. and Pino, L.A., 1987. Ecoepidemiology of *Haemonchus contortus* bahiensis, ecotype present in sheep of arid zones of Venezuela. *Memórias do Instituto Oswaldo Cruz*, **82**: 359-369.
36. Ahmad, M., and Ansari J.A., 1989. Effect of haemonchosis on haematology and non-specific phosphomonoesterase activities in sheep and goats. *Helminthologia*, **26**(4):295-302.
37. Dawkins, H. J. S., Windon, R. G. and Eagleston, G. K., 1989. Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus columbriformis*. *International Journal for Parasitol*ogy, **19**: 199-205.
38. Jain, N.C., 1986. Schalm's Veterinary Haematology, 4th ed. Philadephia Lea and febiger. Pp. 1221.
39. Rahman, W. A. and Collins, G. H., 1990. Changes in live weight gain, blood constituent and worm egg output in goats artificially infected with a sheep derived strain of *H. contortus*. *British Veterinary Journal*, **146**: 543-550.

40. Rahman, W. A. and Collins, G. H., 1991. Infection of goats with *H. contortus* and *Trichostrongylus colubriformis*: histopathology and pH changes. *British Veterinary Journal*, **147**: 569-574.
41. Berghen, P., Hilderson, H., Vercruysse, J., and Dorny, P., 1993. Evaluation of pepsinogen, gastrin and antibody responses in diagnosing ostertagiasis. *Veterinary Parasitology*, **46**: 175-195.
42. Yadav, C. L.; Grewal, H. S. and Banerjee, D. P., 1993. Susceptibility of two crossbreeds of sheep to *H. contortus*. *International Journal for Parasitology*, **23**: 819-822.
43. Hansen, J. and Perry, B., 1994. The epidemiology, diagnosis and control of helminth parasites of ruminants: A handbook. International Livestock Research Institute, Nairobi, Kenya. Pp. 171-172.
44. Radostits, O. M., Blood, D. C. and Gay, C. C., 1994. Veterinary Medicine. A Text Book of the Diseases of Cattle, Sheep, Pig, Goats and Horses 8th ed., Bailliere Tindall, pp. 1228.
45. Barger, I. A., 1996. Prospects for integration of Novel parasite control options into grazing system. *International Journal for Parasitology*, **26**: 1001-1007.
46. Dorchies, Ph., Bergeaud, J. P. and Khanh, N. V., 1997. Reduced egg counts in mixed infections with *Oestrus ovis* and *Haemonchus contortus:* influence of eosinophils. *Parasitology Research*, **83**:727-730.
47. Waller, P., 1997. Sustainable helminth control of ruminants in developing countries, *Veterinary Parasitology*, **71**:195-207.
48. Dacie, J. V. and Lewis, S. M., 1991. Practical Haematology, 7th ed. London, pp. 37-73.
49. Kassai, T., 1999. Veterinary Helminthology; Real Educational & Professional publishing Ltd., Butterworth-Heinemann, pp. 76-81.
50. Omar, F. A., 1999. Pathogenesis of *Haemonchus contortus* in Naturally and Experimentally Infected Sudanese Desert Sheep. Ph.D. Thesis, University of Khartoum.
51. Stear, M. J. and Bishop, S. C., 1999. The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. *International Journal for Parasitology*, **29**: 777-780.
52. Abakar, A.D. El Amin, E.A. and Osman, A.Y., 2000. Clinical Response to Experimental *Haemonchus contortus* Infection in Desert Lambs, *The Sudan Journal of Veterinary Research*, **16:** 1-10.
53. Balic, A., Bowles, V.M. and Meeusen, E.N.T., 2000. The immunology of gastrointestinal nematodes in ruminants. *Advanced Parasitol*ogy, **45**: 181-241.
54. Borgsteede, F. H. M., 2000. Diagnostic expérimental des strongyloses. *Journeé de la Societé Française de Buiatrie 15-17 nov.*Paris, pp. 198-204.
55. Larsen, M., 2000. Prospects for controlling animal parasitic nematodes by predacious micro fungi. *Parasitology*, 120, S121-S131.
56. Sampson, H. A., 1999. Food Allergy. Part 1: immunopathogenesis and clinical disorders. *The Journal of allergy and clinical immunology*, **103**: 717-728.
57. Sharma, D. K., Chauhan, P. P. S. and Agrawal, R. D., 2000. Haematological changes in experimental haemonchosis in Barbari goats. *Journal of Animal Science*, **70**(4):353-355.
58. Ahmed, A. E., 2002. Studies on *Haemonchus contortus* Infection in Desert Goats in South Darfur State, Sudan. A Thesis submitted in accordance with the requirements of the University of Khartoum for the degree of master of veterinary science. Department of Pathology Faculty of Veterinary Medicine University of Khartoum. Pp.1-67.
59. El Hassan, R. O., 2002. Experimental infection of three Sudanese sheep breeds to *Haemonchus contortus*. Thesis. university of Khartoum, Sudan.
60. Van Wyk, J. A. and Bath, G. F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research*, **33**: 509-529.
61. Aiello, E.S. and Mays, A., 2000. The Merck of veterinary manual 3rd ed. Merck and co. inc. White house station, N.J.USA. Pp. 216.
62. Whittier, D., Zajac, A. and Umberger, H., 2003. Control of Internal Parasites in Sheep, Virginia Cooperative Extension, Publication No 410-027.
63. Githiori, J. B., 2004. Evaluation of Anthelmintic Properties of Ethnoveterinary Plant Preparations Used as Livestock Dewormers by Pastoralists and Small Holder Farmers in Kenya. Doctoral thesis ISSN 1401-6257, ISBN 91 576 6666 0.
64. Burke, J., 2005. Management of Barber pole Worm in Sheep and Goats in the Southern U.S. Booneville, AR: Dale Bumpers Small Farms Research Update.
65. Suchitra, S. and Joshi P., 2005. Characterization of *Haemonchus contortus* calerticulin suggests its role in feeding and immune evasion by the

parasite. *Biochimica et Biophysica Acta*, ***1722***: 293-303.
66. O'Connor, L.J., Walkden-Brown, S.W. and Kahn, L.P., 2006. Ecology of the free-living stages of major Trichostrongylid parasites of sheep. *Veterinary Parasitology*, **142**: 1-15.
67. Zajac, A. M., 2006. Gastrointestinal nematodes of Small Ruminants: life cycle, anthelmintics, and diagnosis. *Veterinary Clinic for Food Anim*als, **22**: 529-541.
68. Getachew, T., 2007. Rôle des éosinophiles dans la régulation des populations d'*Haemonchus contortus* chez le mouton, le titre de docteur de l'institut national polytechnique de Toulouse, pp. 1-225.
69. Sissay, M. M., 2007. Helminth parasites of sheep and goats in eastern Ethiopia: Epidemiology, and anthelmintic resistance and its management. *Doctoral thesis*, Swedish University of Agricultural Sciences, Uppsala, Sweden. ISSN 1652-6880, ISBN 978-91-576-7351-0.
70. Sissay, M. M., Uggla, A. and Waller, P. J., 2007. Epidemiology and seasonal dynamics of gastrointestinal nematode infections of sheep in a semi-arid region of eastern Ethiopia. *Veterinary Parasitol*ogy, **143**:311–321.
71. Tariq, K. A., Chishti, M. Z. and Ahmad, F., 2010. Gastro-intestinal nematode infections in goats relative to season, host, sex and age from the Kashmir valley. *Indian Journal of Helminthology*, **84**(1): 93– 97.
72. Williams, A. R., 2010. Immune-mediated pathology of nematode infection in sheep is immunity beneficial to the animal? *Parasitology*, **138**(5): 547-556.
73. Bowman, D., 2003. Parasitology for Veterinary, 8th ed. Elsevier, USA, pp. 162-163.
74. CSA, 2012. Central Statistical Authority Statistical; Report on Livestock and Farm Implement, North Gondar, Agricultural office.
75. Kelkele, F., Tolossa, Y. and Kassa, G., 2012. Experimental infection of Ethiopian highland sheep by different infective doses of *Haemonchus contortus* (L3): haematological and parasitological parameters, serum protein concentrations and clinical responses. *Ethiopian Veterinary Journal* **16**(1): 41-57.
76. CSA, 2013. Agricultural sample survey. Report on livestock and livestock characteristics. The Federal Democratic republic of Ethiopia, Central Statistical Agency (CSA). Private Peasant Holdings. Statistical Bulletin 570, Addis Ababa, Ethiopia, April, 2013.
77. Besier, B.**,** 2009. Sheep worms barbers pole worm. Note 476. Department of Agriculture and Food, Government of Western Australia, pp. 1-4.

10/22/2024