Experimental infection of Ethiopian highland sheep by different infective doses of *Haemonchus contortus* (L_3): haematological and parasitological parameters, serum protein concentrations and clinical responses

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Abstract

The study was conducted to examine changes in haematological and their eventual associations with parasitological parameters and clinical responses in experimentally infected Ethiopian highland sheep by increased doses of Haemonchus contortus infective larvae (L3). A total of 24 male sheep were allotted in to four equal groups: a single dose of 2 000, 4 000, or 6 000 infective larvae of Haemonchus contortus (L3) was orally administered to animals from the first 3 groups whereas the last group not treated and served as negative control. The faecal egg count (EPG), the body weight as well as haematological parameters (haematocrit and eosinophils counts) and proteinaemia were measured throughout the experimental 13 weeks long period and worm burden and sex ratio were assessed at necropsy. The infection was confirmed by the occurrence of the faecal egg excretion and by the presence of adult worms in abomasums. All Haemonchus infected sheep exhibited a progressive and severe anaemia characterized by marked reductions of haemoglobin concentration and haematocrit, associated with weight loss and growth retardation and on the contrary with strongly and transiently increased eosinophil population compared to control animals ($P<0.01$). Except for proteinemia, parasitological, clinical and haematological alterations were significantly proportional to the parasite load. Moreover, the eosinophil counts, the adult worm population and the egg excretion positively and significantly correlated together, whereas they were negatively associated with body weight, proteinemia and the other haematological parameters. All experimentally infected animals appeared to be highly susceptible to the infection by Haemonchus contortus and the severity of the haemonchosis was clearly related to the infective dose.

Keywords: *Haemonchus contortus,* sheep, anaemia, eosinophil, proteinemia, Ethiopia.

http://dx.doi.org/10.4314/evj.v16i1.4

Introduction

In Ethiopia, helminthosis mostly in the form of gastro-enteritis is considered as the major cause of reduced productivity in sheep and goats (Tembely *et al.,*.; 1997). Many studies carried out in different parts of the country have shown that *H. contortus* is one of the most prevalent and economically important parasites of small ruminants (Deasalegn Lidetu, 1999; Abebe Wossene and Esayas Gelaye, 2001; Bersissa Kumssa, 2004; Amenu Asha, 2005; Dereje Shiferaw, 2008).

Infection with the blood-feeding nematode, *Haemonchus contortus* is the major constraint in 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 (Miller *et al.,*., 1998; Tembely *et al.,*., 1997, Waller *et al.,*., 2004). Host responses to infection by nematode parasites vary depending on intrinsic factors such as host breed, sex, age as well as nematode species involved. 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 (Balic *et al.,*., 2000).

Among important clinical responses, 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 and may serve as an index to characterize health status of animals (Albers *et al.,*., 1990). There are quantifiable variations in blood parameters particularly in haemoglobin concentration, RBC counts and PCV values between exotic and indigenous animals but these physiological values were not recorded for different species and breeds of indigenous animals in Ethiopia. Information is also lacking about the effects of GI nematodes infection on these physiological parameters (Bekele Tafesse, 1987; Fufa Abunna, 1999, Yasmin Jibril, 2000). Therefore, the major objectives of the present study were i) to evaluate the responses of Ethiopian highland sheep in terms of changes in haematological, serum protein and parasitological parameters after experimental infection with local isolates of *Haemonchus contortus* ii) to assess the effect of different infective doses of *H. contortus* on the above parameters.

Materials and Methods

Study period and study area

The study was conducted between December 2009 to March 2010 at the Faculty of Veterinary Medicine (FVM), Debre Zeit. The area is located in Oromia National Regional State, about 47 km Southeast of Addis Ababa, at an altitude of 1850 meters above sea level. It has a mean annual rainfall of 350 mm and a mean annual temperature of 170C (CACC, 2003).

Experimental animals

The study was conducted on twenty four male indigenous Ethiopian highland sheep, aged ten to twelve months, weighing 16-20 kg, and reared under traditional management system. Faecal samples from all animals were examined for the presence of any parasite eggs using standard faecal analysis by floatation, sedimentation and faecal culture techniques (MAFF, 1986) Three weeks before the commencement of the experiment, all the animals were treated with Ivermectin (FARQUIMICA, Cerrilos-Santiago-Chile) at 200 µg /kg, Praziquantel (APF, Ethiopia) 15mg/kg and Triclabendazol (Fasinex 250, EAP, Ethiopia) 250mg/25kg body weight to clear any parasite that may present. After ten days, the faecal samples of all animals were examined for the presence of any parasitic eggs, using faecal analysis by flotation, sedimentation and faecal culture techniques (MAFF, 1986) and showed absence of any parasite egg. The animals were weighed, ear tagged and randomly allocated into four groups of six animals each namely, groups G1, G2, and G3 (Haemonchus received) and G4 (Uninfected control) and kept in four separate compartments. Hay with concentrate feed was provided according to schedule and water was provided ad libitum.

Parasites and Experimental infection

H. contortus adult female were collected from naturally infected sheep slaughtered at Elfora export abattoir, Debre Zeit. The infective larvae of H. contortus (L3) were obtained from egg culture by the method of Hansen and Perry (1994). On D0, aanimals from the first group (G1) received a single infective dose of 2000 L3, while animals from the second (G2) and third groups (G3) received 4000L3 and 6000L3 respectively. The animals of group four (G4) were kept free of any infection as non infected control. The artificial infections were performed according to the procedure described in Yacob Hailu et al., (2002).

Parasitological study

Faecal egg output

To assess the H. contortus infection faecal samples were collected for egg counting from the rectum of individual animal from groups G1 (n=6), G2 (n=6), G3 (n=6) and G4 (n=6) twice a week starting from D0 (H. contortus infection) until the end of the experiment (D91). Faecal egg counts (FEC) were performed using a modified McMaster technique described by MAFF (1986).

Worm identification and counts

All sheep were euthanized and subjected to detailed examination on day 91. The abomasums of each animal was collected for parasite retrieval, count, identification of sexes as indicated in Yacob Hailu *et al.,* (2002).

Haematological examination

Blood samples from all animals were collected in the vials containing ethylenediaminetetraacidic acid (EDTA), as anticoagulant, weekly from the day 0 to the end of the experiment (day 91) to determine relative number of eosinophils by *Battlement method* (Kelly, 1974) and packed cell volume according to Uilenberg (1998). The relative percentage of White blood cells (Differential leukocyte count) was determined from stained blood smears using Battlement method (Dacie, 1991). The total serum protein concentration was determined by Biuret method (Dacie, 1991).

Data Analysis

The kinetics of egg excretion, % blood eosinophil, 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). The Pearson correlation test was used for assessing the correlation between parameters.

Results

Parasitological data

No egg excretion was observed in group four (G_4, control) throughout the experimental period. In groups infected with haemonchus (G_1, G_2 and G_3), the egg excretion pattern was similar starting from day 21 post infection

increasing regularly up to day 91 (Figure 1). Meanwhile the EPG level between the three infected groups showed significant difference with egg excretion in group G_3 significantly higher ($P<0.05$) compared to group G_2 and G_1. FECs were negatively correlated with PCV value and total serum protein ($r= -0.43$, $P<0.01$ and $r= -0.12$, $P<0.05$ respectively) and positively correlated with worm burden and blood eosinophilia ($r= 0.27$, $P<0.01$ and $r= 0.36$, $P<0.01$ respectively).

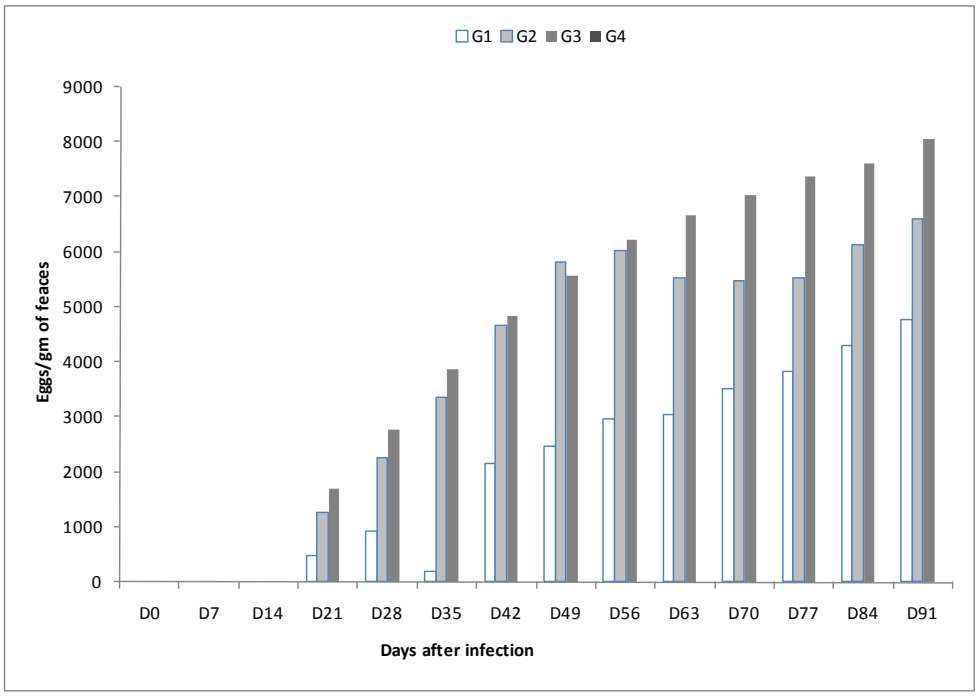

Figure 1: Nematode egg excretion from sheep infected with different doses of H. contortus L3

Total worm burden

The mean number of *H. contortus* recovered from sheep in groups G_1, G_2 and G_3 was 951±37.006, 1962 ±360.43 and 2950±232.02 respectively with significant differences between each group ($P<0.001$). The worm establishment rate for G_1, G_2 and G_3 was 48%, G2 49.05% and G3, 49.16%, respectively (Table 1). Meanwhile, worm burden was positively correlated with level of eosinophilia ($r=0.66$, $P<0.01$) but negatively correlated with body weight, PCV and total protein concentration ($r= -0.16$, $P<0.05$, $r= -0.49$ $P<0.05$ and $r= -0.81$, $P<0.01$ respectively). The mean male to female ratios for group G_1, G_2 and G_3 was 1.3,

1.4 and 1.1 respectively, with no significant differences between the groups (P>0.05).

Table1: Total worm burden, sex ratio and worm establishment of *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	1.4	1.5	1.1
Establishment rate (%)	48	49.05	49.16

Haematological data

Blood eosinophil counts

The mean percentage of eosinophils in the control group (G_4) maintained at physiological level throughout the trial 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 (Figure 2). Meanwhile, the dynamics of eosinophil counts showed similar pattern in all groups infected with Haemonchus with significantly higher values in G3 (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.

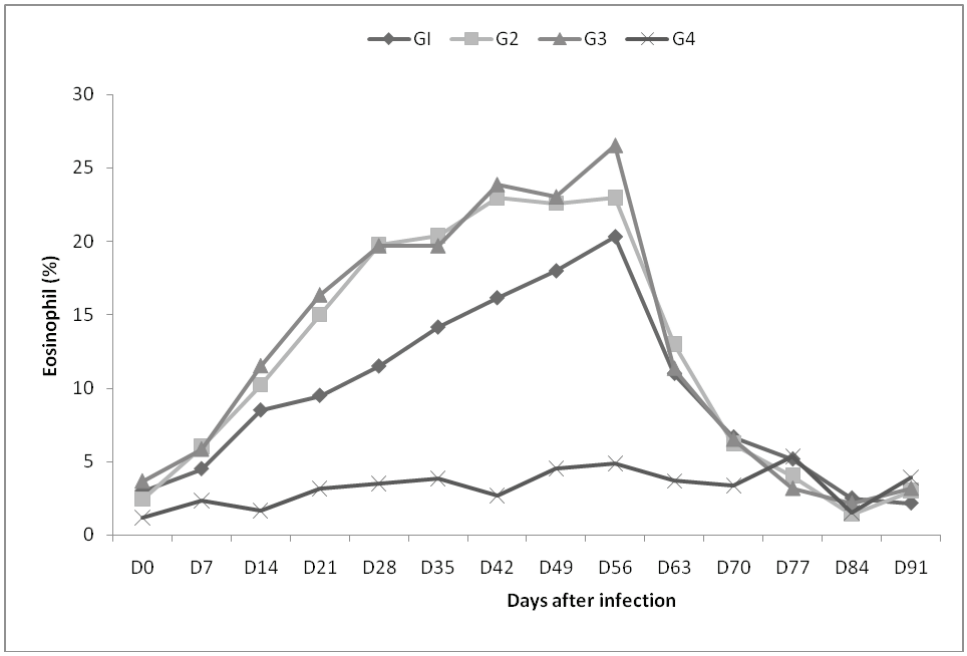

Figure 2: Evolution of mean blood eosiniphils (%) in the four experimental groups. G1, G2 and G3 were infected with different does of *H. Contortus* L3

Packed cell volumes (PCV)

The PCV values of control animals (G_4) were at physiological level throughout the trial period. In the three Haemonchus infected groups (G_1, G_2 and G_3) the mean values significantly decreased until the end of the trial with increasing infective dose (Figure 3) relative to the control group ($P<0.001$). All sheep from group 3 were severely anaemic at the termination of the experiment.

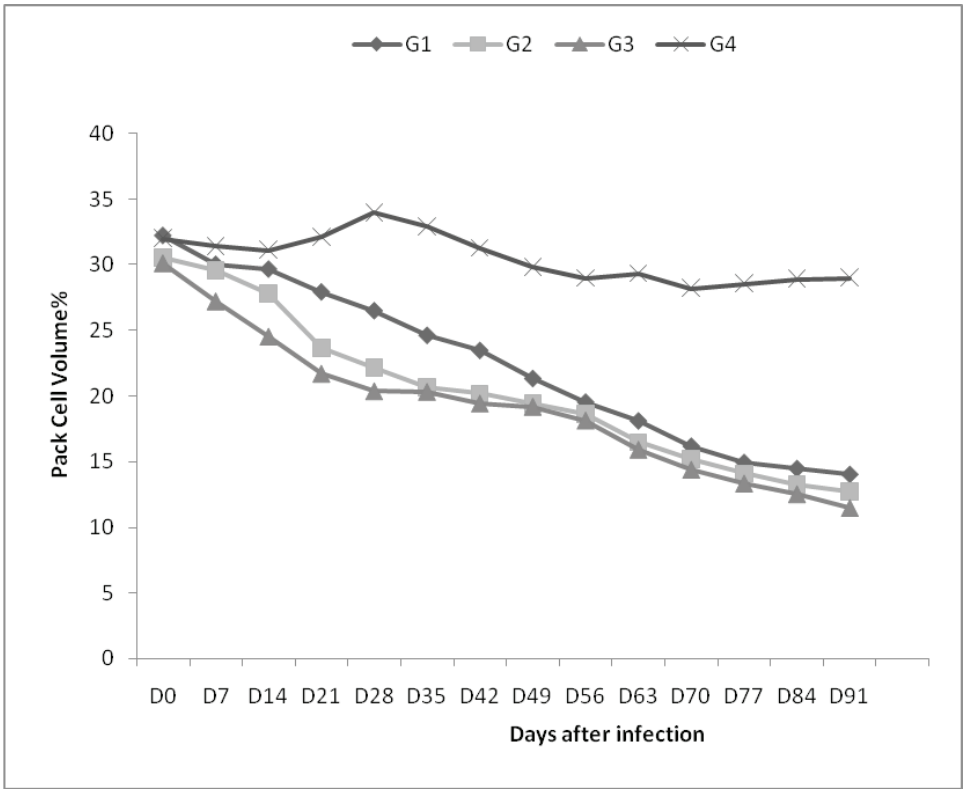

Fig 3: Patterns of mean packed cell volumes (PCV) in the four experimental groups of sheep

Serum protein levels

The highest mean serum protein value was recorded for group G_4 (control) whereas concentrations were relatively lower in the three infected groups compared with control values. There was no significant differences for serum concentration ($p>0.05$) between infected groups (Figure 4). There was positive correlation between serum protein concentration and PCV ($r = 0.19$, $p<0.01$).

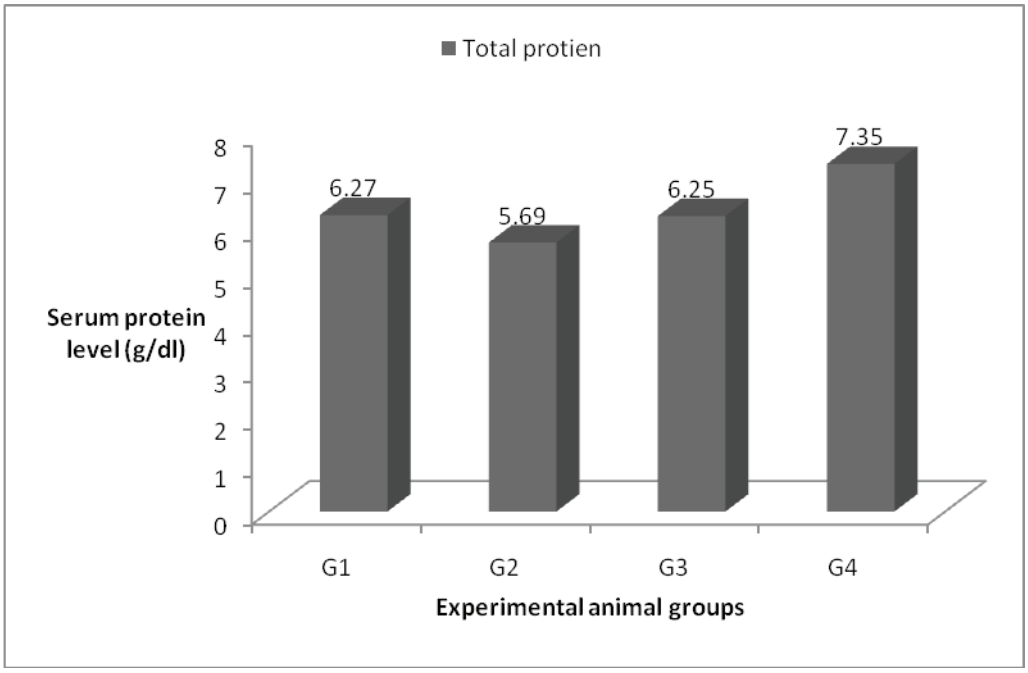

Figure 4: Mean serum protein levels in the four experimental groups of sheep. (data represent mean of all counts from day 0 to day 91).

Clinical responses and body weight changes

All infected animals with nematode 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. On the other hand, animals in the three nematode infected groups, G_1, G_2 and G_3 showed a significant reduction in body weight compared to the initial body weight at the beginning of the experiment with a final mean reduction of 3.77 kg, 3.41kg and 6.44 kg respectively (Figure 5). The differences of reduction in live weight between group G3 and the other two was significant ($P<0.001$). Meanwhile, the body weight was negatively correlated with FEC ($r = -0.28$, $p<0.01$).

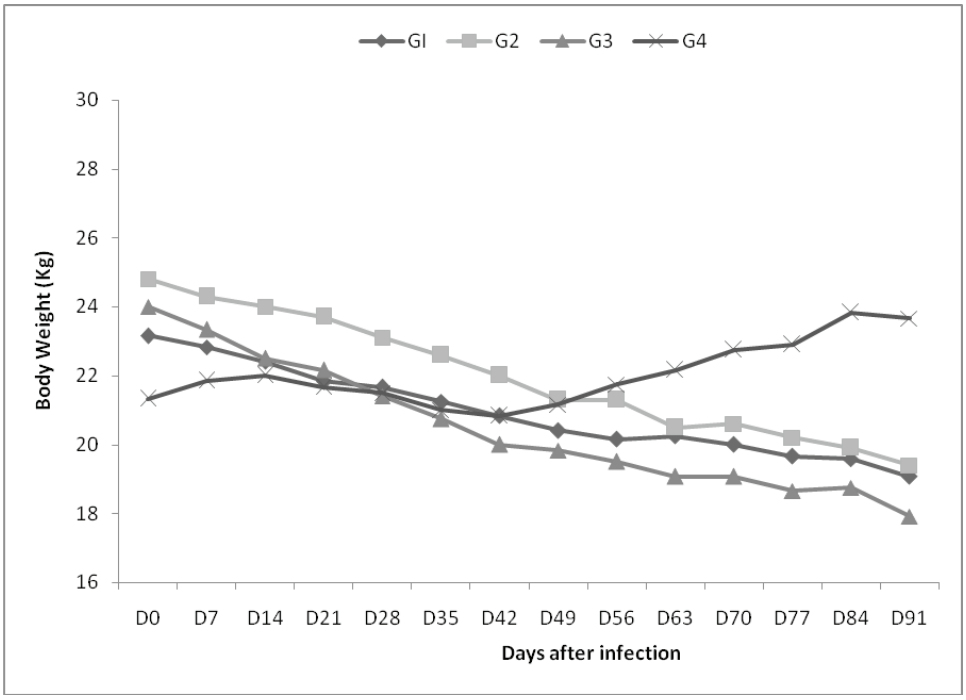

Fig 5: Body weight changes in four experimental groups

Discussion

This study was conducted to assess changes in hematological, serum biochemical and parasitological parameters as well as to observe clinical responses in indigenous Ethiopian highland sheep artificially infected by different doses of *Haemonchus contourtus*. The difference in infective doses of *H. contortus* showed remarkable influence on values of blood cell count and parasitological parameters.

In all infected animals, the first egg excretion was detected on D21 post infection. This was in agreement with earlier reports elsewhere (Yacob Hailu *et al.*,., 2002; 2004; Getache Terefe *et al.*,., 2005). Mean while, the mean EPG level was gradually increased throughout trial period which might be due to an increase in female worms fecundity associated with maturation of existing worm population starting from day 21 post infection This finding is in agreement with Yacob Hailu et al., (2004) but disagrees with Elbihari et al., (1984) who 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 haemonchus infected animals. Faecal egg count was negatively correlated with live body weight, Hb concentration, PCV and total serum protein while positively correlated with worm burden. This is in agreement with the finding of Yacob et al., (2004) who reported moderate association of Faecal egg count with worm burden and Kasali et al., (1988) that indicated low PCV and high EPG levels in nematode infected animals were 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, lower female length and fecundity (Douch et al.,., 1996; Stear et al.,., 1995; Onah and Nawa, 2000). In this study the total mean FEC was generally moderate in all infected groups but with significant difference between the three groups ($p<0.001$). Results from this study demonstrated the presence of strong association between faecal egg count and total adult worm burden in H. contortus infected animals. The direct proportional decrease in Hb level with increased worm burden showing the presence of positive correlation of mean total nematode burden to mean total FEC count in all nematode infected groups which also strongly agrees with the findings of (Craing, 1998; Waruiru et al.,., 2005; Yacob Hailu et al.,., 2004).

Based on the observed parasitological parameters, the lambs appeared to be very susceptible to the infection of *H. contortus*. The establishment rate observed in the current study in all infected groups was comparable with values reported by Dorchies et al.,. (1997); Yacob Hailu et al.,. (2002) and Getachew Terefe et al.,. (2005). Our data provided evidence on parasite development indicating the presence of positive correlation between infective dose and establishment which is in agreement with Amenu Asha (2005) and Yacob Hailu et al., (2004). The results in the present study pertaining to worm burden, their sex ratio, establishment of infection and correlation with FEC were similar to findings obtained from previous experimental trials on abomasal nematode, *H. contortus* (Stear *et al.,*., 1995; Getachew Terefe *et al.,*., 2005).

The evaluations of Haematocrit values in the present study were also consistent with earlier reports by Dorchies (1997) and Yacob Hailu et al., (2002). Haemonchosis has been described as an acute syndrome characterized by severe anaemia associated with the rapid acquisition of large worm burdens (Soulsby, 1986). The pattern of changes in the PCV values of in infected

animals might show sever blood loss. Surprisingly, in the current study, the decline in PCV was detected right from the beginning of infection in nematode received sheep. This might be due to the change in appetite in infected animals attributed to hormonal changes like that of gastrin level which affects feed intake (Hoste, 2001).

The negative correlation between PCV, EPG and worm burden is in agreement with several previous reports (Gauly and Erhart, 2000; Miller *et al.,*., 1998; Yacob Hailu *et al.,*., 2004, Getachew Terefe *et al.,*., 2005) suggesting that blood loss due to the parasite was directly proportional to parasite number. Similarly, the positive correlation with body weight measurement and parasitological parameters was consistent with the findings of Baker et al., (2001) and Nieuwoudt et al., (2002).

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 are blood suckers (Amarante *et al.,*., 2004). Similarly, in the present study observed progressive decline in haematocrit level in groups of sheep infected by the parasite. Meanwhile decline in haematocrit value was more marked group G3 compared to G1 and G2. This might be attributed to the difference in the numbers of established worm which was found proportional to the number of infective dose. The observed course of anaemia in groups infected with different doses of *H. contortus* was in agreement with the works of Yacob Hailu et al., (2009) for anaemia induced by abomasal nematode parasite *H. contortus*. Similarly, Kaufman et al., (1990); Dorchies et al., (1997); Yacob Hailu et al., (2004) and Getachew Terefe et al., (2005) reported that the trend of anaemia in *H. contortus* infection follows rapid fall in haematocrit values in the first 3 weeks post infection followed by further drop in haematological parameters until the end of the experiment.

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 (Pfeffer *et al.,*., 1998; Balic and Cox, 2000). In this study, all animals infected with *H. contortus* showed considerable degree of blood eosinophlia as compared to the non-infected animals indicating the presence of rapid mobilization of circulating eosinophils against *H. contortus* larvae. This was in agreement with Kaufmann and Pfister (1990); Getachew Terefe et al., (2005), and Yacob Hailu et al., (2002). Eosiniphils 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; Dorchies *et al.*,., 1997; Getachew Terefe *et al.*,., 2007). In this study the highest eosinophil count was observed in groups that were infected with 6000 infective haemonchus larvae (G_3). The result of this study also showed the presence of strong positive correlation between blood eosinophilia, worm population and EPG level which was in agreement with the result of (Dawkins *et al.*,., 1989; Balic *et al.*,., 2000; Yacob Hailu *et al.*,., 2004).

In this study all experimentally *H. contortus* infected sheep showed reduced serum protein concentrations which was significantly lower ($P<0.05$) up to the end of the trial compared to the control animals. This agrees with the findings of Yacob Hailu et al., (2004). These changes might be attributed to impairment of appetite.

This study further showed that haemonchosis had a significant effect on live body weight, body condition and health of infected animals at large. Differences in body weight between infected and control groups were statistically significant ($p<0.001$) which agrees with the findings of Barger and Cox (1984); Kaufmann and Pfister (1990) and Yacob Hailu et al., (2009).

The current experimental model showed the susceptibility of the highland sheep to Haemonchosis and effects of these nematodes on the health of the indigenous sheep breed during single exposure. However, these parasitological, haematological and serum biochemical changes illustrated in this experiment should be further compared with concordant data from field observations after natural infection. The way these animals respond to repeated small dose infections, as it commonly occurs in nature, should also be investigated (Deasalegn Lidetu, 1999; Abebe Wossene and Esayas Gelaye, 2001; Bersissa Kumssa, 2004; Amenu Asha, 2005; Dereje Shiferaw, 2008).

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