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ADDIS ABABA UNIVERSTY

FACULTY OF VETERINARY MEDICINE

STUDY ON BASIC HEAMATOLOGICAL, SERUM BIOCHEMICAL AND PARASITOLOGICAL PARAMETERS IN EXPRIMENTALLY INFECTED SHEEP BY DIFFERENT INFECTIVE DOSES OF *HAEMONCHUS CONTORTUS* (L_3)

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FANTU ASHINE

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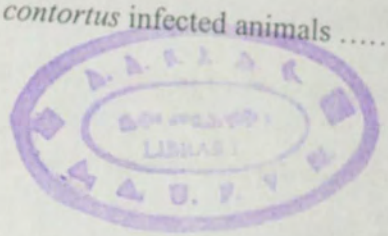

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ABBREVATIONS

ANOVA	analysis of variance
GI	Gastro Intestinal
GIT	Gastro Intestinal Tract
FEC	Faecal Egg Count
PCV	Packed Cell Volume
RBC	Red Blood Cell
WBC	White Blood Cells
g/dl	Gram Per Decilitre
EPG	Egg per Gram of Faeces
IgE	Immunoglobulin E
Th2	Helper T-cell 2
SPSS	Statistical Packages for Social Science

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ABSTRACT

A study to assess changes in haematological, serum biochemical and parasitological parameters in experimentally infected Ethiopian highland sheep using different doses of infective larvae of *Haemonchus contortus* (L_3) was conducted from December 2007 to March 2008. A total of twenty-four sheep aging one to two years were divided in to four groups. The first three groups G_1, G_2 and G_3 were infected orally with 2000, 4000 and 6000 infective larvae of *Haemonchus contortus* (L_3) respectively. The last group of six animals remained uninfected control. Parasitological parameters such as faecal egg count (EPG), and haematological parameters like Packed Cell Volume (PCV), haemoglobin concentration, blood cellular profiles (red blood cell, white blood cell and differential counts), and total serum protein and body weight changes were monitored weekly. All animals were killed humanely 91. Post-mortem examination was carried out to determine worm burden, differentiation of sexes of the nematode. The mean worm burden in groups G_1, G_2 and G_3 was 951± 37.6; 1962 232 ± 360; 2950± respectively. Progressive severe anaemia, hypoproteinemia, loss of live body weight, reduced PCV, Hb, WBC and RBC was observed in all animals infected with nematode while in uninfected control animals all of these parameters remained in the normal physiological range throughout the experimental period. On the basis of the observed clinical, parasitological and haematological parameters experimental animals appeared to be very susceptible to the infection by *Haemonchus contortus* which was proved by significant loss of body weight, development of clinical haemonchosis with severe anaemia and reduced PCV as well as high number of worms recovered from the abomasums of infected sheep.

Key word: Biochemical, Ethiopia, Haematological parameters, *Haemonchus contortus*, Highland sheep, Parasitological parameters, Serum.

1. INTRODUCTION

Ethiopia has the largest livestock inventories in Africa, It is estimated that more than 38 million cattle, 30 million small ruminants, more than 1 million camels, 4.5 million equines and 40 million chickens exist (CSA, 2001). Livestock ownership currently contributes to the livelihoods of an estimated 80 percent of the rural population. In the arid and semi-arid extensive grazing areas in the eastern, western and southern lowlands cattle, sheep, goats, and camels are managed in migratory pastoral production systems (CSA, 2001).

Ruminants are major sources of food protein, income savings, skin, fiber and manure. The full exploitation of this huge resource is hindered in the tropical environment and particularly in Africa due to a combination of factors such as drought, poor genetic potential of the animal, traditional system of husbandry, management and the presence of numerous diseases (Waruiru *et al.*, 2005). Among diseases that affect small ruminants, gastrointestinal nematodes imposes sever economic impact on sheep and goat production (Kaufmann and Pfister 1990). They cause production losses that are manifested by reduced weight gain, lowered meat and milk production and even death especially in the young (Githigia *et al.*, 2005).

In the field while sharing common pasture, animals are exposed to a variety of parasites among which gastrointestinal nematodes that cause considerable animal health problems in many parts of the world are commonly seen (Clark, 2001; Cox, 2001; Miller *et al.*, 1998; Tembely *et al.*, 1997; Waller *et al.*, 2004).

The highland of Ethiopia with high rainfall and optimal temperature is also conducive for the development of helminthes. Over stocking and prolonged grazing accentuate infection rate since the number of infective larvae increases with higher animal density on pasture (Barger, 1996). The climatic condition of Ethiopia is also more favourable for development and survival of infective stages of helminthic parasites, helminthosis mostly in the form of gastro-enteritis is ranked as the major cause of reduced productivity on sheep and goat (Tembely *et al.*, 1997). Even though the exact estimation of the economic loss due to helminthosis is hard to obtain, annual losses of 7000 million Ethiopian birr was estimated (Donald, 1999). *Haemonchosis*, causes anaemia, diarrhoea, and emaciation resulting in reduced weight gains, increased morality and increased production cost. Many studies carried out indifferent parts of

Ethiopia have shown that *H. contortus* is one of the most prevalent and economically important parasites of small ruminants (Esayas, 1999, Amenu, 2005, Lidetu, 1999, Abebe and Essayas, 2001, Berrsisa, 2004). Similar studies conducted in African countries (Fritsche *et al.*, 1993, Achi *et al.*, 2003, Nganga *et al.*, 2004).

Under field condition, in addition to the host genetics and acquired immunity, many factors determine the out come of GI nematode infections in domesticated livestock through their influence on host susceptibility and resistance to infection, and the capacity to regulate parasitic development, fecundity and survival. Polyparasitism is the commonest form of livestock parasitism in the tropics (Chiejina, 2001).

Infections with the blood-feeding nematode, *Haemonchus contortus* are a major constraint on sheep and goats health and production in temperate as well as tropical regions. The parasite mainly affects the abomasal mucosa of its host. Adult worms feed on blood and can cause severe anaemia, resulting in poor growth rate and weight loss, and heavy infections can cause death. (Tembely *et al.*, 1997; Miller *et al.*, 1998; Waller *et al.*, 2004). The losses caused by *Haemonchus contortus* are more severe and important due to its extreme pathogenecity, wide geographical distribution, diversified host spectrum and its high prevalence in small ruminants (Troncy, 1989).

Haematological parameters provide an excellent basis for judgment with respect to the nature of the disease, the extent of tissue and organ damage, the response of defence mechanism of the patient in diagnosing the type of possible anaemia, in evaluating the patient before commencing any surgical intervention and to select appropriate treatment (Shalm *et al.*, 1975).

In apparently healthy animal a number of factors may influence the normal blood value. These include variations in sample size, source, age, sex, breed, health and nutritional status of the animal, haematological techniques used, time of sampling, temperature and altitude. Physiological status such as excitement, muscular exercise, pregnancy, oestrus, parturition, water balance (dehydration) and transportation (Sharman and Marry, 1994). The blood parameters such as total count of RBC, WBC, differential leucocytes count, hematocrite (PCV), haemoglobin content may serve as an index to characterize health status of animals Shalm *et al.*, 1975).

Considerable information is available on the normal blood parameters of domestic animals but these values are that of exotic breeds which are somehow different from that of local breeds and there are quantifiable variations in blood parameters particularly in haemoglobin concentration, RBC counts and PCV values. Meanwhile, these recorded physiological values are not available for different species and breeds of indigenous animals in Ethiopia. Moreover, information is lacking about the effects of physiological and environmental factors as well as GI nematodes on these physiological normal values (Bekele, 1987, Fufa, 1999, Yasmin, 2000).

The objectives of this study are therefore to:-

- Assess changes in haematological, serum biochemical parameters in infected host by *Haemonchus contortus* (L_3)
- Determine if difference in infective doses by *H. contortus* have an effect on major parasitological parameters.
- Observe clinical and parasitological responses following the experimental infection
- Generate base line information regarding effect of the parasite on haematological parameters in indigenous sheep

2. LITRETURE REVIEW

2.1. Major GI nematodes of small ruminants

Nematodes (round worms) are unsegmented, hair like, tubular worms ranging in size from a few millimetres to several centimetres. They are a group of worms that are responsible for most of the helminthes disease of vertebrates and even some invertebrates are vulnerable to invasion by them (Brander *et al.*, 1991; Reine, 1983).

The effect of parasites on their host depends upon numerous factors such as the age of the animal, breed, management, nutritional status, and the parasite species involved (Urquhart *et al.*, 1996). Helminth parasites are the causes of very significant morbidity, mortality and economic losses in domestic animals. Most parasitic helminthes infect their host via the oral route, and live either at the mucosal surface of the gastro intestinal tract, respiratory tract or cross these barriers on their way to predilection sites (Mulcahy *et al.*, 2004).

The severity of the disease and effects it cause is prominent in young flocks (lambs) and yearlings (Belschner, 1976) and to those individuals that are stressed (Soulsby, 1986; Urquhart *et al.*, 1996; Radostits *et al.*, 2000). Pregnant, parturient and lactating female are more susceptible than non-reproducing females. Hence the disease deserves special attention as it diminishes the capacity of sheep and goats to achieve their inherent potential level of production for any given feeding and management regimen (Morris, 1988).

Although nematode parasites inhabiting the gastro intestinal tract of ruminants are generally considered luminal dwellers, both larval and adult parasites appear to induce definite immunological responses in their host. These responses may be local and/ or systemic in nature acting dependently or independently of each other and ultimately producing high complex interactions between host and parasite (Waston, 1986).

Various epidemiological factors are affecting the distribution and importance of parasitic disease. One of the factors seriously affecting the pre-parasitic phase of nematode development is the presence of suitable climate, particularly of temperature and moisture. The requirements of the different free-living stages of nematodes in this regard vary from parasites

to parasites. As a result some parasites are more adapted to temperate cool environment while others are adapted to warm tropical environment (Craig, 1998).

Production losses due to nematode parasite infection of small ruminants could be due to changes in feed intake, impaired gastro intestinal tract function, competition for the host's essential nutrients and tissue damages during parasite migration and feeding. These effects could be manifested in severe clinical signs such as anaemia, oedema, diarrhoea, and anorexia; resulting in poor general performance and even mortality particularly in the young and immuno-suppressed individuals (Fox, 1997; Eysker and Ploeger, 2000).

Some GI nematode species cause decreased feed utilization by the host. These include mainly *Teladorsagia spp., Trichostrongylus spp., Cooperia spp., Strongyloides papillosis, Oesophagostomum spp and Nematodirus spp etc.* The pathological changes observed due tothese parasites include: formation of tunnels under mucosal epithelium of abomasum *(Teladorsagia spp., Trichostrongylus axei)*, nodules in the large intestine *(Oesophagostomum spp)* and causing erosions in the epithelium of abomasal and intestinal mucosal, catarrhal enteritis, villous atrophy in anterior small intestine, hyperaemia and oedema. In extreme cases, diphtheritic enteritis and exudates hinder absorption (Soulsby, 1986; Urquhart *et al.*, 1996; Radostits *et al.*, 2000).

Certain GI nematode parasites affect their hosts by sucking blood and damage the host's tissue. These include *Haemonchus spp., Chabertia ovina, Gaigeria pachyscelis, Bunostomum trigonocephalum* and *Trichuris sp.* In most cases, the predominating syndrome is progressive debilitating anemia (Smith, 1999), but hyper acute and acute infection of *Haemonchus contortus* and acute infection of *Gaigeria pachyscelis* could lead to death (Bowman, 1999; Radostits *et al.*, 2000).

Marked tissue reaction manifested by formation of nodules in the intestine of infected small ruminants is commonly observed in infestation by the 3rd stage larvae of *Oesophagostomum columbianum* (Troncy, 1989; Urquhart *et al.*, 1996). In previously exposed sheep and goats due to sensitization, 3rd stage larvae of *Oesophagostomum columbianum* pass into sub mucosa of small intestine and some times under heavy infection to sub mucosa of large intestine with marked inflammatory reaction around each larvae will takes place. These nodules (inflammatory reaction) could be as big as 2.0 cm in diameter and containing greenish eosinophilic pus and fourth stage larvae (Urquhart *et al.*, 1996).

2.2. The parasite: *Haemonchus*

In the field, domestic animals are exposed to a multiplicity of parasites among which is gastrointestinal nematodes that cause considerable animal health problem in many parts of the world. *Haemonchus contorts*, a blood feeding *Trichostrongyle* of the abomasum, is a widely spread nematodes infecting ruminants (Tembely *et el.*, 1997; Miller *et. al.*, 1998; Waller *et al.*, 2004) *Haemonchosis* is an important parasitic disease of sheep, goat, cattle, camel, and others ruminants where ever they are kept but the disease exerts its greatest economic effect in sheep and goats These parasite mainly affects the abomasal mucosal of their hosts it inhabits (Kaufmann, 1996).

Infection with *Haemonchus* species is a major constraint to domestic ruminant's production throughout the world. *H. placei and H. contortus* are the most important species in cattle and sheep respectively (Lichtenfels *et al.*, 1988). *H. contortus* is the species most commonly found in Sheep and Goats and also be found in cattle when these animals graze the same pasture. *H. placei* it the usual *Haemonchus* species in cattle and it can also develop well in sheep and goats and causes clinical disease but causes less severity than that caused by *H. contortus, H. longistipes* is the species that usually affects Camels and Dromedaries and it can also develop in other animals. *H. similis* is the one that usually affect cattle and deer in same countries and it can also affect other animals (Soulsby, 1986; Radostitis *et al.*, 1994, Urquhart *et al.*, 1996).

Haemonchosis was first considered to be primarily a hemorrhagic anaemia; most of the blood loss was accounted by ingestion of the worms themselves and also blood loss further attributed at the puncture site. The most sensitive indicator of the degree of Haemonchosis was considered to be the hemoglobin (Hb) level. The spectacular depression of Hb level, accompanied by weakness and death, are the classical clinical features of Haemonchosis by (Roberts and Swan, 1982).

Haemoncosus is the most common in Tropical and sub tropical areas is encountered usually during the summer and autumn, while *T. axei and Ostertagia* spp are more common in winter rainfall areas haw ever, for a much greater portion of the year, levels of infection are low and a state of chronic infection exists. These low levels of infection have generally been assumed to be unimportant, and the effects on haemoglobin and on productivity over the long term

have received little attention (Roberts and Swan 1982). Clinical Haemonchosis occurred in sheep irrespective of age and previous experience of infection in many endemic areas despite of the existing gross difference in climate, husbandry and breeds of sheep (Miller *et. al.*, 1998).

Haemonchus contortus produce thousands of eggs (prolific egg layers up to 10,000 eggs per day for several months) while others like *Trichostrongylus spp.* produce only a few hundred (Urquhart *et al.*, 1996; Radostits *et al.*, 2000). Some nematode eggs survive outside of their host for considerable period of time (ex. *Trichuris, Ascaris, Nematodirus*), which may be dependable on the thickness of their egg shell thus responsible for longer time contamination of the grazing field (Anene *et al.*, 1994).

2.2.1. Morphology and Biology

The genus is among the largest in the super family ranging from 10-13 mm in length. In fresh specimens the worms can be easily seen due to their bright homogeneously red colure and considerable size. In both sexes there is pair of wedge shaped cervical papillae in the oesophageal region and a tiny lancet inside the bucal capsule used for piercing small blood vessels (Kaufmann 1996, Urquhart, 1996).

In fresh specimens the most obvious feature females is that the white egg filled uterus winding spirally around the blood filled intestine-giving rise to the so- called Berbers pole effect. The vulva is located about a quarter body length from the tail and may or may not be guarded by variously shaped cuticular inflations (vulvar flaps). The form of the vulvar flaps may range from an extreme linguiform shape to a knob shape or a complete absence the female worms are classified in to three depending of the type of their vulvar morphology: Linquiform, females with a supra-vulvar flap; knobbed, females with well-developed knob like process and smooth, females with no vulvar flap process (Rose, 1978). The prevalence of these various vulvar flap configuration varies among species and subspecies (Soulsby, 1986; Bowman, 1995; Urquhart *et al.*, 1996).

In males the bursa is large enough to be seen by the naked eye. The most important diagnostic features are the barbed short and wedge shaped specula's and small dorsal lobe and small lateral lobes (Reinecke, 1983; Solusbay, 1986). The total lengths of the spicules, the distance

from the barb to the tip of the right and left spicule are the three basic morphmetric parameters for species, differentiation (Jacquiet. *et al.*, 1997).

In nematode, the sexes are separate and the males are generally smaller than the females, which lay eggs, or larvae, during development, a nematode molts at intervals shedding it is cuticle. In the complete life cycle there are five moults, the successive larval stages being designated L_1, L_2, L_3, L_4 and L_5, which is the immature adult (Urquhart *et al.*, 1996). One feature of the basic nematode life cycle is that immediate transfer of infection from one final host to another really occurs some development usually takes place, either in the faecal pat or in different species of animal the intermediate host before infection can take place (Urquhart *et al.*, 1996). In common form of direct form of life cycle the free living larvae undergoes two molts after hatching and infection is by ingestion of free infective L_3. There are some importance exceptions however, infection some times being by larval penetration of the skin or by ingestion of the egg containing larvae. In indirect life cycle, the first two moults usually takes place in an intermediate host and infection of the final host is either by ingestion of the final host or by inoculation of the L_3 when the intermediate host such as blood sucking insect, feeds, after infection two further moults takes place to produce the L_5 or immature adult parasites, following copulation further life cycles initiated (Urquhart *et al.*, 1996)

2.2.2. Pathogenesis and Symptoms

The Pathogenic effects of gastro intestinal parasites depend on their localization, feeding habit, the dose of ingested larvae and immune status of the host (Urquhart *et al.*, 1996). Haemonchosis is characterized by produce three types of clinical signs depending of the degree of infestation. Thus in hyper- acute cases, small ruminants die suddenly from hemorrhagic gastritis. Acute haemonchosis is characterized by anaemia, which cause polar of the skin and mucous membranes and haematocrit reading decries. Generalized oedema due to loss of plasma proteins and progressive weight loss. But diarrhoea is not a common feature of haemonchosis; the lesion is those associated with anaemia. The abomasum become oedematous and the usual manifestation of the disease is chronic phase the PH increase causing gastric dysfunction, anaemia, reduced appetite, weakness (Urquhart *et al.*, 1996; Hansen and Perry, 1994).

When large number of larvae infects sheep, death can occur suddenly while the sheep still appear to be in good health (Hansen and Perry, 1994, Urquhart *et al.*, 1996). In these animals large red mass of worms are clearly visible in the stomach. The stomach contents are often brown because of has pin-point blood spots on it. The blood of the sheep is watery due to anaemia (Urquhart *et al.*, 1996).

At peak infection, naturally acquired population of *Haemonchus contortus* can remove one fifth of the circulating erythrocyte volume per day over the course of nonfatal infection lasting two months. *H. contortus* with it is blood sacking nature each worm removes about 0.05 ml of blood per day by ingestion and see page from lesions so that a sheep with 5000 *H. cotortus* may lose about 250 ml of blood daily. The pathogenic effect of *H. contortus* results from the in ability of the host to compensate for blood loose (Radostitis *et al.*, 1994; Urquhart *et al.*, 1996). Because of the spectacular depression of Hb, anemia due to haemonchosis is generally considered to be moderately macrocytic normocromic in nature (Roberts and Swan, 1982). Infection with *Trichostrongylus axei, Ostertagia ostertagia and O. circumcincta* have also been reported to be associated with anaemia but it is much more likely to be a marginal nutritional anaemia associated with inappropriate and excessive loss of plasma proteins in to the alimentary tract (Jennings, 1987; Urquhart *et al.*, 1996).

The development of anaemia in *Haemonchus contortus* infection is directly attributable to the blood sucking activity of the parasite. The first, which occurs during the prepatent period the first 3 weeks, is characterized by a dramatic fall in PCV but serum iron is normal at this stage. This is considered to be the result of blood loss due to the developing but immature parasite (Dargie *et al.*, 1979, Al-Qyaisy *et al.*, 1987).

Although at this stage the blood loss at absolute volume is not large as when the parasite is mature. The haematopoietic system of the host is not fully mobilized to produce red cells in quantities sufficient to meet the needs of the animal (Jennings, 1987). The second stage of the anaemia occurs at the early post patent phase of the disease. During this period quantitative measurement showed a maximum blood loss, not necessarily associated any further drop in pcv due to the mobilization of the haemopoetic systems and the high serum iron levels hav ever, the capacity of the animal infected with *H. contortus* to reabsorb haemoglobin iron is limited the iron reserve of the animal become seriously depleted, and led progressively to the third stage of the anaemia, the low serum iron is accompanied by a marked drop in PCV

indicating failure in haemopoiesis due to iron deficiency. A persisting protein losing gastroenteropathy possibly complicates this condition (Dargie *et al.*, 1979).

A number of factors have been shown to influence the course of *H. contortus* infection and the severity of the associated anaemia's. In addition to the nutritional status the ability of the animals to respond quickly to the haematological stress, the immunological status of the animals and the innate resistance determines the proportion, if any of a given dose of infective larvae, which will become established (Jennings, 1987).

Roberts and Swan (1982) demonstrated a strong relation ship between faecal egg counts and total adult worm counts of *H. Contortus*. Further to this, it was considered necessary to investigate the relation ship between worm counts and Haemoglobin levels in an attempt to characterize ovine *Haemonchosis* more fully and provide additional criteria on which the significant of faecal egg counts could be assessed. A directly proportional depression of hemoglobin (HB) level to linear increase in worm burden.

The losses caused by *Haemonchus contortus* are more severe and important due to its extreme pathogenicity, wide geographical distribution, diversified host spectrum and its high prevalence in small ruminants (Troncy, 1989).

2.2.3. Laboratory Diagnosis

Coproscopy: It is the basic method for parasitological diagnosis. It is based on observation of the number of eggs laid by adult female parasites present within the gastrointestinal tract and released with faeces. Factors such as species of parasites, animal species, and reaction of the host determine the number of eggs. However, the quantity of eggs is roughly proportional to the number of worms present and hence reflects indirectly the degree of parasitism. Hansen and Perry, 1994; MAFF, 1977).

Faecal culture: It is the only practical method available for obtaining an indication ante-mortem of the worm genera with which ruminants are affected and enables to identify the different species and genera of gastrointestinal nematodes present. It is indicative for degree of pathogenecity and egg production by different species of gastrointestinal nematodes Distinguishing features are the shape of the "head" (cranial extremity) of the larvae or the

length of the sheath" tail" (the extension of the sheath from the tip of the larval caudal extremity to the tip of the sheath (Georgi, 1985; Wyk Van and Michael, 2004).

The coproculture enable to easily detect the infective larvae (L_3) which is the only free larval stage easily identifiable. It is based on the development of eggs into L_3 by keeping the faecal sample at 22-23°c, humidity of 85-90% and optimal oxygen. After 10 to 13 days it is possible to collect the larvae by using a sedimentation apparatus of Baermann. Then the genera of the larvae could be identified (Hansen and Perry, 1994; MAFF, 1977).

Necropsy: it is the method that gives the most valuable information burden. It enables to detect and count the worms, identifies the larval stages and differentiates the two sexes. Its inconvenience is that expensive to kill animals and is time consuming procedure (Hendrix, 1998).

For parasite counts, the gastrointestinal tract from abomasums to rectum is required. The adult and larval nematodes are carefully washed out collected, counted and identified. Most of the parasite species are identified immediately on the basis of their shape, colour, size and their localization in the GIT. (Hansen and Perry, 1994; MAFF, 1977).

2.3. Host parasite interaction

Interaction refers to the interdependent operation of factors to produce effect (Thrust field, 1995) the out came of disease occurrences depends on the inter play of the host, the agent (the parasite) and environmental factor. By adding or modifying the factors the frequency of the occurrence of the disease can be changed (Thrusfild, 1995).

2.3.1. Characteristics of immune response to gastrointestinal nematodes

In all domestic species of animals, there is variation of responses to parasitic infections. In ruminants, the level of resistance to parasitic infections is generally determined and significant differences have been observed among breeds. These variations could be inborn or acquired. Genetic variation can be exploited to improve the capacity of the animals to resist parasitic infections (Stear and Wakelin, 1998). Distinct immune responses often occur to antigens exposed at different stages of the parasites life cycle. The immune response to

parasites is extremely complex with variations related to the parasite species, environmental factors and the physiological status of the host (Waston, 1986).

Helminth immunity is usually less sufficient and more transient than to the immunity to micro-organisms; possibly because they do not reproduce in the host as do the bacteria, viruses, and protozoa. The immunity produced by helminths that migrate in the host appears to promote a greater immunological response than those confined to the intestine. Larvae acquired by immuned animals may become established but are later destroyed (Benz *et al.*, 1985). Immune response in ruminants to *Trichostrongyloid* infections is very complex. Somatic migrations except in the case of *Bunostomum* are not usually part of the normal developmental cycle of trichostrongyle nematodes and therefore these parasites do not have intimate contact with the host's internal immunologically responsive tissue Both larval and adult stages however, are in contact with the epithelial surface of gastrointestinal tract (Benz, 1985).

Responses to gut-duelling stages are often polarized Th2 responses, characterized by eosinophilia, mastocytosis and IgE production. One effect of such a response in the gastrointestinal tract is stimulating of smooth muscles, increased gut motility, and diarrhoea (Vallance and Collins, 1998). The extent into which the antibody reacts on the parasite ranges from slight interference with the development and reproduction to complete elimination of them from the host.

Researchers presently believe that mucosal inflammation may be an important initial effecter mechanism of expulsion of worm burdens during self-cures and that immunoglobulin of the IgE class and mast cells play roles in the process (Wakelin, 1978). Smith and Christie (1978) noted that globule leucocytes were more numerous in the abomasal walls of sheep resistance to infection by *Haemonchus contortus* whereas worm-free animals had few.

Some recent investigations indicate that, selected animals for resistance to parasitic infections could be more susceptible to other pathologies. Accordingly, it has been proved that genetically selected sheep for their resistance to strongylids are more susceptible to *O. ovis* and other parasites of upper respiratory tract (Dorchies *et al.*, 1997; Jacquiet *et al.*, 1999 and Yacob et *al.* 2002,).

Resistance to gastrointestinal strongylids is associated with immunological and inflammatory mechanisms. One of the main mechanisms is an increase in the number of eosinophils, mast cells and globule leucocytes in the mucosa of digestive and upper respiratory tracts (Huntley, 1984).

Blood eosinophilia and increased numbers of eosinophils in the parasitized gastrointestinal mucosa are typically seen during gastrointestinal nematode infections (Rothwell, 1989; Yacob *et al.*, 2004). Eosinophils are attracted to sites of nematode invasion by chemotactic factors released by degranualation of mast cells. These factors also mobilize the bone marrow eosinophils in the circulation. Once they arrive at the site of parasitic invasion, eosinophils attach to the parasites through IgE and IgG to the helminth`s cuticle. The lethal effects of eosinophils on helminths or nematodes are enhanced by mast cell derived factors such as histamine as well as complement and by factors derived from T-lymphocytes and macrophages (Meeusen, 1999).

Eosinophilia has been shown to be positively correlated with resistance to gastrointestinal nematode infections in sheep (Buddle *et al.*, 1992), and recent studies have shown that eosinophils can damage and kill infective L3 larvae of the gastrointestinal parasites, *H. contortus*, both *in vitro* (Rainbird *et al.*, 1998, Terefe *et al.*, 2007) and *in vivo* (Balic *et al.*, 1999).

One of the most marked features of a gastrointestinal nematode infection is the recruitment and hyperplasia of mucosal mast cells. Mucosal mastocytosis, including the presence of intra-epithelial globule leucocytes, is invariably associated with gastrointestinal helminthosis (Huntley *et al.*, 1992), suggesting that type I immediate hypersensitivity reaction is important in worm expulsion (Miller, 1984).

3. MATERIALS AND METHODS

3.1. Study area

The experimental study was carried out at the compound of fattening project of the Faculty of Veterinary Medicine in Debre-Zeit in sheep obtained from Koka market of lume worda and it district. Debre-Zeit is located at a distance of about 45km South East of the capital, Addis Ababa. The area is located at an altitude of 1850 meters above sea level and has a total human population of 95,000. 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 800 mm. The area has an average maximum and minimum temperature of 27.7 °C and 12.3 °C, respectively (CACC, 2003). Debre-Zeit is the center of Ada'a Liben woreda, and the Woreda has a total land area of about 161,056 he and is divided in to three agro-ecological zones namely mid land 94%; high land 3% and low land 3% (Ada'a Woreda Agricultural and rural development office).

3.2. Study type

Experimental study involving randomized experimental trial to assess changes in basic haematological, serum biochemical and parasitological parameters in experimentally infected Ethiopian highland sheep by different infective doses of *Haemonchus contortus* (L_3).

3.3. Study Period

The study was conducted from September 2007 to June 2008 at the campus of the Fattening project of Faculty of Veterinary Medicine, Debre Zeit.

3. 4. Experimental design

3.4.1. Experimental animals

This study was carried out on twenty four clinically healthy sheep purchased from Koka markets, weighing between 18-25 kg were used for the study. All animals were housed in an

isolation area 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. Animals were allowed to feed on locally dried hay feed and water *ad libitum* with sufficient quantity of concentrates throughout the adaptation and experimental period. Animals were screened for internal parasites. Faecal samples from each animal was collected and examined for the presence of any parasite ova using flotation method (Hansen and Perry 2004). All the purchased animals were dewormed with ivermectin at 200µg/kg, praziquantel 15Mg/kg and triclabendazol 250mg/25kg body weight to clear any parasite that may present. The anthelmintic doses were calculated individually according to body weights and were administered orally. A second faecal examination 10 days later showed all animals to be negative for nematode eggs. All animals were allowed to acclimatize for one month before the experiment commenced.

At the end of the adaptation period animals weighed, ear tagged for easy identification and a randomized complete block design was used where sheep were randomly allocated into four blocks. Parasitological parameters such as faecal egg count (EPG), and haematological parameters like Packed Cell Volume (PCV), haemoglobin concentration, blood cellular profiles (red blood cell, white blood cell and differential counts), and total serum protein and body weight changes were monitored weekly from the start to the end of the trial. All animals were humanly killed on day 91.All abomasums were taken and processed for parasite counts of nematodes as indicated by Hansen and Perry (2004).

3.4.2. Parasites and Experimental infection

The infective larvae of *Haemonchus contortus* (L3) were obtained by culturing eggs from adult *Haemonchus contortus* female collected from the abomasums of naturally infected sheep and slaughtered at Debre Zeit HASHEM export abattoir. The worms were thoroughly washed in tap water in order to remove the adhering materials. Then the abomasum contents were diluted with 2 ml volume of water in large glass trays. The worms, which were actively swimming in the contents, were transferred with a pair of plastic forceps to saline in Petri dishes. The nematodes were washed repeatedly and examined under a stereomicroscope. The collected adult female worms were crashed in a small volume of saline to extract the eggs. The eggs harvested from females were cultured on helminthologically sterile horse faeces to obtain the third larval stages in jars at incubator (27°C) for 7-10 days. Larvae (L3) harvested

using Baerman technique from faecal cultures were stored in small volumes of water in aerated, flat-bottomed flasks at 4°C until used (MAFF, 1986, Hansen and Perry 2004). The first three groups G_1, G_2 and G_3 were infected orally with 2000, 4000 and 6000 infective larvae of *Haemonchus contortus* (L_3) respectively. After counting and through determination of viability and motility of larvae. The last group of six animals remained uninfected control.

Table 1. Experimental design of scheduled infections

Groups	Days of infection with *H. contortus* (L_3) and experimental period		
	D0 Infection	**D+7 to D+91**	**D+91**
		Sampling and assessing parasitological and blood parameters	Necropsy
G1	2000 L_3		
G2	4000 L_3		
G3	6000 L_3		
G4	control		

3.5. Parasitological study

3.5.1. Nematode Egg excretions

Faecal samples were collected weekly from the rectum of individual animals from all groups starting from D0 (*H. contortus* infection) until the end of the experiment (D91) to measure nematode egg excretion pattern by nematodes. The collected faeces were placed in airtight plastic sterile bottles and stored at 4°C. Faecal egg counts (FECs) were performed using a modified McMaster technique with a sensitivity of 50 eggs per gram of faeces after counting eggs on both chambers using 33% saturated zinc sulphate solution as a floatation medium. (MAFF, 1979).

3.5.2. Worm identification and counts

At necropsy, abomasums were removed and processed for parasite counts to determine worm burden. The abomasums were legated at both ends, separated and opened along the greater curvature, the contents were removed by successive washings with tap water and collected in a plastic bucket and passed through a 150µm sieve to eliminate coarse materials. The contents, washings and digested materials, were preserved in 10% buffered formalin. These

materials were put into separate containers and then adjusted to 2 litres volumes. The population of worms in the abomasums of each animal was determined from a 10% aliquot using the classical counting procedure indicated by MAFF (1979). Each suspension was stirred vigorously and a sample of about 200 ml obtained by plugging a 50 ml beaker into the suspension. An alcoholic solution of iodine (5%) was added to colour the material in the samples. Before collecting the worms, sodium thiosulphate solution was added to the samples until dark colour disappeared. This procedure gave the worms a brown colour, which enabled easy collection and counting. Worms were differentiated according to sex and counted using a stereomicroscope. The number of adult worms

3.6. Haematological examination

Blood samples were taken weekly from jugular vein of each animal in to plains test tubes and containing ethylene diamine tetra acetic acid (EDTA) to obtain uncoagulated blood for haematological studies and serum for biochemical analysis from the start to the end of the experiment (D91). Sera were stored at –20 °C until analysis. Blood samples in EDTA tubes were gently mixed using an automated blood mixer (KJMR-IV) and were used for the determination of total red blood cell (RBC) number, white blood cell (WBC) number, packed cell volumes (PCV), haemoglobin (Hgb) concentration and differential leukocyte counts.
The concentrations of RBC, WBC, Hgb and PCV were determined using an Automated Hematology Analyzer (Poch-iV Diff, Kobe, Japan). Differential leukocyte counts were made by the Battlement method on freshly prepared thin blood smears stained with Wright's stain. Microscopic examination of the prepared smears was conducted under oil immersion magnification (X100) and at least 200 white blood cells were counted for differential determination. Values were expressed in percentage. For the purpose of this study, the proportional percentage of lymphocytes, neutrophils and eosinophil counts were only considered.

For determination of total plasma protein concentration the Biuret method was employed, using a photometer, which is the simplest chemical quantitative analytical method (Dacie and Lewis, 1991).

3.7. Clinical observations and body weight record

All lambs were clinically examined daily for any clinical appearance of the disease and also Weighed weekly for the whole duration of the experiment using a dairy scale spring balances (Hansen, Model 603, U.S.A.). Any observed clinical signs and body weight of each animal was recorded starting from one week before the start of experimental infection.

3.8. Data analysis

Raw data obtained were recorded and entered into Microsoft Excel database system. Using SPSS15.0 for windows program, data was summarized and analyzed. The data analysis were the kinetics of egg excretion, blood eosinophil, neutrophil, lymphocyte counts, haemoglobin concentration, packed cell volumes, serum total protein level worm burden and body condition score were compared between the four groups of sheep using analysis of variance (ANOVA) with repeated values (using SPSS software programme). The different variables were compeered by ANOVA.

4. RESULTS

4.1. Clinical observation

All infected animals showed signs of varying degrees of anaemia which were observed pale conjunctiva and a loss in body weight gain. One sheep died in group 1 on day 56 post infection, death was attributable to *Heamonchosis* and its associated clinical sign such as anaemia, weight loss, bottle jaw and emaciations.

4.2. Parasitological data

4.2.1. Egg excretion of *Haemonchus contortus*

No egg excretion was observed in group G4 (control) throughout the experimental period. In groups infected by haemonchus (G_1, G_2 and G_3), the egg excretion patterns were similar starting from day 21 post infection increasing regularly up to day 91. The total mean FEC in these three groups were 3098.81 ± 1063.13, 3824.29 ± 408.23 and 5579.76 ± 892.63 respectively. Meanwhile the differences between each groups in EPG level was significant ($P<0.001$). The egg excretion in group G_3 was significantly higher ($p<0.05$) compared to group G_2 and G_3. FEC were negatively correlated with body weight, Hb concentration, PCV and total serum protein ($r= -0.28$, $P<0.01$, $r= -, 0.42$ $P<0.01$, $r= -, 0.43$ $P<0.01$, $r= -, 0.12$ $P<0.05$) and positively correlated with worm burden and blood eosinophil ($r= 0.27$ $P<0.01$, $r= 0.36$ $P<0.01$,). [Fig.1]. indicates the mean EPG values among different experimental groups inoculated with different dose of *H. contortus* L3 infective larvae.

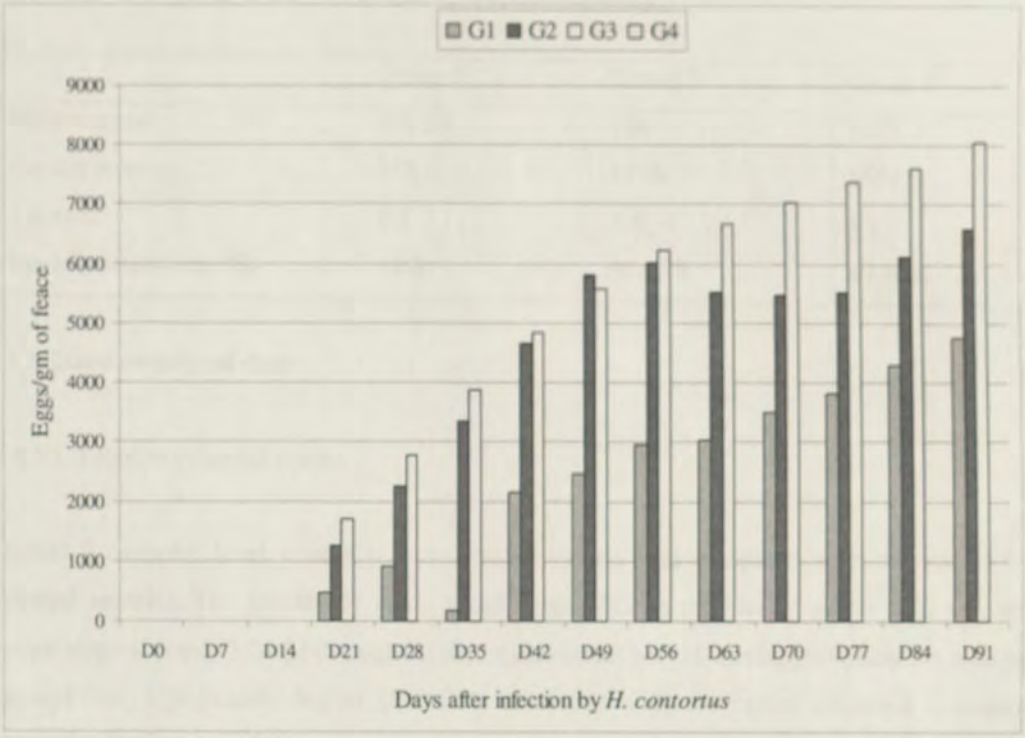

Fig. 1. Nematode egg excretion from *H. contortus* infected sheep

4.2.2. Total worm burden.

The mean number of *H. contortus* recovered at necropsy from abomasums of sheep in groups G_1, G_2 and G_3 was 951±37.006, 1962 ±360.43 and 2950±232.02 respectively. Meanwhile, the differences between each group was significant ($P<0.001$). Percentage of establishment of infection for G_1, G_2 and G_3 was 48%, G2 49.05% and G3, 49.16% respectively. Meanwhile, worm burden was positively correlated with level of blood eosinophilia, FEC ($r=0.66$, $P<0.01$, $r= 0.27$ $P<0.01$) and nematode establishment rate but negatively correlated with body weight and PCV, Total protein ($r= -0.16$, $P<0.05$, $r= -, 0.49$ $P<0.05$, $r= -0.81$, $P<0.01$). The mean male to female *H. contortus* ratios for group G_1, G_2 and G_3 were 1.3, 1.4 and 1.1 respectively, with no significant differences between the groups ($P>0.0$).

Table. 2. Total worm burden in *H. contortus* infected sheep

	Group 1	Group 2	Group 3
Male worms	396.66	786	1383.3
Female worms	555	1176	1566.6
Sex ratio	1.3	1.4	1.1
Establishment rate (%)	48%	49.05%	49.16%

4.3. Haematological data

4.3.1. Blood eosinophil counts

Blood Eosinophil level counts was monitored weekly and compared with the values for control animals. The number of mean blood eosinophils in the control group (G_4) was at a physiological level (3.24%) throughout the experimental period. Eosinophil counts in infected groups was significantly higher ($P<0.001$) compared with the values observed in control animals. Peak eosiniphilic values were recorded on D56 for all infected groups (Fig.2). Meanwhile, the dynamics of eosinophil counts showed similar pattern in all infected with *Haemonchus* groups. The level of blood eosinophils observed in group G3 was significantly higher ($P<0.05$). Compared to groups G_1 and G_2. Towards the end of the experiment, a gradual fall in the level of eosinophilia was seen in all three infected groups. Eosinophilia was also positively correlated with FEC and worm burden, ($r= 0.36$ $P<0.01$, ($r=0.66$, $P<0.01$).

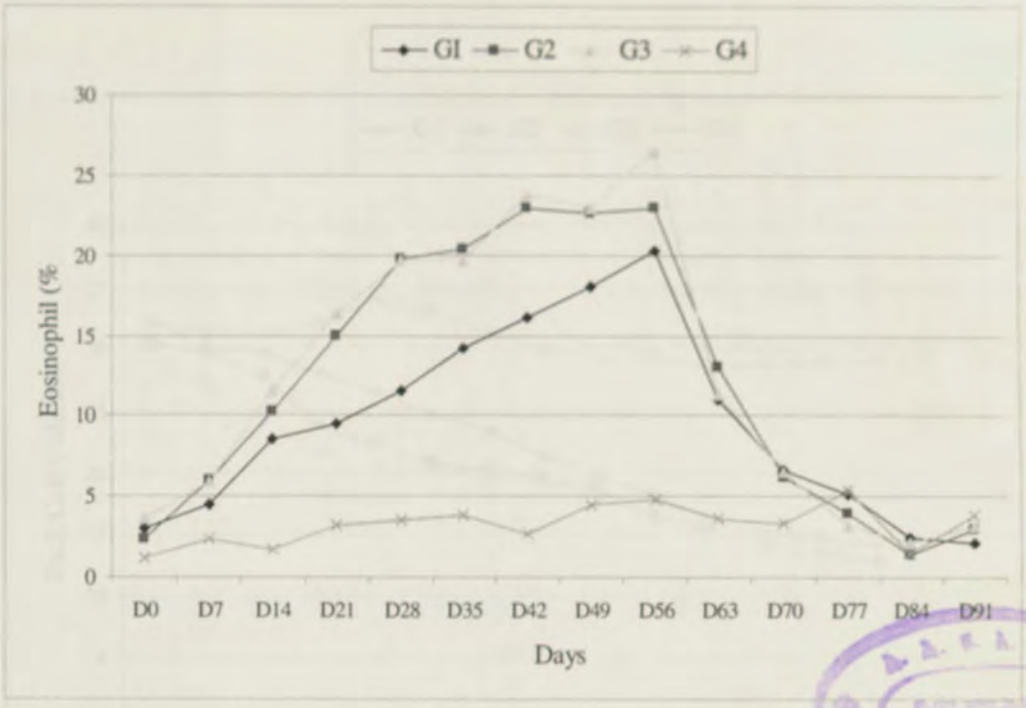

Fig. 2. Evolution of mean blood eosiniphils in the four experimental groups

4.3.2. Packed cell volumes (PCV)

The PCV values of control animals (G_4) were at physiological level (29±.527) throughout the experimental period. For the three nematodes infected groups G_1, G_2 and G_3 the mean values were 22, 25 ±.701, 20.25 ±.737 and 19.21 ±.608 respectively. In all groups of animals infected with *H. contortus* significant reduction ($P<0.001$) in PCV values was observed compared with uninfected animals. In all infected groups G_1, G_2 and G_3 the PCV values were decreased to 11.5%, 14% and 12.7% respectively. Meanwhile, all sheep from group 3 were severely anaemic at the termination of the experiment. PCV was also negatively correlated with FEC, worm burden and blood eosinophilia ($r=-0.43$, $P<0.01$, $r=-0.49$, $P<0.01$, $r=-0.27$, $P<0.01$) respectively.

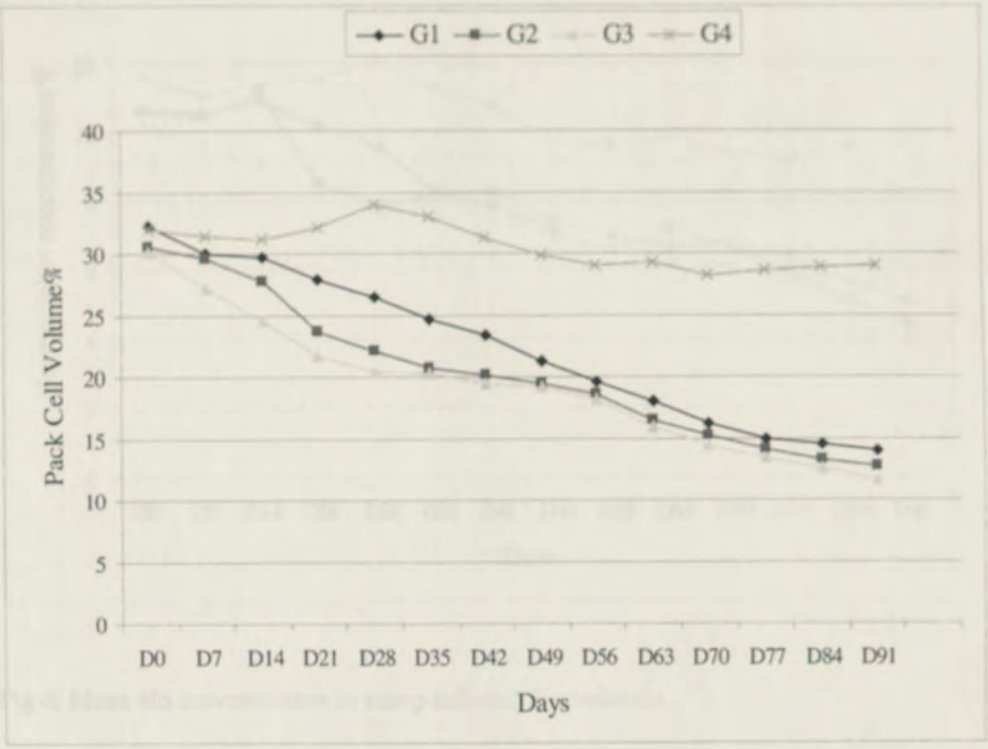

Fig. 3. Patterns of mean packed cell volumes (PCV) in the four experimental groups

4.3.3. Haemoglobin concentration

The Hb concentration pattern of all groups is shown in Fig.4. All animals infected with *H. contortus* (L_3) showed significant reduction ($P<0.001$) in Hb values when compared with uninfected controls.

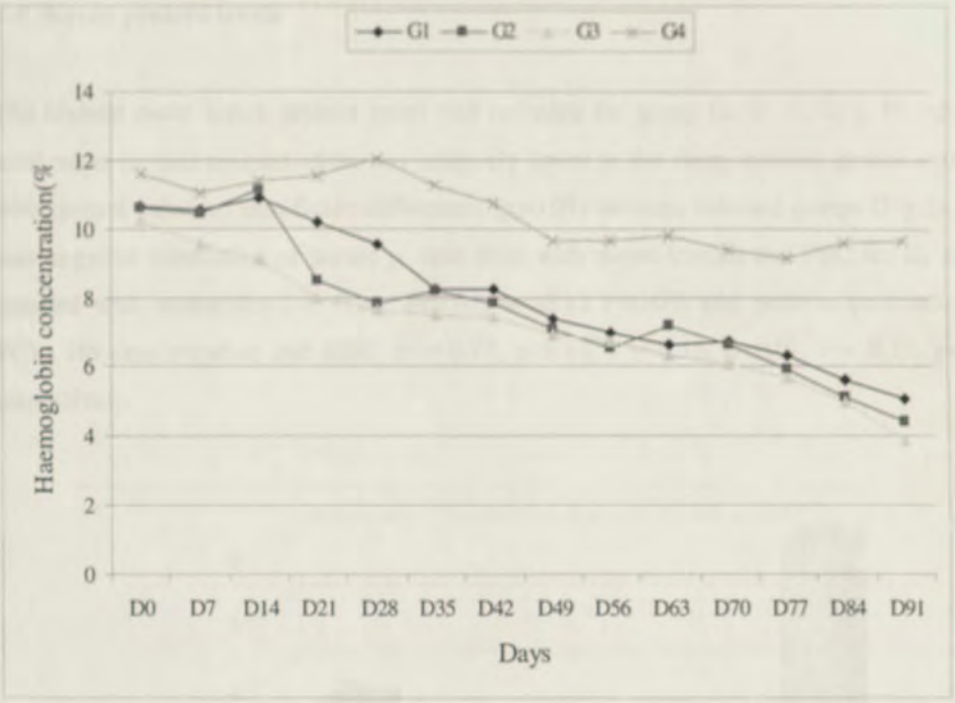

Fig.4. Mean Hb concentration in sheep infected *H. contortus*

4.3.4. Red blood cell and White blood cell counts

The total red blood cells were in normal physiological level in control animals throughout the experimental period. Meanwhile, the mean RBC count for groups G_1, G_2 and G_3 was 5.5, 4.35 and 3.18 respectively showing significant reduction throughout the trial period. There was no significant differences observed ($p>0.001$) between groups pertaining to the mean RBC level. For groups G_1, G_2 and G_3 values for WBC count were also lower 3.1, 2.94 and 3.2 respectively with no significant differences ($p<0.001$) between groups infected by nematode. Differential WBC counts of lymphocytes, showed a significant difference ($p<0.001$) between *Haemonchus* infected groups with higher level observed in group three (G_3). The mean RBC count was negatively correlated with EPG, total worm burden, blood eosinophilia and the total protein (r=-0. 46, P<0.01, r=-0.49, P<0.01 r=-0. 31, P<0.01, r=-0.19, P<0.01) and positively correlated with Hb concentration and PCV(r=0. 89, P<0.01, r=0.87, P<0.01) respectively.

4.4. Serum protein levels

The highest mean serum protein value was recorded for group G_4 (6 ±1.725). However the total mean protein concentration was relatively lower in the three infected groups compared with control value no significant differences ($p>0.05$) between infected groups (Fig.5). There was negative correlation of serum protein level with worm burden and FEC for all animals infected with nematodes ($r= -0.81$, $P<0.01$, $r= -0.12$ $P<0.05$) and positive correlation with PCV, Hb concentration and RBC ($r = 0.17$, $p<0.01$, $r = 0.16$, $p<0.01$, $r = 0.19$, $p<0.01$,) respectively.

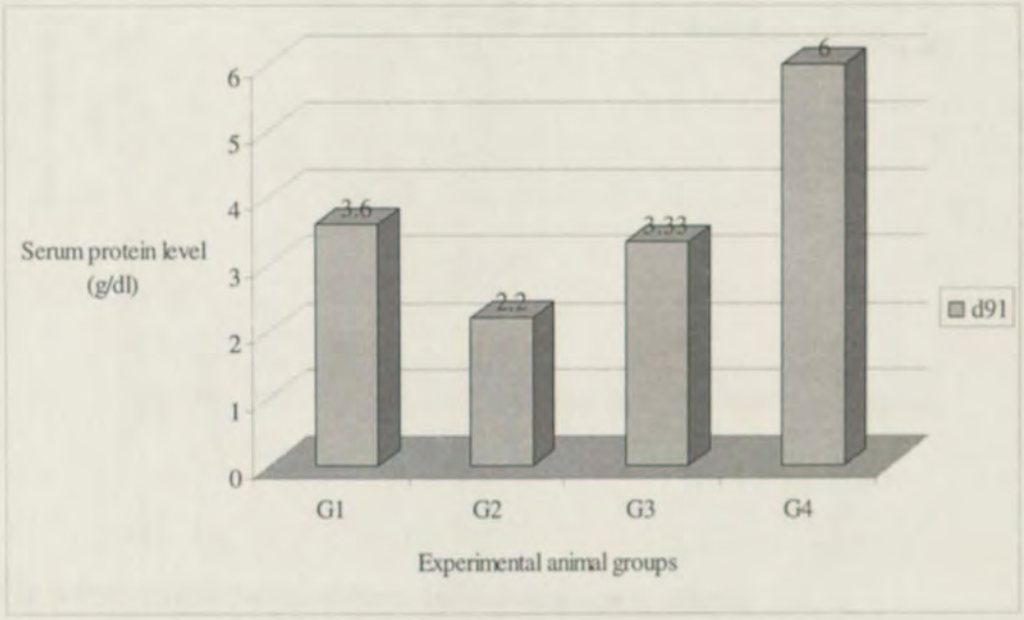

Figure. 5. The mean serum protein levels in the four experimental groups

4.5. Clinical responses and body weight changes

All infected with nematode animals showed signs of depression, pale mucous membrane and loss of body condition. Control animals have shown a progressive gain in body weight throughout the trial period. Animals in the three nematode infected groups G_1, G_2 and G_3 showed a significant reduction in body weight at the rate of 3.77 kg 3.41kg and 6.44 kg respectively. The difference of reduction in live weight between groups was significant

($P<0.001$). [Fig. 5]. Meanwhile, the body weight was negatively correlated with FEC and worm burden ($r = -0.28$, $p<0.01$, $r = -0.16$, $p<0.05$) and positively correlated with PCV, Hb concentration and RBC ($r = 0.43$, $p<0.01$, $r = 0.46$, $p<0.01$, $r = 0.33$, $p<0.01$).

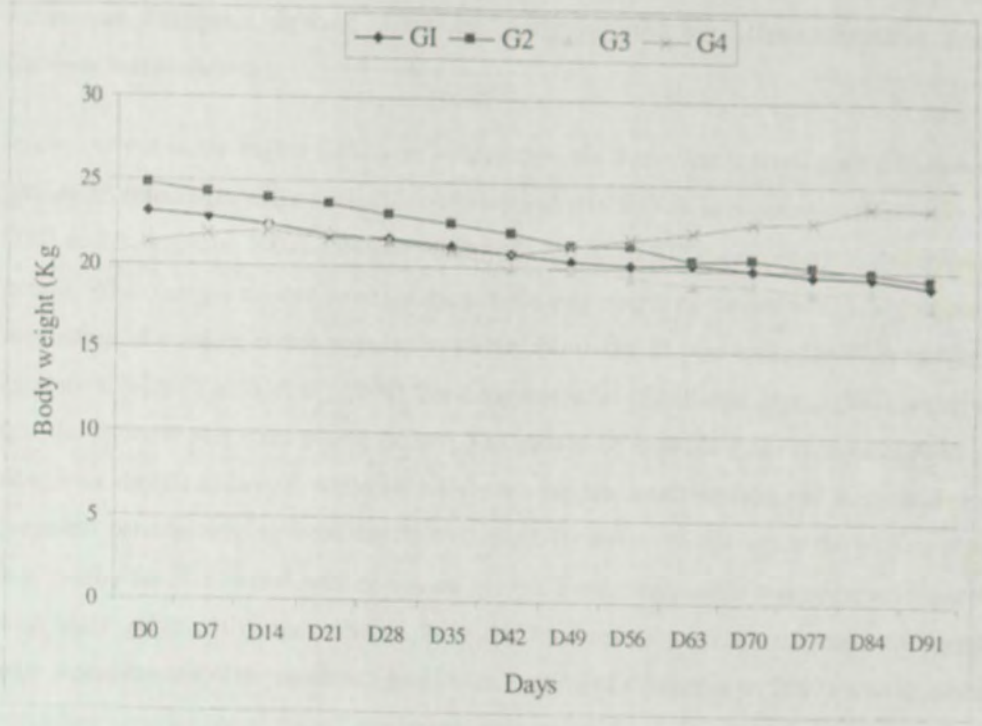

Fig. 6. Body weight changes of four experimental groups of animals

5. DISCUSSION

This study was conducted with the aim of determining changes in the hematological, serum biochemical and parasitological parameters as well as to observe clinical responses in indigenous Ethiopian highland sheep artificially infected by different infective doses of *Haemonchus contourtus.*

In the current study higher EPG was observed in the first fourth week post infection in all groups of animals infected with *H. contortus* and this was in agreement with (Yacob *et al.*, 2002 and Basazinew, 2007). Gradual increase in the mean EPG was observed throughout trial period. Which might me due to an increase in the egg output of female worms associated with maturation of existing worm population started from day 21 post infection This finding is in agreement with (Yacob et al., 2004) but disagrees with (Elbiharils *et al.*, 1984*)* which states that the shortest prepatent period occurred in groups of animals that received high infective dose from day 22 to day 28, while animals receiving low infective doses of larvae had average prepatent periods ranging from day 35 to day 60. However, in this study the prepatent period was similar for all infected with nematode groups. Faecal egg count was negatively correlated with body weight, Hb concentration, PCV and total serum protein and positively correlated with worm burden. This result is agreed with finding of (Basazinew, 2007) who reported the moderate association of feacal egg count with worm burden and also (Teklay and Kasali, 1989) finding reported that low PCV and high EPG levels in nematode infected animals in significant association with poor body-condition scores and low bodyweights.

Resistance of sheep to nematodes has been associated with lower faecal egg count which may in turn be related to lower larval establishment rate, worm burden development, lower female length and fecundity (Douch *et al.*, 1996, Stear., *et al,* 1995, Onah and Nawa, 2000). Significant difference in FEC was reported between the relatively *H. contortus* resistant Florida Nativa and the susceptible Rambouillet breeds of sheep (Amarante *et al.*, 1999) in Corriedale and Crioula Lanada breeds (Bricarello *et al,* 2004). This shows that lower FEC can be a potential indicator for the development of some degree of resistance against the parasites but in this study the total mean FEC was generally moderate in all groups but the difference among the three different groups was statistically significant ($p<0.001$). This result is contrary to the result of (Teklay and Kasali, 1989) who stated that highly prolific worms like *H.*

contortus had low EPG counts, this might be associated with some factors where *H. contortus* was not established and/or was removed after infection due to self cure (Preston and Allonby, 1977; Kasali *et al,* 1988) and/or other environmental factors.

Our data provided evidence on parasite development indicating the presence of positive correlation between the total worm burden and establishment rates which is in agreement with (Amenu, 2005; *Basazinew*, 2007). In the contrary, delay in parasite development has been shown after repeated exposure of lambs to *H. contortus*, or *Trichostrongylus circumcincta* (Schalling *et al.*, 1995, Stear *et al.*, 1995, Dorchies *et al.*, 1997, Yacob *et al.*, 2004).

In all infected with *Haemonchus* animals, (G_1, G_2 and G3), female worms were more abundant in number than males contributing to 58.35%, 59.93 % and 53.68%, respectively and the corresponding figures for male warms were 41.6%, 40.06 % and 46.88 %, respectively. This finding disagrees with finding of (Basazinew, 2007) which reports the abundance of male worms compared with female ones.

(Allonby and Urquhart, 1975, Robertes and Swan, 1982) demonstrated that there is a strong relation sheep between faecal egg count and total adult worm counts in H. *contortus* infected animals and direct proportional decrease in Hb level with increased worm burden. In our study, a positive correlation of mean total nematode burden to mean total FEC count was observed in all groups. The correlation of mean EPG and worm burden recorded in this study agrees with the findings of (Tembley *et al.*, 1997, Roberts and Swan, 1982; Craig, 1998; Silvestre *et al.*, 2000; Waruiru *et al.*, 2005; Amenu, 2005 and Basazinew, 2007) who reported the presence of positive corelation between fecal egg count and the worm burden of nematodes such as *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Ostertagia columbianum*. This has also been demonstrated for different species of trichostrongyles from sheep and horses in which the number of eggs was directly proportional to the worm growth (reflected in the length of worms). In this study also seen higher establishment rate of infective larvae manifested by higher worm population together with higher nematode egg out put.

In the present study, the clinical signs of Haemonchosis, parasitological values and the haematological changes are generally similar to the result of (Bradley *et al.*, 1973). The evaluations of Haematocrit values in the present study are in agreement with those of (Dargie

and Allnby, 1975). Haemonchosis has been described as an acute syndrome characterized by severs anaemia associated with the rapid acquisition of large worm burdens (Solusbay, 1986). The haematological parameters reported in the infected animals indicate the development of anaemia, which was directly attributable to the blood sucking activity of developing larvae and adult nematodes. This could also indirectly a refraction of deficiency of the hosts' erythropoietic response to cope with the haemorrhage. The PCV in this study was negatively correlated with mean total EPG count and worm burden which is in agreement with the result of (Gauly and Erhardt; 2000, Basazinew, 2007; Burke and Miller, 2008). However the positive correlation with body weight measurement was consistent with the findings of (Teklaye and Kasali, 1989; Baker *et al.*, 2001; Nieuwoudt *et al.*, 2002).

There is strong positive correlation between PCV, Hb, RBC count, observed in our study allows inter changeable use of these parameters to determine anaemia in infected animals. As it was indicated by Coles (1986) who reported the presence of a significant association between lower RBC and higher worm burden during helminth infection indicate development of anaemia due to sever parasitism or malnutrition are reflected by smaller number of circulating RBC containing less haemoglobin.

The haemotocrit is an essential parameter, which may be used besides faecal egg counts to describe resistance against nematode parasites in sheep in situations where the dominant nematode species sucks blood (Amarante *et al.*, 2004). The present study indicated that progressive decline in RBC, PCV land Hb levels were more marked in group of sheep infected by the nematode mainly significant decreases observed in animals from group G3 than G1 and G2 groups this might be attributed to the difference in infective dose, the numbers of established worm, assuming that large number of parasites feed more blood than smaller number ones. In all infected sheep the mean PCV levels, mean RBC counts and mean Hb levels were lower than the control group throughout the whole observation periods ($P<0.001$). This result is in agreement with those of (Allonby and Urquhart, 1975, Dargie and Allnby 1975, Arzoun *et al.*, 1984, Barger and Cox, 1984).

The progressive depression of haematological parameters (PCV, RBC and Hb) during *H. contortus* infection has been described in different breeds of cattle, sheep, goats and camels and is the result of chronic haemorrhage with secondary iron deficiency (Dargie and Allonby, 1975). Anaemia is the main feature of this disease. All infected animals were found to be

anaemic at the end of the experiment and this was similar to the typical anaemia of ovine *Haemonchusis* as reported by (Roberts and Swan, 1982; Arzoun *et al.*, 1984). The result of the mean PCV, mean RBC count and mean Hb concentration of groups infected with different dose of *H. controtus* indicate that there was a dramatic fall of PCV, RBC and Hb, after the first three weeks post infection as a result of blood loss in agreement with the findings of (Roberts and Swan, 1982). Results of (Dargie *et al.*, 1979) indicated that as infection progresses from week 4 on words the animals continue to loss a maximum blood by mature adult parasites and the associated haemorrhage when the actively feeding parasites frequently change their feeding sites resulted leak in the abomasal mucosa. The light fall in PCV, RBC, and Hb levels observed during this time may be caused by the mobilization of the haemopoeitic system and the higher serum iron levels. The course of anaemia in groups infected with different dose with *H. contortus* was in agreement with that was described by (Dargie,*et a*l., 1979; Arzoun *et al.*, 1984) for anaemia induced by abomasal nematode parasite *H. contortus,* (Kaufmann *et al* .,1992 and Basazinew, 2007) reported similar finding where the trend of anaemia in *H. contortus* infection follows rapid fall in the first 3 weeks post infection followed by further drop in haematological parameters for a week, were observed until the end of this experiment

It is now known that resistance of sheep to *H. contortus* is to some extent genetically controlled, and is related to such factors as the breed and Hb types of the host. For example, experimentally infected Scottish black face sheep develop less sever anaemia and have lower egg counts and worm Burdens (Altaif and Dargie, 1978). The reason for the apparent advantage of one breed and Haemoglobin type over another is not yet clear, but it would appear that differences in the severity of anaemia are almost entirely attributable to the existence of innate ability to the resist the establishment of infection but in this study in all infected groups animals were anaemic, with high worm burden and higher egg counts observed showing high susceptibility to haemonchosis which might be attributed to the malnourishment, mineral deficiency and probably due to poor genetic potential.

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. (Dawkins *et al.*, 1989; Pfeffer *et al.*, 1996, Balic *et al.*, 2000, Yacob *et al.* 2004). In this study, animals infected with different dose of *H. contortus* larvae showed considerable degree of blood eosinophlia as compared to the non-infected animals indicating the presence of an

increased mobilization of circulating blood eosinophils against *H. contortus* larvae. This was in agreement with Arzoun *et al* (1984); Kaufman *et al* (1992); Yacob *et al* (2002, 2004); Basazenew (2007); Terefe *et al* (2005,). Moreover; the highest eosinophils count was observed in groups that were infected with 6000 infective larvae (G_3). In this study, eosinophil counts were the positive correlation of blood eosinophilia with worm population and EPG was in agreement with the result of (Dawkins *et al*.,1989, Balic *et al*., 2000, Basazenew, 2007). Eosinophis mobilized against specific parasites were frequently found to cause immobility and death of larvae of homologous or heterologous parasites often in association with antibodies and/or other factors (Emery *et al*., 1993; Kazura and Grove, 1978; Rainbird *et al*., 1998, Terefe *et al*., 2007). Even though our result didn't include any humoral and tissue cellular data, we assume that activated circulating eosinphils might have influenced the development of H. contortus in the abomasums. This may be through migration into the abomasum and acting directly on the worms in association with antibodies and other inflammatory cells or by releasing various toxic protein/factors (Wardlaw, 1996) into the blood circulation, which could be in contact with haematophagous stage of H. contortus

The experimentally infected sheep with different doses of *H. contortus* showed reduced serum protein concentrations compared to the control animal which was observed continuously to the end of the experiment. This agrees with the findings of (Arzoun *et al*., 1984, Abebayhu, 2006 and Basazinew, 2007) these changes might be attributed to the blood loss, impairment of appetite, digestion and absorption.

This study showed that haeminchosis had a significant effect on live body weight, body condition and health of infected animals at large. All infected with nematode lambs showed signs of depression, pale mucous membrane, oedema (bottle jaw), emaciation and loss of body condition. Progressive decline in mean body weight of all treatment groups and weight gain for control groups was observed through out the study period. Differences in body weight were found to be statistically significant ($p<0.001$) which is similar to the findings of (Barger and Cox, 1984, Kaufman *et al*., 1992, Urquhart *et al*., 1996, Haile-leul, 2002).

Our results illustrate the basic value of experimental infection model of with abomasal nematode to further study the effect of *Haemonchus contortus* on relatively less stressed and well managed animals enabling to compare some haematological and clinical parameters with uninfected and healthy animals.

6. CONCLUSIONS AND RECOMMENDATIONS

All artificially infected animals by the nematode suffered from haemonchosis which was manifested by typical clinical pictures such as anaemia, submandibular oedema, emaciation and gradual loss of condition. Infections by *Haemonchus contortus* have affected major haematological parameters, resulting in significant reduction of red blood cell and hemoglobin concentrations and PCV values as well as serum protein level. *H. contortus* infection was also found to reduce body weight gain. The observed pronounced excretion by nematodes and higher worm burden suggested the full establishment of the parasite. The difference in infective doses of *H. contortus* showed remarkable influence on values of major parameters obtained during the experimental study.

The current model of study has also applied implications, for instance to understand what are the effects of these nematodes on the health of the host enabling to gradually and closely follow up the clinical manifestation of haemonchosis dynamics of infection as to prevue specific treatment and control against the disease and also to establish data base bank in haematological parameters for indigenous sheep.

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

- These parasitological, haematological and serum biochemical changes illustrated during this experiment should be further compared with concordant data from field observations.
- This model of study should be rationalized and conducted in animals of different sex, age group but with known genetic and agro ecological origin.
- Researches in genetic selection of sheep for their resistance to nematodes should be encouraged in locally available breeds to control the problem of parasitism in the country.
- Since studies on such infection could have major consequences on the epidemiology and control of these parasitism further studies should be conducted after natural challenge of animals.
- It is important to create awareness and initiate professionals to conduct clinico-haematological research in various local breeds of animals.

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8. ANNEXS

Annex 1

Parasitological Examination

Recovery of nematodes from the abomasums

Procedure:

- Separate the abomasum from the intestine for wash
- Open the stomach in bowl and collect the contents
- Wash the stomach wall thoroughly under stream of water from the tap and rub the mucous membrane carefully with the fingers to remove any adhering to it.
- Pour the contents of the bowl a little at a time to a wire mesh screen with an aperture of 0.15mm and then wash with a stream of water from a rubber tube attached to a tap until no more coloured matter or feed particle pass through.
- Flush the content on the sieve with a jet of water from the tap.
- Fill the content of the bucket to 2 litters.
- Agitate the whole content vigorously and take an aliquot of 200 ml by using a beaker and place in a glass Petri dish and examine under a stereomicroscope.
- Adding few drops of iodine solution and allowing standing for 35 minutes can facilitate examination. Adding sodium thiosulphate leaving the parasites stained can also make decolorization of the stained debris.
- Count the number of each species and multiply by a factor to arrive at the total parasite burden. (MAFF, 1977, Hansen and Perry, 1994).

Annex 2

McMaster egg counting technique

Procedure:

- Weigh 3 gram of faeces
- Break up thoroughly in 42ml of water in a plastic container

- Pour through a fine mesh sieve (aperture 250microns)
- Collect the filtrate, agitate and fill a test tube, preferably 15ml and flat bottomed
- Centrifuge at 2000 rpm for 2 minutes
- Pour off supernatant, agitate sediment and fill the test tube to the previous level With flotation solution
- Invert the test tube 6 times and remove fluid with pipette to fill both chambers of McMaster slide
- Let the slid stand for 3 minutes and then start counting all the eggs under the ruled square.
- Examine one chamber and multiply number of eggs under one eched area by 100 or two chambers and multiply by 50 to arrive at the number of eggs per gram of Faeces (epg). (MAFF, 1977, Hansen and Perry, 1994).

Annex 3

Larvae Culture

Procedure

- The collected female worms were Tinley sliced by scalpel blade in 0.8% NaCl solution to release their eggs. The egg suspension was spread over finally broken up horse faeces that were sterilized for two hours at 140 °c.
- Moist and crumbly consistency was obtained by adding dry faeces or water to ensure sufficient moisture for the development of the infective larvae (MAFF, 1977, Hansen and Perry, 1994).
- Then the mixture were transfer to Petri dish and placed in an incubator running at 27 °c and left there for 7-10 days to get 3rd infective stage.
- The cultured Petri dish was stirred each day to avoid the growth of fungi and errat the lower layers of the culture and water was added to culture every 1-2 days. Sufficient number of infective larvae was obtained freshly collected female worms (Hansen and Perry, 1994; Bowman, 1995).
- To harvest infective larvae the cultures were removed from the incubator and the faeces was tipped out from petridish in to a 300 Ml wide mother jars. Then

lid of the culture Petri dish and it is cover was washed with small quantity of water so as to remove any migrated larvae. Then water was added to the culture until the jar was full to the brim.

- A standard Petri dish was inverted over the mouth of the jar and this was tureen in the Petri dish and allowed to stand over night. The next day the fluid in the Petri dish containing many L3 will be pipetted in to a conical centrifuge and concentrate by centrifugation
- The larvae was passé once in the sheep before transit in this isolate of *H. contortus* was served as initial infective larvae source and maintained in apparently nematode free sheep for production of subsequent source of infective larvae for experimental infection the L3 used for experimental infection will be obtained through faecal culture at 27 °c for 7-/10 days baerminization of faeces as above purification by sedimentation and recover of the clean larvae. The larvae harvested were stored in tap water at plus 4 °c until use.

Annex 4

Preparation and counting of larval inoculation

Freshly harvested larvae were used for experimental infection as recommended by Wood, *et al* 1995. The larves suspension was removed from refrigerator and it is volume was measured, recorded and then transferred to a tightly stoppered and then transferred to a tightly stoppered flask and was vigorously shaken for several minutes to set a homogenous suspension.

- Then 100ml of the larval suspension was immediately with drawn with a micro pipette and transferred to a microscope slide the drop was then spread out and covered with a 38x22mm cover slip. The slide was methodically searched under a microscope and the larvae counted. This shaking, sampling and counting was repeated five five times and the final count was based on the mean count of these five samples.
- Then the number of viable L3 per millilitre was established. Then the desired number of L3, which was 72000 in the present study, in an inoculums volume

of 15 ml per animal was dispended in to syringes that were labelled with the ear tag number of the recipient animals.

- Then the inoculation was given orally and the syringe was rinsed with 20 ml water and the washing administered. (Wood., *et al* 1995, MAFF, 1979).

Annex 5

Haematological examination

Sample collection

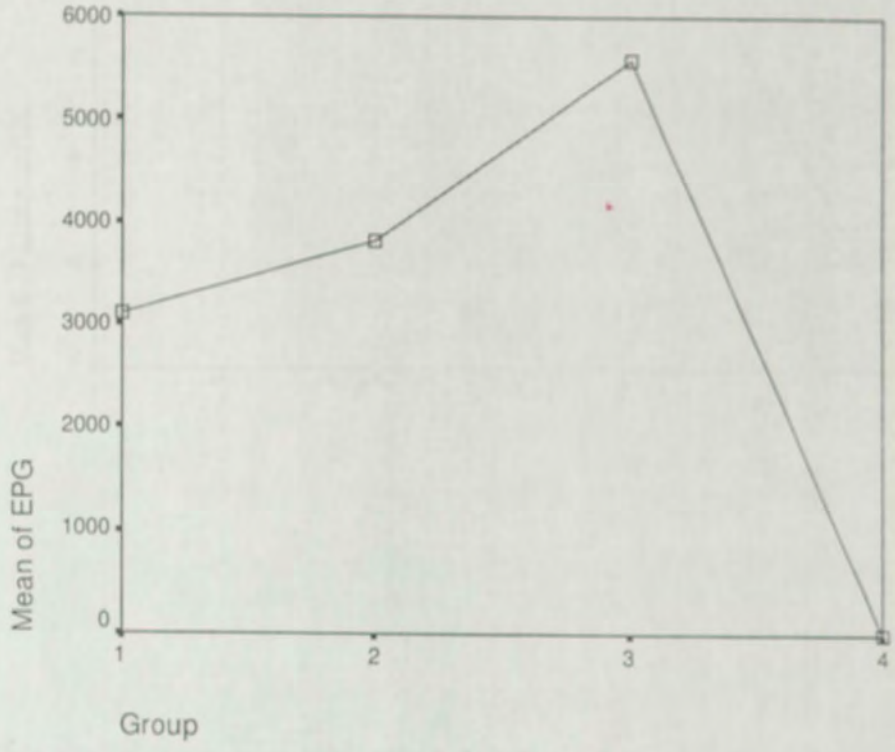
A needle was introduced first through the Skin and then in to the distended jugular vein and the blood was drawn to a deserved level. At lest 4 0r 5 Ml of the blood in a dry and clean vacutainer tube to which Ethylene Diamin Tetra Acetic Acid (EDTA) was formerly added. The Vial was tightly coppered and immediately tipped back and forth a dozen time to dissolve the anticoagulants and mixing of blood was done gently to avoid rough handling which may lead to the rupture of erythrocytes in the haematological investigation, a definite procedure was followed, and all tests were completed with a reasonable time as stated by Coles (1986), and Jain (1993).

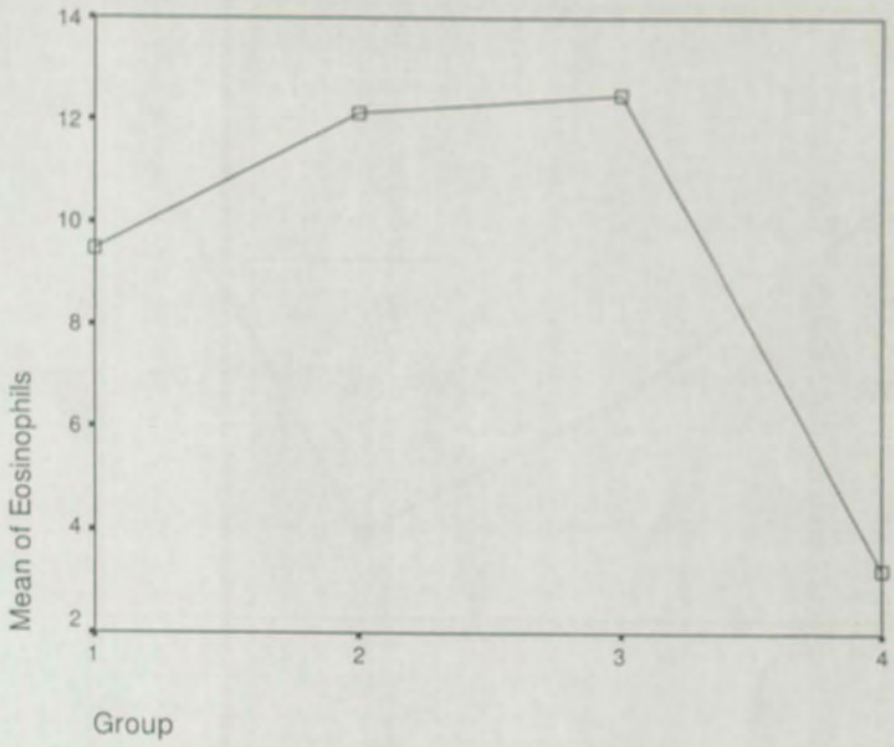
Laboratory procedure

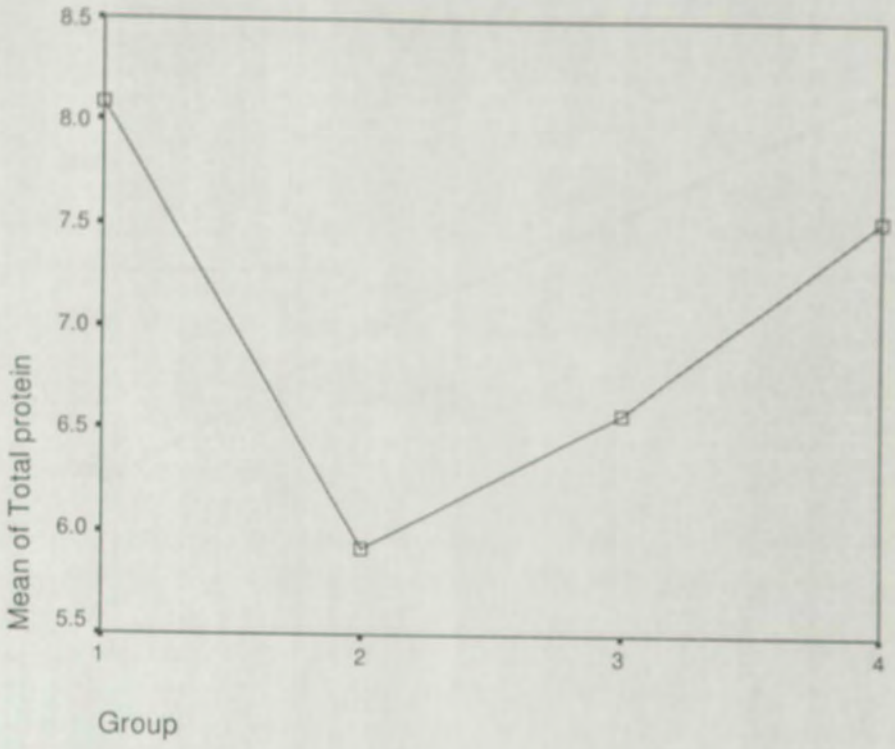
Blood samples in EDTA tubes were gently mixed using an automated blood mixer were used for the determination of total red blood cell (RBC) number, white blood cell (WBC) number, packed cell volumes (PCV), haemoglobin (Hgb) concentration and differential leukocyte counts. The concentrations of RBC, WBC, Hgb and PCV were determined using an Automated Hematology Analyzer (Poch-iV Diff, Kobe, Japan). Differential leukocyte counts were made by the Battlement method on freshly prepared thin blood smears stained with Wright's stain solution. Microscopical examination was conducted under oil immersion magnification (X100) and at least 200 white blood cells were counted for differential determination. Values were expressed in percentage. For the purpose of this study, the proportional percentage of lymphocytes, neutrophils and eosinophil counts were only considered.

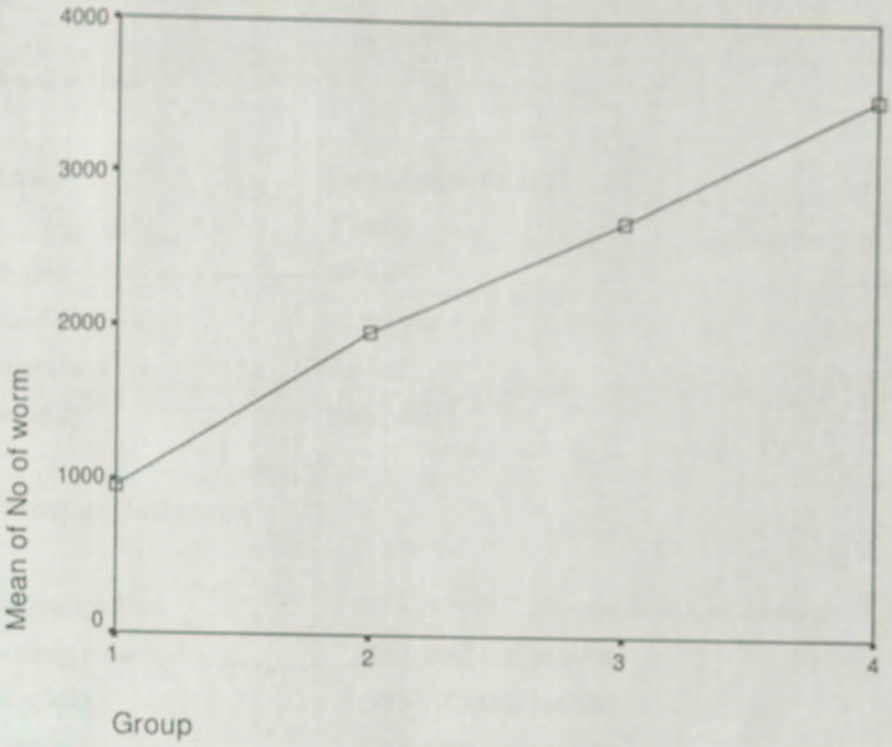
Serum Samples obtained using plain vacationers will be subjected to standard biochemical procedures to determine serum protein.

Annex 6 Plots of Mean EPG, PCV, HB, RBC, Eosinophile, body weight,Total protein and worm count




14
12
10
8
6
4
2
Mean of Eosinophils
1
2
3
4
Group


8.5
8.0
7.5
7.0
6.5
6.0
5.5
Mean of Total protein
1
2
3
4
Group


4000
3000
2000
1000
0
Mean of No of worm
1
2
3
4
Group

9. CURRICULEM VITEA

1. Personal Data

Full name	Fantu Ashine Kelkele
Sex	Female
Birth Day	1968 GC
Place of Birth	Addis Ababa
Martial status	Married
Nationality	Ethiopian

2. Educational background

Elementary school	1974-1979 G.C Sheromeda School Addia
Secondary school:	1980-1982 G.C in cuba
High school	1983-1985 G.C in Cuba
University	1985-1990 G.C in Cuba
Post graduate studies	2006-2008 G.C in Addis Ababa University
3. Work Experience	1990-2006 G.C in DireDawa Veterinary Clinic
	From1994-2006 in Dire Dawa Zonal Laboratory

4. Language ability

Spanish	Spoken and written
English	Spoken and written
Amharic	Spoken and written

5. Paper and publications

10. SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work, has not been presented for a degree in any other university

Name: Fantu Ashine

Signature ----------

Date of submission 25 June 2008

This has been submitted for the examination with my approval as the university adviser

Dr: Yacob Hailu ----------

Dr: Gezahegn Mamo----------

Fan.
2008

Study on basic heamato-
logical, Serum Biochemical
and Parasitological - - -

Fantu Ashine.

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