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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDY ON CONCURRENT *T. CONGOLENSE* AND *H. CONTORTUS* EXPERIMENTAL INFECTION IN GOATS: INTERACTION AND PATHOGENIC EFFECTS

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University, in Partial fulfillment of the Requirements for the Degree of Masters of Science (MSC) in Tropical Veterinary Medicine

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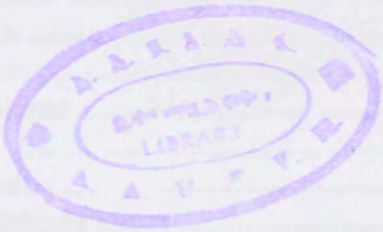

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LIST OF ABBREVATIONS

DIC	Disseminated intravascular coagulation
EDTA	Ethylenediaminetetraaceticacid
FEC	Faecal Egg Count
FFA	Free Fatty Acids
fl	femitoliter
GI	Gastro intestinal
GIT	Gastro intestinal tract
Hb	Haemoglobine
IFN_γ	Interferon gamma
IgE	Immunoglobuline E
IgG	Immunoglobuline G
Ig G_1	Immunoglobuline G_1
IgG_2	Immunoglobuline G_2
Ig M	Immunoglobuline M
IL	Interluekine
ILRAD	International Laboratory for Research in Animal Diseases
LT	Lymphotoxine
MCH	Mean Corpuscular Haemoglobine
MCHC	Mean Corpuscular Haemoglobine Concentration
MCV	Mean Corpuscular Volume
OIE	Office de international epizootics
PCV	Packed cell volume
RBC	Red Blood Cell
Th1	T-helper lymphocytes
Th2	T-helper lymphocytes
VAT	Variant Antigenic Type
VSGs	Variant Surface Glycoprotiens
WBC	White Blood Cell

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ABSTRACT

Study on concurrent *Trypanosoma congolense* and *Haemonchus contortus* infections to assess the interaction and pathogenic effect of single and mixed experimentally induced infection were conducted in goats. A total of 25 goats of approximately one year old were divided into five groups and each group was infected either with *H. contortus* one week after a preceding infection with *T. congolense* or with primary *H. contortus* infection one week prior to *T. congolense* or infection with either the nematode or trypanosome alone, and the fifth group was served as non infected control. For experimental purpose each experimental animals were infected with *T. congolense*, derived from the donor goat after one passage in mice with total intravenous inoculation of $5x10^{4}$ *T. congolense*. The total infective dose of *H. contortus*, which was given orally to each animals was, 10,000 3rd stage larvae. Parasitological observations such as prepatent period, faecal egg count, worm burden and trypanosome parasitaemia; and hematological parameters like packed cell volume, haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cell counts, white blood cell counts, differential leukocyte counts (eosinophil, lymphocyte and neutrophils), total protein and also clinical parameters including body weight change, mortality rates among various treatment groups were made in mixed infected groups and in animals singly infected with either parasites. It was revealed that the order of infection in the preceding or subsequent infection was very important in which the most harmful combination was a primary *T. congolense* infection one week prior to *H. contortus* super infection, that resulted in progressive and sever anaemia accompanied by leucopenia, hypoproteinemia, loss of weight and short prepatent period of *H. contortus*. Except animals mono infected with *H. contortus*, which have shown macrocytic hypochromic anaemia, all other treatment groups have revealed normocytic normochromic anaemia throughout the whole observation period. The result also showed that animals with concurrent *T. congolense* and *H. contortus* run high risk of succumbing during the infection period. Higher rate of mortality and more pronounced pathological effects were observed in combined infections than single ones. It was concluded that the increased *H. contortus* egg excretion observed in animals infected with both parasites might significantly increase the risk of

nematode infections. The interaction between these two highly potent anaemia causing pathogens should be considered whenever attempts are made to control these two diseases.

Keywords: Goat, Concurrent infection, *Trypanosoma congolense*, *Haemonchus contortus*, Interactions.

1. INTRODUCTION

Although animals are known to harbour single parasitic infections, mixed parasitic infections with varies species or several different types of parasites is a common phenomenon in most cases of natural infections (Clark, 2000; Cox, 2001). In the field, animals are exposed to multiplicities of parasites among which are trypanosomes and gastrointestinal nematodes that cause considerable animal health problems in many parts of the world (Kaufmann *et al.*, 1992; Dwinger *et al.*, 1994).

Trypanosomosis is still a major constraint to livestock production in Sub-Saharan Africa. In Ethiopia, trypanosomosis is one of the major diseases, which contribute to direct and indirect economic losses to livestock production. Cyclically transmitted animal trypanosomosis is a serious constraint to livestock production and agricultural development in southern and southwestern Ethiopia (Ford *et al.*, 1976; Abebe and Jobre, 1996). Trypanosomosis is classically associated with profound suppression of the immune responses in affected animals, which shows reduced cellular and antibody responses, usually become increasingly susceptible to concurrent bacterial and viral infections and often die from fulminating parasitaemia and or other complications (Onha, 1992). A practical and important consequence of trypanosome immunosuppression in livestock includes: down regulation of immune response to vaccines used to control important diseases (Scott *et al.*, 1977; Rurangirwa *et al.*, 1993). They also are known to suppress immune response to a number of helminth parasites resulting in increased fecundity, adult worm burden, and failure of immune mediated expulsion of adult worms (Scott *et al.*, 1977; Rurangirwa *et al.*, 1993).

In Ethiopia where farm animals are kept on pasture throughout the year and climatic conditions are favorable for development and survival of infective stages, helminth parasites are recognized as major causes of economic loss. Helminthosis mostly in the form of gastroenteritis is ranked as the major constraints on sheep and goat production. Parasitic gastroenteritis including haemonchosis, causes anaemia, diarrhea, and emaciation resulting in reduced weight gains increased morality and increased production cost (Mulugeta *et al.*, 1989).

A blood feeding trichostrongyle nematode parasite, *Haemonchus contortus* found in the abomasum, is regarded as the most prevalent, pathogenic and economically important parasite of small ruminants (Jacqueit *et al.*, 1992; Fristche *et al.*, 1993; Tembely *et al.*, 1997; Baker *et al.*, 1998; Chiejina, 2001; Bersisa, 2004) in humid and subhumid tropics including Ethiopia.

Anaemia is the predominant sign of trypanosomosis; therefore whenever it is observed in tsetse-infested areas, it has mainly been attributed to trypanosome infections. There are however, other equally potent and highly prevalent anaemia causing pathogens that affect the ruminant production. One of these is gastrointestinal helminth infection (Kaufmann and Pfister, 1990).

The interaction between two or more parasites is inevitable situation, which may lead to a significant biological change in the body tissue, and fluids, which could not be, attributed either of these parasites individually. There are evidences that suggest the existence of synergistic or antagonistic effects between two or more parasites in the given host. It has been reported that mixed infections of trypanosomes and GIT nematodes lead into more severe worm infections (Dwinger *et al.*, 1994). Antagonistic interaction has been reported to exist between *Strongyloides ratti* and *Trypanosoma brucei* in which the two parasites interact in a manner that ameliorate their pathogenic effects, resulting decrease in the level of parasitaemia, intestinal worm burden and increased life span of infected mice's (Onha *et al.*, 2004). The infection of *T. evansi* in goats lowered down the body resistance to *H. contortus* infection and making the animal more susceptible to it (Sharma *et al.*, 2000).

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

Concurrent infections between trypanosome species and GI nematodes is of particular relevance and interest in trypanosome endemic regions of Africa, in view of the powerful depression effect

of the former on the immune response of their host (Holmes *et al.*, 1974; Scott *et al.*, 1977). Comprehensive data on the interaction between trypanosomes and GI nematode infections in the small ruminant population of the tropics are scanty and little attention has been given to assess such interaction. Therefore the objective of the current study was to assess the interactive and pathogenic effects of concurrent experimental *T. congolense* and *H. contortus* infections in goats. The specific objectives include:

- To assess haematological and parasitological parameters associated with single and mixed infections.
- To depict the dynamics of total protein and body weight changes as the consequence of single and mixed infections.
- To determine the prepatent period of *H. contortus* and the first date of detection of trypanosomes in mixed infections in goats.

2. LITERATURE REVIEW

2.1. Overview of trypanosomes:

2.1.1. Trypanosomes

Trypanosomes are flagellated protozoa, which belongs to the family trypanosomatidae. The family consists of several genera and many species. The species, which parasitize vertebrates, require a vector for transmission (Adam *et al.,* 1979). Trypanosomes live and multiply in the blood stream, lymphatic vessels and tissues, including the cardiac muscle and the central nervous system. Trypanosomes of veterinary and medical importance are classified into four subsections namely, Duttonella (*T. vivax, T uniforme*); Nannomonas (*T. congolense, T. simae*); Pycnomonas (*T. suis*) and Trypanozoon (*T. brucei, T. rhodensiense, T. gambiense, T. evansi, T. equiperdum*). (Mulligan, 1970; Adam *et al.,* 1979).

2.1.1.1. Pathogenesis:

The pathogenesis of the disease caused by trypanosomosis differs according to the species causing the infection. *T. vivax* and *T. congolense* appear to be parasite of the blood plasma and produce tissue injury primarily by the anaemia associated with infection. *T. brucei* is more widely distributed in the host affecting the intercellular fluid of the body cavities (Robertson, 1976). Here although anaemia occurs, it is considerably to be of secondary importance to the extensive, degenerative, necrotic and inflammatory changes. The pathogenesis of trypanosomosis includes; chancre, lymphadenopathy, anaemia and tissue damage (Mulligan, 1970).

Chancre: is a raised cutaneous inflammatory swelling produced within few days at the site of inoculation of trypanosomes when the metacyclic forms multiply locally as typical blood forms. The fly bite, inoculates metacyclic trypanosomes in dermal connective tissue of the skin and here a local inflammatory reaction, the chancre develop (Urquhart *et al.*, 1996).

Lymphadenopathy is the enlargement of the lymph nodes draining the chancre area. Following the enlargement of the lymph nodes draining the chancre, generalized enlargement of the lymph nodes and splenomegally develop. This is associated with marked proliferation of lymphoid cells in these organs. Numerous large active germinal centers are present and there is a marked increase in the number of plasma cells in the medullary cord of the lymph nodes and in the splenic red pulp. Marked gamma globulinaemia accompanies these changes (Morrison *et al.*, 1981).

Anaemia: development of anaemia is a well-recognized and inevitable consequence of trypanosome infection in domestic animals (Losos and Ikede, 1972). In most early cases, there is an acute on set of anaemia, corresponding clearly with the detection of the parasite in the blood stream (ILRAD, 1987). The initial fall in packed cell volume is associated with the first wave of parasitaemia in the blood. During this period anaemia is extravascular and is possibly the result of increased red blood cell destruction by erthrophagocytosis in the spleen, lungs haemal nodes and bone marrow as a result of direct traumatic effect on red cells there by increasing red cell fragility (Mamo and Holmes 1975, ILRAD, 1995). In cattle subjected to a single needle or fly challenge, the packed cell volume (PCV) progressively decreases by about 40-50% over the first 4-6 weeks (Morrison *et al.*, 1981). During this period the anaemia is hemolytic due to production of toxins (lytic factor) associated with protein of low molecular weight, which has anaphylatoxin actively and released by autolysing trypanosome of endogenous phospholipases (Holmes and Jennings, 1975; Morrison *et al.*, 1981).

Cattle that survive the acute phase, progress into chronic anaemia. This may still result in death or in either spontaneous recovery or survival with persisting low grade anaemia. This chronic phase is characterized by low and transient parasitaemia or complete absence of detectable parasites in the blood. Death results from congestive heart failure (Morrison *et al.*, 1981). Bleeding disorder is commonly associated with trypanosome infection and may become a major aspect of pathogenesis trypanosomosis in cattle with a hemolytic syndrome associated with *T. vivax* infection (Holmes, 1987; Wellde *et al.*, 1983; Olubayo and Mugera, 1985).

Tissue damage: from blood borne nature of trypanosome infections most tissues and organs are damaged during course of infection although some are more consistently and severely infected than others. While necrosis is not the major feature of the disease in cattle, tissue cell damage and degeneration may be marked. The nature of the cellular infiltrate and possibly the mechanisms involved in cell injury would appear to depend on the difference in tissue invasiveness between the species of trypanosomes. One vital organ, which is consistently damaged by all three species of trypanosome, is the heart. The changes, which occur in the heart also, reflect to some extent what occurs in other tissues and organs. Other vital organs, which are commonly affected, include the skeletal musculature, central nervous system, endocrine organs and reproductive tract (Robertson, 1976; Morrison *et al.,* 1981; Hall, 1985; Abebe 1991).

2.1.1.2. Clinical features

Acute infections may be occasionally occurring, in all domestic animals notably with *T. vivax* in cattle leading to death after 1-3 weeks (Robertson, 1976). Regardless of species of trypanosome and the species of the host, the principal clinical sign are intermittent fever, an increasing degree of anaemia and progressive loss of condition and the disease is seen more commonly as a chronic form (Hall, 1985; Robertson, 1976).

Infected animals are dull, have staring lusterless coat, loss weight and are easily exhausted. They also lag behind the herd, their superficial lymph nodes are enlarged and prominent, cattle infected with *T. vivax* often show photophobia and excessive lacrimation (Hall, 1985; Lossos, 1986, Murray *et al.,* 1982; Robertson, 1976).

2.1.1.3. Pathology

The pathology of trypanosomosis differs according to the species of trypanosome causing the infection and the stage of the disease. *T. vivax* and *T. congolense* appear to be strictly parasites of the blood plasma and produce tissue injury primarily by anaemia associated with infection . *T. brucei* is more tissue parasite infecting the intracellular fluids of the body cavity (Abebe, 1991).

Post mortem examination of animals after acute trypanosomosis may show extensive small haemorrhage involving mucus and serous surfaces, area of emphysema in the lungs and mild gastroenteritis, after more chronic infection the carcass may be anaemic and emaciated with an enlarged spleen and lymph nodes (Robertson, 1976). Subcutaneous edema and accumulation of pericardial and thoracic fluid containing trypanosome are found particularly in horse and dog infected with *T. brucei* (Seifert, 1996). Degenerative changes have been observed in testis and epididymis of sheep, goats and cattle infected with *T. brucei, T. congolense* and *T. vivax* (Morgan, 1990). Pathological changes of pituitary and adrenal glands with associated endocrine dysfunction have been observed in Boran (Bos indicus) cattle infected with *T. congolense* (Abebe, 1991).

2.1.1.4. Epidemiology

The distribution of tsetse transmitted trypanosomosis limited to the continent where the tsetse vectors are found. Mechanically transmitted trypanosomes are found in Africa, Asia, Middle East and South America (ILRAD, 1987). Among the salivarian group only *T. vivax* and *T. evansi* are found beyond the confines of tsetse fly belt by mechanical transmission (Hall, 1985). Tsetse transmitted trypanosomosis affects 37 sub Saharan countries; an estimate of 160 million cattle and 260 million sheep and goats are kept in this area of risk extending over 10 million Km^2 of land (Erkelens *et al.*, 2000). Five species of tsetse are found in Ethiopia: *Glossina longipennis, G. morsitans submorsitans, G. pallidipes, G. fuscipes fuscipes and G. tachnoides* and are confined to southern and southwestern region of the country (Langridge, 1976). *G. m. submorsitans* is usually found in the deciduous woodland and wooded grassland, often interspaced with evergreen species. *G. longipennis* is found in dry acacia, thorn-bush and is very active after sunset and before nightfall. *G. f. fuscipes* and *G. tachnoides* inhabit gallery forest, thickets and fringing vegetation on streams, rivers and lakeshores (Ford *et al.*, 1976, Langridge 1976).

Tsetse transmitted trypanosomosis infect various species of mammals but, from an economic point of view, it is practically important in cattle. It is mainly caused by *T congolense, T. vivax*

and to lesser extent, *T brucei brucei, T. uniforme*, *T simiae* and *T sius* are other, less common tsetse transmitted species (OIE, 2002).

2.2. General overview of helminthes

Gastro intestinal nematodes of the order Strongylida are the most common cause of clinical helminthosis, which is caused by the infection of digestive tract due to the presence and development of nematodes in the wall or the lumen of the abomasums, the small intestine, and/or large intestine (Brander *et al.,* 1991; Bouman, 1995).

The trichostrongyloid nematodes are small often hair like worms in the group, which are especially common and pathogenic in grazing ruminants, but horses, swine, cats and birds are also host important species. The abomasums and intestine are the usual locations in ruminants, but dictyocaulus reach maturity in aberrant locations in the air passages (Dunn, 1978; Soulsby, 1986; Bouman, 1995). Structurally they have few cuticular appendages and the buccal capsule is vestigial. The male have a well-developed bursa and the two spicules, the configuration of which is used for species identification. The life cycle is direct and usually non migratory and ensheathed L_3 is the infective stage (Soulsby, 1986; Radostitis *et al*., 1989; Urquhart *et al*., 1996).

2.2.1. Haemonchus

H. conturtus is the species most commonly found in sheep and goats and it can also be found in cattle when these animals graze the same pasture. *H. placei* is the usual Haemonchus species in cattle and it can also develop well in sheep and goats and cause clinical disease but causes less severity than that caused by *H. contortus*. *H. longistipes* is the species that usually affects camels and it can also develop in other animals. *H. similes* is the one that usually affects cattle and deer in some countries and it can also affect other animals (Soulsby, 1986; Radostits *et al.,* 1994; Urquhart *et al*., 1996). Hamonchus species mainly affect the abomasal mucosa of their hosts. Adults and the late L_4 stage larvae ingest blood which causes sever anaemia. Heavy infections

can result in the death of the host (Eysker and Ogunsusi, 1980). Haemonchus is a voracious blood sucking abomasal nematode and is responsible for the extensive losses in sheep, goat and cattle especially in the tropics (Urquhart *et al.*, 1996).

Haemonchus is for the most part a primary parasite, predisposing cause for infestation including overcrowding, lush pasture and hot humid climatic conditions. However, development of clinical illness is favored by a fall in the plane of nutrition particularly in young animals (Radostitis *et al.*, 1989). Haemonchus is the most common in tropical and sub tropical areas or in the areas with summer rainfall, while *T. axei* and Ostertagia are more common in winter rainfall areas (Urguhart *et al.*, 1996).

Haemonchosis is characterized by cardinal signs like anaemia, which causes pallor of the skin and mucus membranes and haematocrit reading of less than 15%, in generalized edema of haemonchosis the lesions are those associated with anaemia. The abomasums becomes oedematus and in the chronic phase the PH increases causing gastric dysfunction (Bouman, 1995; Urquhart *et al.*, 1996). At peak infection, naturally acquired populations of *H. contortus* can remove one fifth of the circulating erythrocyte volume per day from lambs and may average one tenth of the circulating erythrocyte volume per day over the course of non fatal infections lasting two month. Thus each worm removes about .05ml of blood per day by ingestion and seepage from lesions so that a sheep with 50000 *H. contortus* results from the inability of the host to compensate for blood loss (Radostitis *et al.*, 1989). Observation of phenomenon called self cure is found to be the characteristic feature of haemonchosis in endemic areas in which the major part of the adult worm burden is expelled resulting in sharp drop in egg out put to near zero after the advent of a period of heavy rain (Urquhart, 1996)

2.2.2. Epidemiology of Haemonchus species:

Several factors are known to determine the epidemiological pattern of parasitic diseases condition. These include weather condition, season, husbandry practice and physiological status of animals. The development of eggs to infective larvae and the survival of the larvae on pasture

are influenced by temperature, rainfall and other environmental conditions, (Mulugeta *et al.*, 1989; Urquhart *et al.*, 1996).

The predominance of GI parasites, in the wet season, identifies rainfall as the main climatological factor influencing the occurrence of GI parasites. Adequate moisture contribute to the development and proliferation of infection stages of the parasite on the contrary the dryness and desiccation characteristics of the dry season is unfavorable for development and survival of extra host stages of the parasites, resulting in long rates of GI parasite infection during this period (Fristche *et al.*, 1993).

Haemonchus species are distributed world wide but most important in warm tropical areas, because larval development of haemonchus occurs optimally at relatively at high temperatures, since high humidity, at least in the microclimate of the faeces and herbage is also essential for larval development and survival, the frequency and severity of outbreaks of the disease is largely dependent on rainfall in any particular area (Urquhart *et al.*, 1996).

Haemonchosis is regarded as the most pathogenic, and economically important nematode of small ruminant. Studies carried out in different parts of Ethiopia have shown that *H. contortus* is one of the most prevalent nematode of small ruminants (Bersissa, 2004; Abebe and Essayas, 2001). Similar studies conducted in African countries (Fritsche *et al.*, 1993; Jacqueit *et al.*, 1992) have shown the existence of large spectrum of nematodes, *H. contortus* being one of the most prevalent species.

The circulation of haemonchus species among wide range of hosts have impact on survival of low probability for an infective larvae to be ingested by a susceptible host in conditions of low breeding pressure and continuous host migration. A wide host spectrum could be a considerable advantage for a haemonchus species which exist as low intensity infection only (*H. placei)* where as it would be a limited important for haemonchus species which show high prevalent and intensity within their usual hosts as shown in *H. longistipes* and to the lesser extent *H. contortus* (Jacqueit *et al.*, 1998).

The survival of *H. contortus* infection on tropical pasture is variable depending on the climate and degree of shade, but the infective larvae are relatively resistance to desiccation and some may survive for 1-3 months on pasture or in faeces (Urquhart *et al.*, 1996). *H. contortus* out lived the dry environment (unfavorable climatic condition) as inhibited larvae in the abomasal mucosa, while other nematode species survived as adults with reduced fecundity. Egg production per adult worm was found to depend heavily on season and egg counts in dry season did not correspond to the size of the worm burden (Fristche *et al.*, 1993).

2.3. Anaemia

Anaemia is the reduction in the number of erythrocytes, haemoglobine or both in the circulating blood. Many classifications for anaemia have been proposed and the two most common and widely accepted are morphologic and etiologic classifications. Anaemia is classified morphologically utilizing MCV, MCH, MCHC, (Mean corpuscular volume, Mean corpuscular haemoglobine and Mean corpuscular haemoglobine concentration) (Coles, 1986).

Morphologically anaemia can be Normocytic normochromic, Macrocytic normochromic, Macrocytic hypochromic, and Microcytic hypochromic. Etiologically anaemia may be placed in four general categories including blood loss (Haemorrhagic anaemia) anaemia due to excessive destruction of erythrocytes or shortened erythrocyte life span, anaemia due to depression of bone marrow and nutritional deficiency anaemia (Coles, 1986).

2.3.1. Trypanosome induced anaemia

The mechanisms involved in trypanosomosis to induce anaemia may be complex and different mechanisms have been described as the cause of anaemia during trypanosome infections. The major cause of which has been attributable mainly to extravascular haemolysis due to phagocytosis of erythrocytes by an expanded mononuclear phagocytic system (MPS) resulted from massive intravascular presence of living, dying, and dead trypanosomes as well as from resulting antigen antibody complexes (Anosa, 1997; Murray and Dexter, 1988).

Intravascular haemolysis may be of a minor feature (Esievo *et al.*, 1982), haemolytic factors (trypanosome haemolysins, such as lipid soluble factor, plasma factor, enzymes including trypanosome proteases having proteaolytic activity and sialidase activities (Esievo *et al.*, 1982, Knowles *et al.*, 1989), autoerythrocyte antibodies (Assoku and Gardiner, 1989) have been reported to play varies roles in erythrocyte lysis and /or destruction during trypanosomosis.

Active phospholipase A has been shown to be generated during autolysis of trypanosomes and have direct effect on red cell membrane and cause the release of large quantities of free fatty acids (FFA) particularly linoleic acids are haemolytic. Apart from the possible localized harmful effect of FFA; trypanosomal phospholipase could have a direct pathological effect on red cell membrane (Murray and Dexter 1988).

Trypanosoma congolense attach sialic acids on red cells and that sialic acids are constraints of carbohydrate moieties of variable surface glycoprotiens of *T. congolense*. The early anaemia, which occurs in infected animals, could be attributed to the activity of trypanosome sialidase which might cleave the surface sialic acid, rendering erythrocyte more prone to phagocytosis directly, by immunoglobuline and complement opsonization or by activation of classical or alternate pathway of complement. Furthermore, cleavage of sialic acids from red cell membrane would expose new epitops on the surface of affected cells that could lead to antibody production against these exposed epitops and increased phagocytosis (Murray and Dexter 1988).

There is evidence that immunological mechanisms might be involved in the anaemias of African trypanosomosis. Auto agglutination, increased sedimentation rate in cattle and also in humans with trypanosomosis has been reported (Murray and Dexter 1988).

Immunoglobulins and complements on the red cells of patients infected with trypanosomes have been reported IgM, IgG and C3 were demonstrated on red cells of cattle infected with trypanosome (Murray and Dexter 1988). It has been proposed that soluble trypanosome antigens, released from dying trypanosomes, is adsorbed on the red cells with subsequent opsonization of antibody and complement. Auto antibodies of a variety of cells or their products have been

widely reported in animals infected with trypanosome and have been hypothesized to play a role in trypanosome induced anaemia (Murray and Dexter 1988).

Parasite derived factors were the primary cause of red cell damages and that by exposing hidden epitops on the red cell membrane had induced the production of autoantibodies which then play a role in maintenance and progression of anaemia. Immunoglobulins and complements play a role in the anaemia that occure during the parasitaemic phase of trypanosome infection by facilitating red cell destruction through phagocytosis via FC and complement receptors on the macrophage. Disseminated intravascular coagulation (DIC) can lead into a form of haemolytic anaemia termed microangiopathic haemolytic anaemia in which the red cells are damaged by widespread fibrin deposition in the microvasculature, the red cells then appear as distorted cells or shistocytes which are liable to lysis or phagocytosis. Fever has also a role in the anaemia of African trypanosomosis, even a small elevation in temperature have a major effect on red cells (Murray and Dexter 1988).

2.3.1. Haemonchus induced anaemia

The abomasal parasites of ruminants that have been associated with anaemia are, *H. placei* in cattle and *H. contortus* in sheep and goats. Infection of *Trichostrongylus axei, Ostertagia ostertagi* 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 into the alimentary tract (Jennings, 1987; Urquhart *et al,* 1996).

Haemonchus contortus is blood sucking abomasal parasite in which the single mature adult parasite removes by ingestion and seepage about 0.05ml of blood per day (Urquhart *et al.*, 1996). The anaemia *H. contortus* infection follow three stages. The first, which occurs during the prepatent period during the first 3 weeks, was characterized by a dramatic fall in PCV; serum iron at this stage is normal. This is considered to be the result of blood loss due to the developing but immature parasite (Dargie *et al.*, 1979).

Although at this stage the blood loss at absolute volume is not as large as when the parasite is mature, the haemopoeitic system of the host is not fully mobilized to produce red cells in quantities sufficient to meet the needs of the animal (Dargie *et al.,* 1979; 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 haemopoeitic system and the high serum iron levels. However, the capacity of the animal infected with *H. contortus* to reabsorb haemoglobine iron is limited the iron reserve of the animal became 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 a dyshaemopoiesis 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* infections and the severity of the associated anaemias. In addition to the nutritional status the ability of the animal to respond quickly to the haematological stress, the immunological status of the animal and the innate resistance determines the proportion, if any, of a given dose of infective larvae, which will become established (Jennings, 1987).

2.4. Immunity to trypanosomes and helminth parasites:

2.4.1. Immunological responses of ruminants to gastrointestinal nematodes:

The immune responses against intestinal nematodes have been studied extensively in humans and rodents. Immunity against these parasites comprises the production of specific IgE antibodies, eosinophlia, and mucosal mastocytosis and that it is dependent on the activation of T- helper 2 cells. As far as ruminants are concerned most studies have been mainly restricted to studying systemic and peripheral antibody responses (Schallig, 2000).

Different cell types are thought to be involved in the recognition, processing and presentation of antigens in the gut, epithelial cells, macrophages and B-cells. After presentation of parasite antigens to T lymphocytes, the T cells further regulate the host response against the GI nematodes (Claerebout and Vercruysse, 2000)

The importance of T lymphocytes for protection against GI nematodes has been demonstrated in the laboratory animals infected with *Trichinela spirallis*, *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus*, *Trichuris muris*, and *Strongyloides stercoralis*. In each of these model systems the T cell subset to which this protection has been ascribed is the CD4+ bearing T helper cell. In mice CD4+ cells can be segregated into T helper1 (Th1) and T helper2 (Th2) cell subsets, based on the cytokines they secret. The Th1 cells secrete interferon (IFN)γ

interterleukine (IL)-2 and lymphotoxine (LT)-α whereas, Th2 cells secrete, among others, IL-4

IL-5, IL-9, IL-10, and IL-13. Products of one subsets negatively regulate the other subset. Whether the Th1 and the Th2 subsets gains dominance in the immune response depends not only on a number of host factors, such as the antigen presenting cell type, co-stimulatory molecules and cytokine environment, but also on the nature and dose of the parasite antigen (Claerebout and Vercruysse, 2000; Schallig, 2000).

The phenomena that are typically seen during nematode infection, such as eosinophilia, mucosal mastocytosis, and IgE responses are controlled mainly by Th2 cytokines. A Th2 type response in the gut has been associated with expulsion *T. muris*, *N. brasiliensis*, and *H. polygyrus*, *T. spirallis*. *Invivo* studies have demonstrated the importance of specific cytokines in controlling expulsion of *T. muris*, *N. brasiliensis*, *H. polygyrus*, *T. spirallis*, and *S. stercoralis*. Overall, IL-4 and IL-14 promote protective immunity against GI nematodes, while IL-12 and IFNγ promote the survival of the parasite (Claerebout and Vercruysse, 2000; Schallig, 2000).

In sheep, lymphocytes have been shown to be important in immunity against GI nematodes. Transfer of gastric lymph lymphocytes from lambs, immunized against *H. contortus* or *T. circumcincta* to their genetically identical uninfected twins, transferred protection against a

homologous challenge infection. T helper cells have a crucial importance in the protective immune response against *H. contortus invivo* depletion of CD4+ T cells abrogated immunity to *H. contortus* in genetically resistance sheep. More recently, *invivo* depletion of CD4+ cells partially abrogated immunity to *H. contortus* induced by gut immunization. There is yet no proof the existence of two distinct subsets of T helper cells in ruminants. However, despite the lack of data on Th1/Th2 bias in ruminants, there are many features of ruminants which would be considered a Th2 type response if they had occurred in the mouse. Eosinophilia and mast cell hyperplasia are often observed during GI nematode infection in ruminants. Unlike rodents the role of specific cytokines in regulating the immune response against GI nematode infection has not yet been determined in ruminants. However, a study demonstrated that the prolonged administration to sheep of a monoclonal antibody to IFN_{γ} resulted in significantly increased protection against *T. columbriformis*. This later result suggests that IFN_{γ} negatively influences a protective response against *T. columbriformis* (Claerebout and Vercruysse, 2000; Schallig, 2000).

2.4.2. Immunity to trypanosomes

The immune responses to an African trypanosome infection are dominated by two overwhelming phenomena: a massive non-specific polyclonal activation of B cells and a generalized immunosuppression of some humoral (B cell) and cellular (T cell) immune functions (Sileghem *et al*, 1994). The polyclonal B cell activation results in a large production of IgM, the first class of antibodies to be generated by the appearance of new foreign antigens. This activation is not triggered entirely by the continually changing epitopes of the different VSGs, however, the newly synthesized antibodies do not react solely with the VSGs and other trypanosome antigens. They frequently are heterospecific in their reactivity and can be autoantibodies directed against the proteins and nucleic acids of the host. It has been proposed that an unknown non-VSG molecule of trypanosomes (Sileghem, *et al*., 1994), or perhaps even the VSGs themselves (Diffley, 1983), serve as the mitogen causing this massive non-specific expansion of B cells and the subsequent increase in Immunoglobulin concentration.

The greatly elevated levels of IgM and resultant antigen–antibody complexes in turn cause hyperplasia of the reticuloendothelial system, especially the spleen and lymph nodes, and are

likely to be responsible for many of the pathogenic characteristics of the infection. The other striking immune feature of African trypanosome infections, a dramatic suppression of immune responses other than the initial B cell activation, results in an increased susceptibility to opportunistic infections. This generalized immune suppression has been reported to affect a large variety of both B cell and T cell functions and seems to inhibit many secondary immune events. For example, the enormous IgM production is not followed by the normally concomitant increase in IgG and the other antibody classes, and T cell proliferation is severely suppressed (Sileghem, *et al.*, 1994).

However, the concentration of IFN_γ is greatly increased in experimental animals infected with *Trypanosoma brucei*. Macrophage activity is enhanced, perhaps by the increase in IFN_γ, and the elimination of trypanosomes by antibodies is thought to be mediated by opsonization and destruction by liver macrophages, rather than by complement-mediated lysis (Jokiranta *et al* 1995; Dempsey and Mansfield, 1983). The levels of some cytokines such as IL-2 decrease during trypanosome infections that may contribute to the lack of T cell proliferation. Although the co-existence of massive polyclonal B cell expansion and profound immunosuppression appears to be a paradox, both phenomena obviously generate an environment conducive to perpetuation of the infection (Jokiranta *et al* 1995; Dempsey and Mansfield, 1983).

2.4.2.1. Antigenic Variation

Antigenic variation is a phenomenon where by blood steam trypanosomes switch from one VSG on their surface to another. The only known function of the VSG is to serve as a protective barrier against the attack of immune system on the outer membrane constitutes (Donelson *et al.*, 2003).

Since trypanosomes are extracellular parasites they are exposed to specific and non specific immune attack. To hide invariant surface antigens from the immune response trypanosomes shroud themselves in a variant surface glycoprotien (VSG) coat whose molecular identity they periodically change, allowing a part of the parasite population to avoid antibody-mediated killing throughout an infection (Donelson *et al.*, 2003).

2.4.2.2. Immunodepression

Immunodepression is a well-characterized feature of trypanosomosis in cattle, humans and mice (Sileghem, *et al.*, 1994). Depressed B and T cell responses to trypanosome and non-trypanosome antigens have been documented in most hosts, with the exception of trypanoresistant wildlife (Schleifer, 1993).

Trypanosome infection has been shown to result in a reduced capacity to mount primary humoral responses to non-trypanosome antigens. In addition, T cell mitogen and antigen-driven proliferation and cytokine production are also reduced in infected animals. Despite these observations, a relationship between the severity of disease and the depression of B cell (reduced antibody response to non-trypanosome antigens) and T cell (reduced proliferation and cytokine production) responses has not been established in either mice or cattle during the early stages of infection. However, T cell and antibody responses appear to be restored as trypanotolerant breeds of mice reduce numbers of parasites during later stages of infection (Kobayashi, 1986).

2.5. Concurrent parasitic infections

Trypanosomosis is classically associated with profound suppression of immune response of the affected animals (Goodwin, 1970; Goodwin *et al.*, 1972), which show reduced and antibody responses, usually become increasingly susceptible to concurrent bacterial and viral infections and often die from fulminating parasitaemia and/or other complications (Onah, 1992). In addition, African trypanosomes are also known to suppress responses to a number of helminth parasites, resulting in increased fecundity, worm burden and failure of immune-mediated expulsion of the adult worms.

The process immune expulsion of primary and secondary *Nippostrongylus brasiliensis* infections in mice and rats (Urquhart *et al.*, 1973) and *Trichuris muris* infection from mice (Phillips et *al.*, 1974) is suppressed by *Trypanosoma brucei* super-infection. Moreover, mice vaccinated against the gastrointestinal nematode parasite, *Trichinella spiralis* using homogenate antigen

preparations express strong protective immunity against infection with the infective third stage larvae (L3) of the parasite. This protective usually evidenced by early expulsion of the adult worms from the intestine, female worm fecundity, strong parasite-specific IgG1, IgG2 and IgM responses strong interleukin-4 (IL-4) and IL-5 responses (Onah and Wakeline, 1999) protective immune responses are significantly suppressed if the mice were infected *T. brucei* either after primary and secondary vaccination (Onah and Wakeline, 1999).

Both nematode parasites and trypanosomes may enhance the pathogenicity of each other as it was also demonstrated that while deaths occurred Djallonke sheep given dual infection of *Haemonchus contortus* and *T. congolense*, singly infected with either of the parasites survived (Goossens *et al.*, 1997). Concurrent *H. contortus* and *T. congolense* infection in N'Dama calves resulted reduced prepatent period, increased weight loss, severe anaemia and high mortality (Kaufmann, 1992).

Animals infected with trypanosomes have usually found to be frequently infected with strongyles and the egg counts of trypanosome infected cattle have been found to be higher than those where no trypanosomes were detected in the blood (Dwinger *et al.*, 1994). High mortality rates, low PCV levels and increased weight loss in goats have been reported due to concomitant experimental infections with *T. congolense* and H*aemonchus contortus* (Griffin *et al.*, 1981). Shilima (2005) indicated a significant increased effect of trypanosome infection on PCV in donkeys of southern Ethiopia when animals are affected by both trypanosome and nematode infections rather than mono infection with either parasite.

Similarly Zewde *et al.* (2001) suggested that concurrent trypanosome and fasciola infection is the most harmful combination of parasitosis that indicated an increased pathogenicity when helminthosis is superimposed on trypanosome infections. The pathogenic effects of concurrent infections due to trypanosoma and fasciola species were predominantly influenced by trypanosome infections.

However, there are indications that some stereotypic immunological responses to parasitic helminths may be beneficial in providing animals with protection against related and unrelated pathogens thereby, ameliorating their pathogenic effects. For instance, some studies have demonstrated *Schistosoma mansoni*, which induces strong T helper 2 (Th2) immune responses of the host into Th2 dominance against unrelated antigens normally induce Th1 type responses (Curry *et al.*, 1995; Sabin, 1996). Trypanosomes induce strong Th1 type responses in infected animals, and enhance parasite growth and render the infected animals incapable of controlling resulting parasitaemia (Olsson *et al.*, 1993; Schleifer *et al.*, 1993; Bakhiet, 1996). In contrast, murine strongyloidiasis (*Strongyloides ratti* and *S. venezuelensis*) induces strong Th2 type responses, resulting in the expulsion of the adult parasites 2 weeks of a primary infection (Ishiwata *et al.*, 1999) and in the conferment of strong protective immunity against subsequent challenge infections (Sato and Toma, 1990).

Pre-existing infection with *O. ovis* larvae induced adverse effects on subsequent, unique infection with either *T. columbriformis* (Yacob *et al.*, 2002) or the abomasal worm, *H. contortus* (Dorchies *et al.*, 1997). Similarly, repetitive *O. ovis* infections have a dramatic influence on pre-existing populations of *T. columbriformis* (Yacob *et al.*, 2004). The presence of *O. ovis* in co-infected sheep was associated with significant decreases in egg excretion and in the number of worms found at necropsy. However, the extent of the changes for both parameters appeared more important when the sheep were infected with *O. ovis* before the nematodes (Yacob *et al.*, 2002).

Similarly, the significantly lower FEC and in utero egg counts registered in concurrently infected group of animals with *O. ovis* and *H. contortus* as compared to group infected with *H. contortus* alone indicates that a previous *O. ovis* infection has led to limited fecundity of *H. contortus*. (Terefe *et al., 2005*) There was no significant difference in the total worm burden as well as in the number of female and male worms between the two groups. Hence, this difference in the number of eggs might be directly related to the reduced length of female *H. contortus* in group with *O. ovis* and *H. contortus* and/or retardation in worm development and suppression in egg production (Terefe *et al., 2005*).

3. MATERIALS AND METHODS

3.1. Study area

The experimental study took place between October 2005 and March 2006 at the compound of fattening project of the Faculty of Veterinary Medicine in DebreZeit on goats obtained from Metehara market some 190 kms from the capital. This is marketing area for the pastoralist people of Afar, Somali and Oromo. DebreZeit 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 800mm. The area has an average maximum and minimum temperature of 27.7 ^{o}C and 12.3 ^{o}C, respectively (CACC, 2003). DebreZeit is the center of Ada'a Liben woreda, and , the Woreda has a total land area of about 161,056he and is divided in to three agro-ecological zones namely midland (94%) highland (3%) and lowland (3%) (Ada'a Woreda agricultural and rural development office).

3.1.1. Experimental animals and management:

Taking into account the approximate uniformity on estimated body weight, age, fourty five goats born and reared around Metehara area, were purposively selected and purchased from Metehara market some 190 km from the capital Addis Ababa, where livestock marketing from pastoralists of Afar, Somali and Oromo people took place. The areas are known to be free from cyclically transmitted animal trypanosomosis (Abebe and Jobre, 1996), which is prevalent in south and southwest part of the country. Therefore these animals could not have been infected with *T. congolense* before, and all the experimental animals purchased were reared under traditional management conditions.

All the purchased animals were vaccinated against pasteurollosis before transportation and upon arrival they were treated with long acting oxy tetracycline and with Albendazole at 10mg/kg

body weight to remove any burden of the parasites and then checked for three consecutive faecal egg count performed on the 1st, 2nd and 3rd weeks during the 21 day adaptation period and at the same time treated with Berenil 3.5mg/kg of body weight to clear any parasite that may present.

All animals were housed in an isolation area with raised concrete based units and a solid partition separated adjacent pens and care was taken to avoid contamination of pens with nematode larvae from outside. All the animals were allowed to fed on concentrates consisting of 25% Nug cake and 75% of wheat bran out of the total 250 gram daily intake of concentrate that was provided to each animal. Water and locally dried hay was provided *adlibitum* throughout the adaptation and experimental period to preclude any parasitic infection. At the end of the adaptation period animals were weighed, ear tagged for easy identification and randomly assigned into five groups consisting five animals each. All animals were bled twice before the start of the actual experiment and the data was served as the pre infection data.

Before the resumption of the experimental trail all animals were evaluated for base line haematological values and the absence of blood and nematode parasites, and all were found to be free from trypanosome and any nematode infection. Only 25 animals were used during the actual experiment the rest were withdrawn during the adaptation period.

3.2. Experimental design

A randomized complete block design was used and goats were randomly allocated into blocks based on their approximate similarities on estimated body weight (18-24 kg), age (1 year old), and sex (all are males). Within blocks experimental animals were randomly assighned into one of the five treatment combinations: Group T, *T. congolense* infected group, GroupTH, *T. congolense* infection followed by super infection of *H. contortus* one week later, Group H, were infected by *H. contortus* only, Group HT, one week pror ifection of *H. contortus* followed by super infection of *T. congolense* and Group C, animals kept free of any infection and served as negative control group.

The *T. congolense* isolate used in this experimental study was obtained from the blood of naturally infected cattle at Gibe valley. The blood samples were collected in the capillary tubes and immediately centrifuged and the capillary tube was cut 1mm below the buffy coat zone and expressed onto a slide and covered with a cover slip and examined for the presence of the parasites. Then 0.2 ml of a blood samples that were positive for *T. congolense* were immediately inoculated into a donor mice and then transported to the laboratory. After 3 weeks of inoculation period in the mice, the mice were anaesthetized with chloroform and 1ml of blood sample was obtained intracardially from infected mice and immediately inoculated into donor goats. That serves as a source of infective material for the experimental animals. Each experimental animals were infected with the total intravenous inoculation of $5x10^4$ *T. congolense.*

Infective larvae of female worms were obtained from goats slaughtered at DebreZeit ELFORA export abattoir. Worm free donor sheep were infected with *H. contortus* 3rd stage infective larvae and have been used as a source of experimental infection. The total infective dose of *H. contortus*, which was given orally to each animals was, 10,000 3rd stage larvae. The details ofexperimental designs are indicated in table 1 and 2.

Table 1: Experimental animal partition in single and mixed infections.

Group	Number of Goats	Infection	Super infection
T	5	*T. congolense*	
H	5	*H. contortus*	
TH	5	*T. congolense*	*H. contortus*
HT	5	*H. contortus*	*T. congolense*
C	5	Negative controls	

Where,

Group T: In this group each animal was infected with $5x10^4$ *T. congolense* during the initiation of the experiment at week 1.

Group H: Each animals assigned in this group were infected with 10,000 third stage *H. contortus* larvae at week 1.

Group TH: All animals assigned in this group were infected with $5x10^4$ *T. congolense* at week 1 and super infected one week later with 10,000 3rd stage larvae of *H. contortus*.

Group HT: Each animals of this group were infected with 10,000 *H. contortus* third stage larvae at week 1 and super infected one week later with $5x10^4$*T. congolense* at week 2.

Group V: Animals of this group were maintained free of infection and served as an overall negative control group. (See table 2).

Table 2: Design of the experiment for evaluating interaction between experimental infections of *T. congolense* and *H. contortus* in one-year-old goats.

Group	Number of Goats	Week of experiment								
		0	1	2	3	4	5	6	7	8
T	5	_	T*							_
H	5	_	H*							WC*
TH	5	_	T*	H*						WC*
HT	5	_	H*	T*						WC*
C*	5	_	_	_	_	_	_	_		

Where,

T* Group of animals intravenously inoculated with *T. congolense* at a dose rate of $5x\ 10^4$ at week 1.

H* Animals infected with 10,000 3rd stage larvae of *H. contortus* orally at week 2.

TH* Group intravenously inoculated with *T.congolense* at a dose rate of $5x\ 10^4$ at day 1 and super infected with 10,000 3rd stage larvae of *H. contortus* orally at week 2 post infection.

HT* Animals orally infected with a primary *H.contortus* infection with infective dose of 10 000 L_3 at week 1, followed by intravenous inoculation of a dose 5x 10^4 *T. congolense* at week 2.

C* Animals of group were maintained free of infection and served as an overall negative control group,

WC* Animals slaughtered to determine total worm count at week 8 post infection by either parasites

At the end of the experiment on the interaction of the two anaemia causing diseases (at the end of week 8), 2 animals from each of the *H. contortus* infected groups (Groups H, TH, HT, N=2 for each Group) were slaughtered for determining worm burden. All the remaining animals assigned in *T. congolense* infected group have been treated with Berenil (3.5 mg/kg) to clear the parasite and animals infected with *H. contortus* were treated with Albendazole (10mg/kg) at the end of treatment trail.

3.3. Measured parameters

All experimental animals were monitored weekly for the following parameters: Body weight, Winterobe index (Haemoglobin determination, Packed Cell Volume, Red Blood Cell count, MCV, MCHC), Total White Blood Cell Count, Differential Leukocyte count, Trypanosome Parasitaemia, Total protein, Egg per gram of faeces (EPG), Total worm burden. However, EPG was taken on daily basis 2 weeks post infection.

3. 3. 1. Packed Cell Volume

Blood samples were collected by bleeding animals from marginal ear veins into paired heparinized microhaematocrit capillary tubes up to ¾ of their length. One end of the tubes were then sealed with cristaseal (Hawaksly, England). The tubes were symmetrically loaded in the haematocrit centrifuge, with the sealed end outwards, centrifuged at 1200 rpm for 5 minutes.

PCV levels of individual samples were determined on haematocrit reader (Hawaksly, England) and the values were expressed in percentages (Woo, 1970).

3. 3. 2. Parasitaemia

All centrifuged samples in the capillary tubes used for PCV level determination were subsequently used for measuring the weekly levels of trypanosome parasitaemia in all treatment groups. Each microhaematocrit tube was cut 1cm and 1mm below the buffy coat layer. The content then expressed on clean slide, examined under a 40x objective microscope by the buffy coat method and the intensity of infection was reported as described by Murray *et al.*, 1977.

3 .3. 3. Faecal egg output

Fresh faecal samples were collected directly from the rectum of all the experimental animals during the weekly sampling occasions. Specimens were transported to the laboratory in a screw cap bottle under airtight condition, egg per gram (EPG) of faeces was determined employing the standard Mc Master techniques using saturated salt floatation medium (Kaufmann, 1996).

3. 3. 4. Differential leukocyte count

Thin blood samples were prepared for the purpose of carrying out differential leukocyte count. Thin smears were first air dried and fixed with methanol for 3-5 minutes, and stained with Giemsa for 30 minutes, washed with distilled water and dried on the air. Thin smears were microscopically examined under oil immersion magnification (X100) and counting and classifying of 200 leukocytes were made using Battlement method and finally values were expressed in percentage and then converted into numbers using the total WBC counted for that particular study period (Dacie and Lewis, 1991).

3. 3. 5. Body weight change

Body weight changes of individual animals have been measured weekly using the weighing balance. Animals were tied in their chest and then hanged in the weighing balance and then the reading of their body weight was registered.

3. 3. 6. Total White and Red Blood Cells, and Haemoglobin concentration

Blood samples were taken weekly from the jugular vein with anticoagulant, ethylenediaminetetraaceticacid (EDTA) coated vacutainer tubes, and prepared for automated electronic counting of total red cells, total white cells and haemoglobine concentration by using Coulter Diluer Dispenser (Coulter Electronic limited Ltd; UK). Other erythrocyte induces of interest were derived by computation and include mean corpuscular volume (MCV) mean corpuscular haemoglobine concentration (MCHC).

3. 3. 7. Total plasma protein

For determination of total plasma protein concentration the Biuret method that uses a photometer was employed, which is the simplest chemical quantitative analytical method (Coles, 1986).

3. 3. 8. Worm burden count

At week 8 post experimental infection 2 animals from *H. contortus* infected groups (Group H, TH, HT) were slaughtered after the end of the trail to assess the worm burden. The abomasum was separately ligated and the contents examined to count for the number of adult and immature parasites using the classical counting procedure indicated in (MAFF, 1977; Hanson and Perry, 1994).

3. 4. Statistical Analyses

All statistical analyses were conducted using SAS package version 9 (SAS institute 2000) for windows. All parameters measured more than a single day were analyzed by the general linear model as repeated measurement, using a fixed model, (GLM procedure) considering time as the

within subject factor and treatment as between subject factor. The following main effects were included: Infection (control versus infected), (mono infected versus mixed infected) block, period (acute week 0-8) goats (nested within treatment and block) and the interaction between the (infection) two parasites across period. Considering the design of the experiment, joint effect of infection by both trypanosome and nematode is of high interest and tested in the model by the interactions between infection by both parasites and period. The animal effect was considered as random. The means were evaluated for significant using Duncan's multiple range tests (Duncan, 1959). Probabilities ($P<0.05$) less than 0.05 were considered significant.

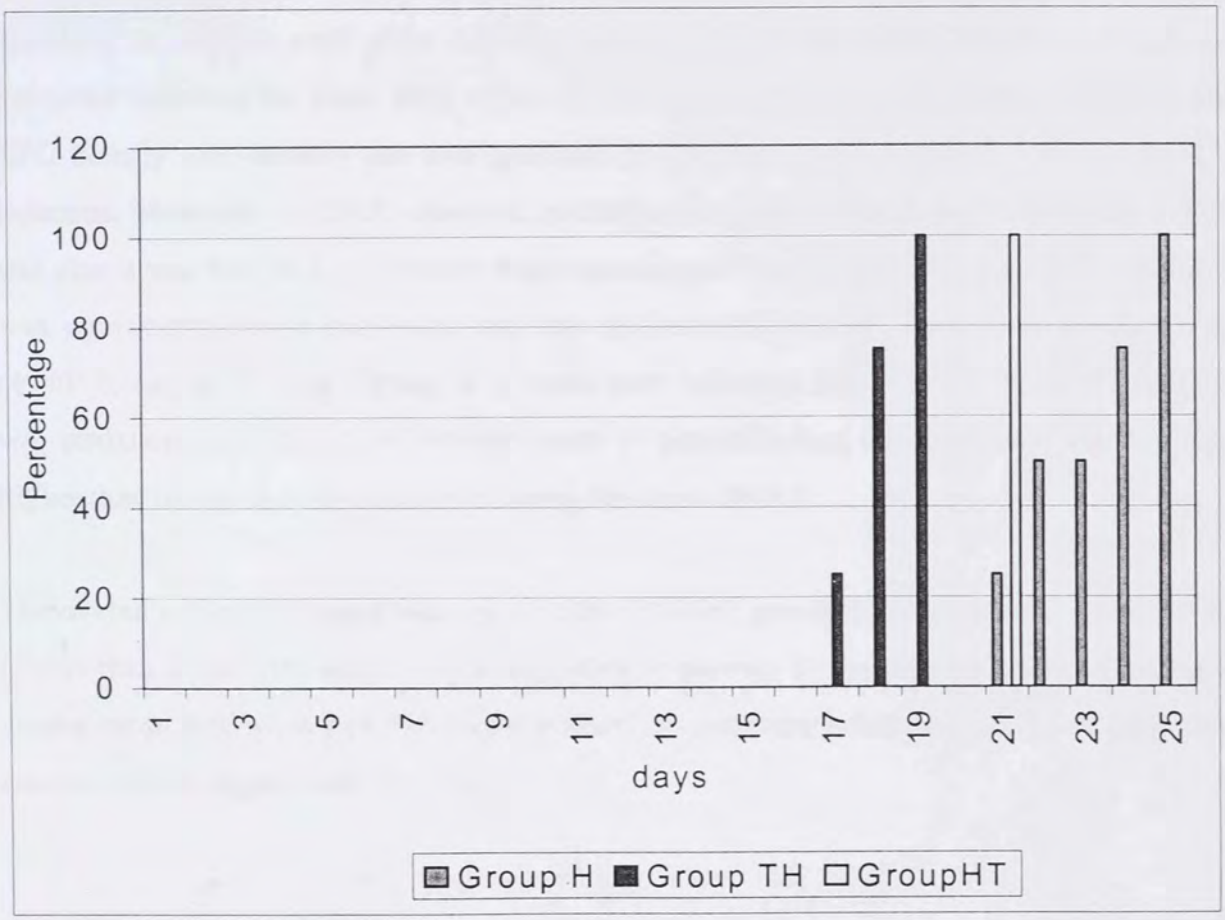
4. RESULTS

4.1. Parasitological parameters

4.1.1. Egg excretion of *Haemonchus contortus*

All goats experimentally infected with *H. contortus* 3rd stage larvae and assigned into different experimental groups started shedding of eggs on different days post infection. Figure 1 indicate the percentage of animals in relation with time where shedding of eggs commenced post infection.

Figure 1. The proportion of animals shedding eggs in relation with time post infection.

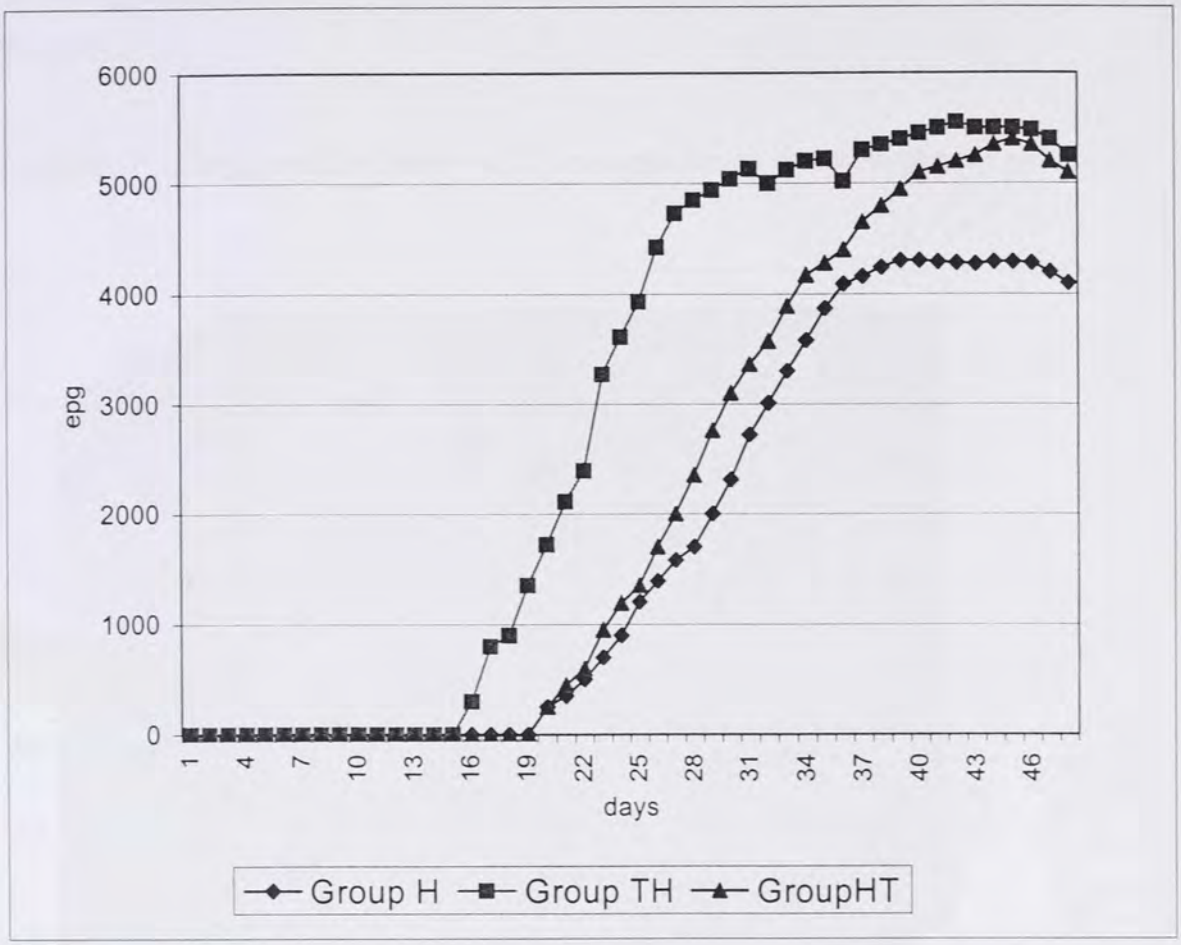


All the animals assigned in Group H started to excrete eggs gradually and at the beginning, the percentage of animals passing eggs on day 21 post infection was only 25%. %. Following day 21 post infection the proportion of animals shedding eggs increased, and on day 25 post infection all animals in this group have shown positive faecal samples for *H. contortus* eggs (Fig1). Animals in Group TH were commenced shedding of eggs in the faeces on day 17 post infection where 25% of the animals found to contain *H. contortus* eggs. The number of infected animals on day 18 reached to 50 %. By day 19 all animals in this group found to discharge *H. contortus* eggs in the faeces (Fig1). In Group HT all (100%) the animals began to excrete eggs on day 21(Fig. 1).

Figure 2 indicates the mean EPG values among different treatment groups. In animals infected with *H. contortus* alone the mean EPG steadily increased from 250 on day 21 post infection and reached to peak of 4300 on day 42 (Fig 2). In primary *T. congolense* infection the mean EPG values of 300 were recorded on day 17 post infection and the EPG sharply rose initially and then gradually to reach a peak value of 5500 on day 42 post infection (Figure 2). In primary *H. contortus* infection the mean EPG values of 300 were recorded on day 21 post infection and the EPG sharply rose initially and then gradually to reached a peak value of 5200 on day 42 post infection. Mean epg of (2000) observed in Groups H at week 4 was lower than Group TH (4940) and also it was statistically different from mean epg of this group ($P<0.05$). Similarly this value was significantly lower than mean epg seen in Group HT (2760). Though mean weekly epg of (4940) in Group TH was highest in 4 weeks post infection, than that of Group HT (2760), this was statistically similar at week 5 and week 6 post infection, but this value was significantly higher than the mean epg in Group H during this time ($P<0.5$).

The overall mean EPG value was statistically ($P<0.05$) greater in animals with mixed infections (3350) than those with single infection (2400). Preceding *T. congolense* infection resulted in a greater mean EPG values (4250) than a primary *H. contortus* infection (3150) and the difference was statistically significant ($P<0.05$).

Figure 2. The mean EPG values among different treatment groups of the experiment in relation with time post infection.



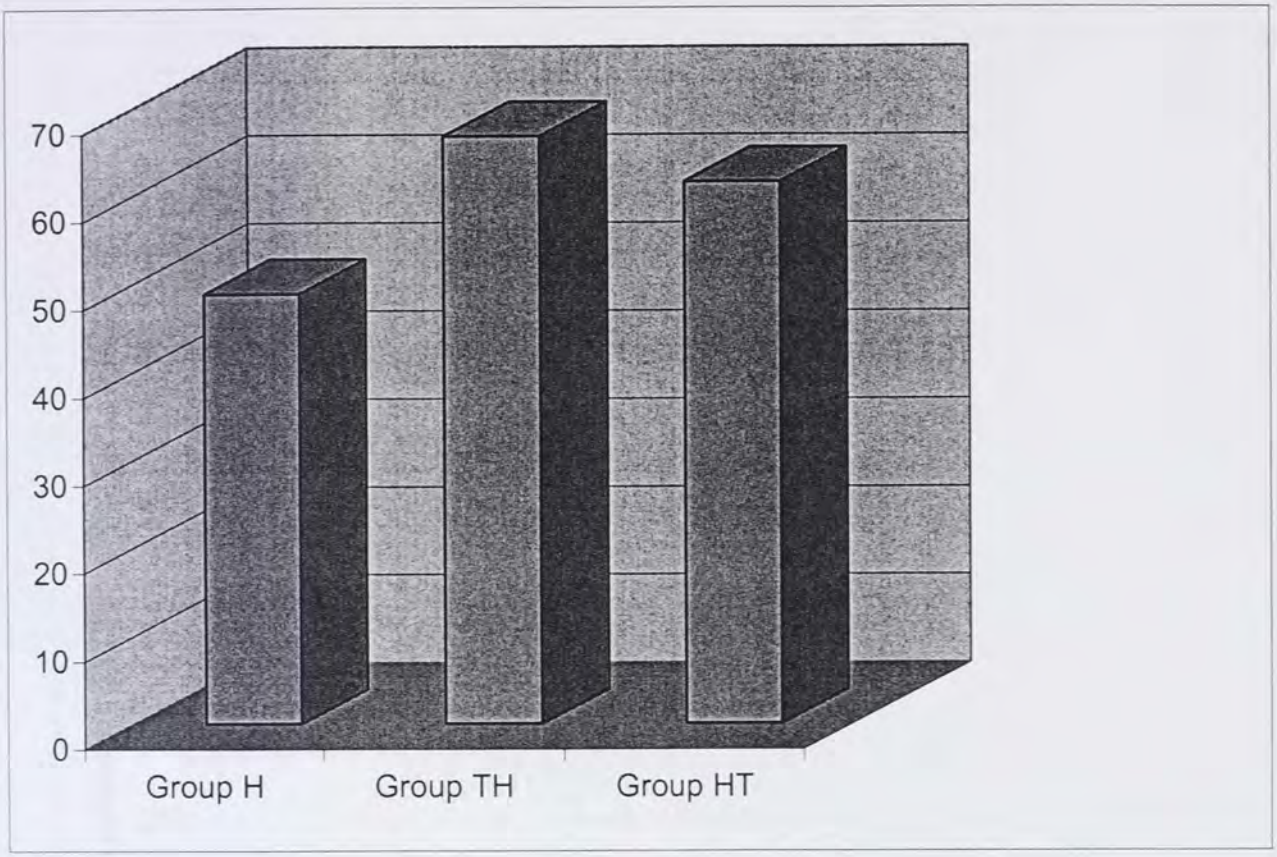
4.1.2. Total worm burden

The highest mean worm burden was observed in goats that received trypanosome and nematode mixed infections. There was a statistically significant difference ($P<0.05$) in the mean worm burden between mixed (6422.5) and mono infected groups (4882). Although proceeding *T. congolense* infection resulted higher mean worm burden than *T. congolense* subsequent infection, the difference was not statistically significant ($P>0.05$).

Higher percentage in the establishment of *H. contortus* 3rd stage larvae was observed in Group TH (66.70%) followed by Group HT (61.75%) and Group H (48.82%), respectively (Fig. 3).

There was a statistically significant difference ($P<0.05$) in the establishment of the larva between primary *H. contortus* infection and *H. contortus* mono infection. However, the difference between preceding and subsequent infections of *T. congolense* was not statistically significant ($P>0.05$).

Figure 3: Percentage establishment of *H. contortus* larvae among treatment groups.

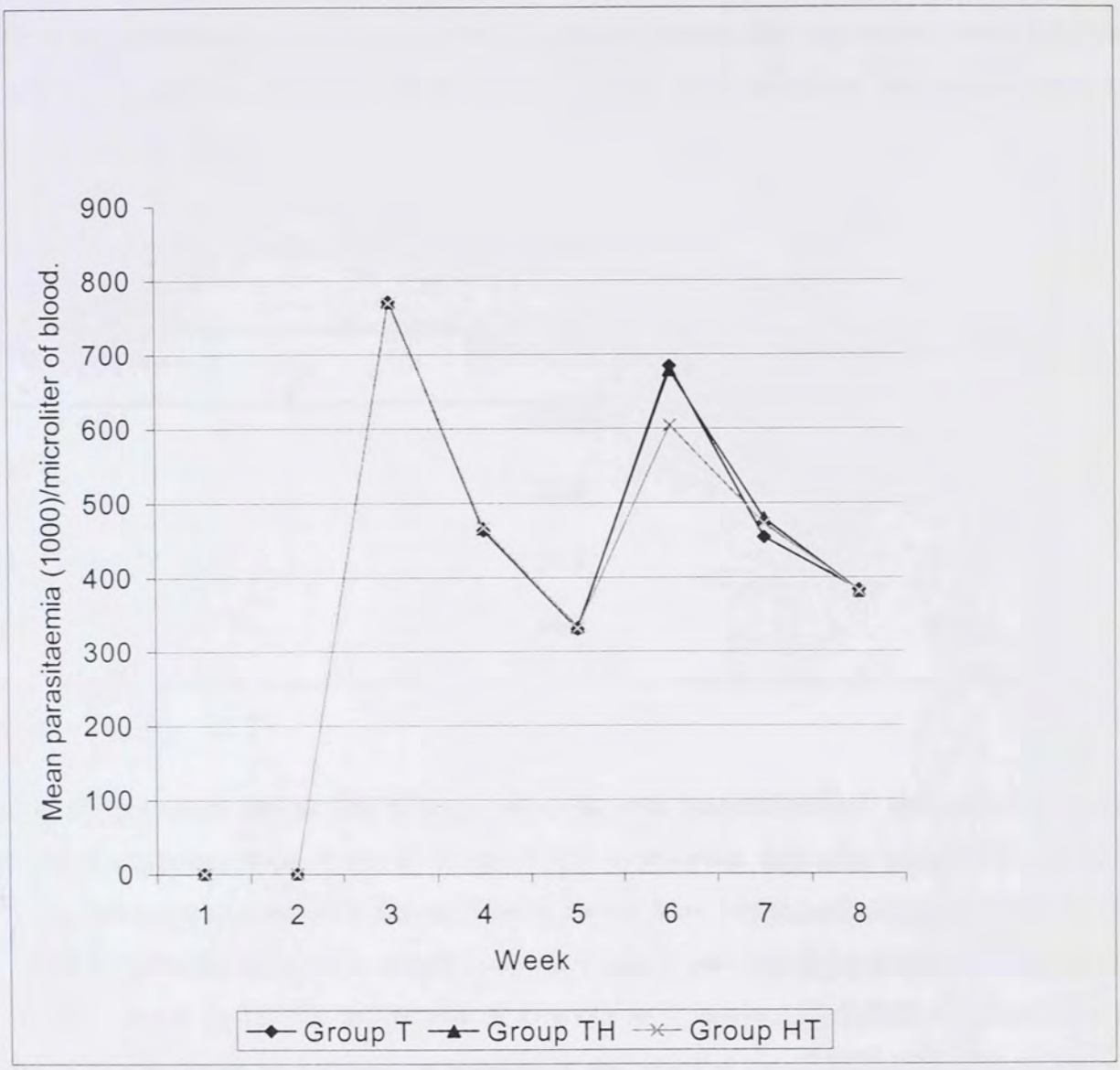


4. 1. 3. Trypanosome parasitaemia

In all trypanosome-infected groups, the progression of trypanosome parasitaemia was similar. *T.congolense* parasitaemia follows a time course in which all infected goats become parasitaemic within one week (day 7) post infection and have shown increasing trend before reaching a peak of 771×10^3, 771×10^3, and 769×10^3 mean trypanosomes per milliliter of blood at day 14 post *T. congolense* infection for groups T, TH and HT, respectively. There was no statistically significant difference ($P>0.05$) in the overall mean trypanosome parasitaemia per µl of blood between mixed

infected groups (407.95 x 10^3) and mono infected with *T. congolense* (370.15 x 10^3). The above result shows that *H. contortus* either preceded or superimposed by *T. congolense* did not affect the progression of trypanosome parasitaemia (Figure 5).

Figure 4. Mean trypanosome parasitaemia among trypanosome infected groups in relation with time post infection.



4. 2. Haematological parameters

4. 2. 1. Packed cell volume

Figure 5 indicate the mean PCV values among treatment and control groups across time. PCV levels were significantly ($P<0.05$) lower in all infected groups than the control group (29.24%). Details of the analysis of Least square means of PCV from treatment and control groups is presented in table 2 below.

Table 3: Least square means of PCV among treatment groups.

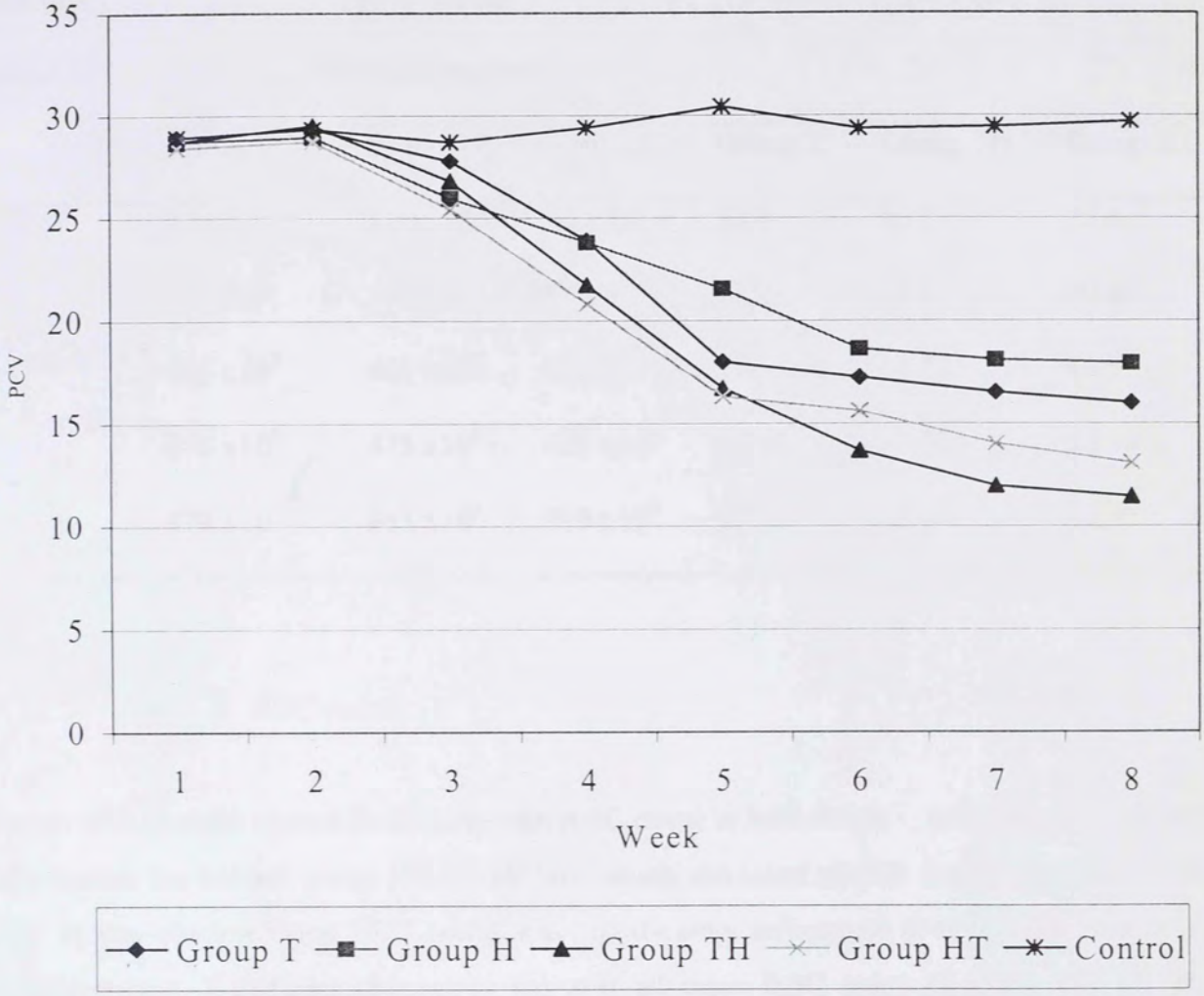
Group	Mean PCV (%)	Absolute mean difference (%)
C	29.243	-
T	21.6	7.63
TH	20.1	9.143
TH	20.3	8.283

During the prepatent period (the first 3 – 4 weeks post infection) of *H. contortus*, in animals infected *H. contortus* alone, dramatic fall in PCV was observed, then after progressive decline in PCV was seen up to the end of the experimental period. Similarly mixed infection of *H. contortus* one week prior to infection of *T. congolense* had shown to decrease from 28.72% before the start of the experiment to 12.95% at the end of experiment 7 weeks post infection. There after the mean PCV was found to decrease progressively and reached mean PCV of 12.5% at week 7. Similar trend of PCV decline was observed in Group HT in which the PCV falls slightly at the start of the experiment between weeks 1 and 2 from 28.72 % to 25.4%.

Animals infected only with *T. congolense* have also shown similar progressive decrease in PCV starting between weeks 1 and 2 at which the PCV slightly falls from 29.3% to 27.6%. This progressive decline in PCV has been shown to continue until week 7 by the time the PCV recorded was 15%. Thus the PCV changed with time across all treatments and was lowest in the two treatment groups infected with both *T. congolense* and *H. contortus*.

The overall decrease in PCV was significantly ($P<0.05$) greater in goats with mixed infections (20.2%) than those with single infections (22.28%). The mean PCV depression caused by a primary *T. congolense* was (absolute mean difference of 9.143%) greater than the mean PCV depression (absolute mean difference of 8.943%) resulted from a preceding *H. contortus* infection but the difference was not statistically significant ($P>0.05$). Although animals infected with *T. congolense* alone showed a greater PCV decline (absolute mean difference of 7.6%) than the loss induced by *H. contortus*, (absolute mean difference of 6. 3%) the difference was not statistically significant ($P>0.05$). The mean PCV of control group remained similar throughout the whole observation period (29.2%). The difference among control group and animals infected either singly or with mixed infections were statistically significant ($P<0.05$) (Fig.5). Two animals were died in Group TH, one in Group HT, but non in group of animals infected only with a single parasite.

Figure 5: The mean PCV values of among treatment groups in relation with time post infection.



Acute phase of trypanosome infections was characterized by progressive anaemia accompanied by parasitaemia and the initial fall in PCV is associated with the first wave of parasitaemia (Table 3).

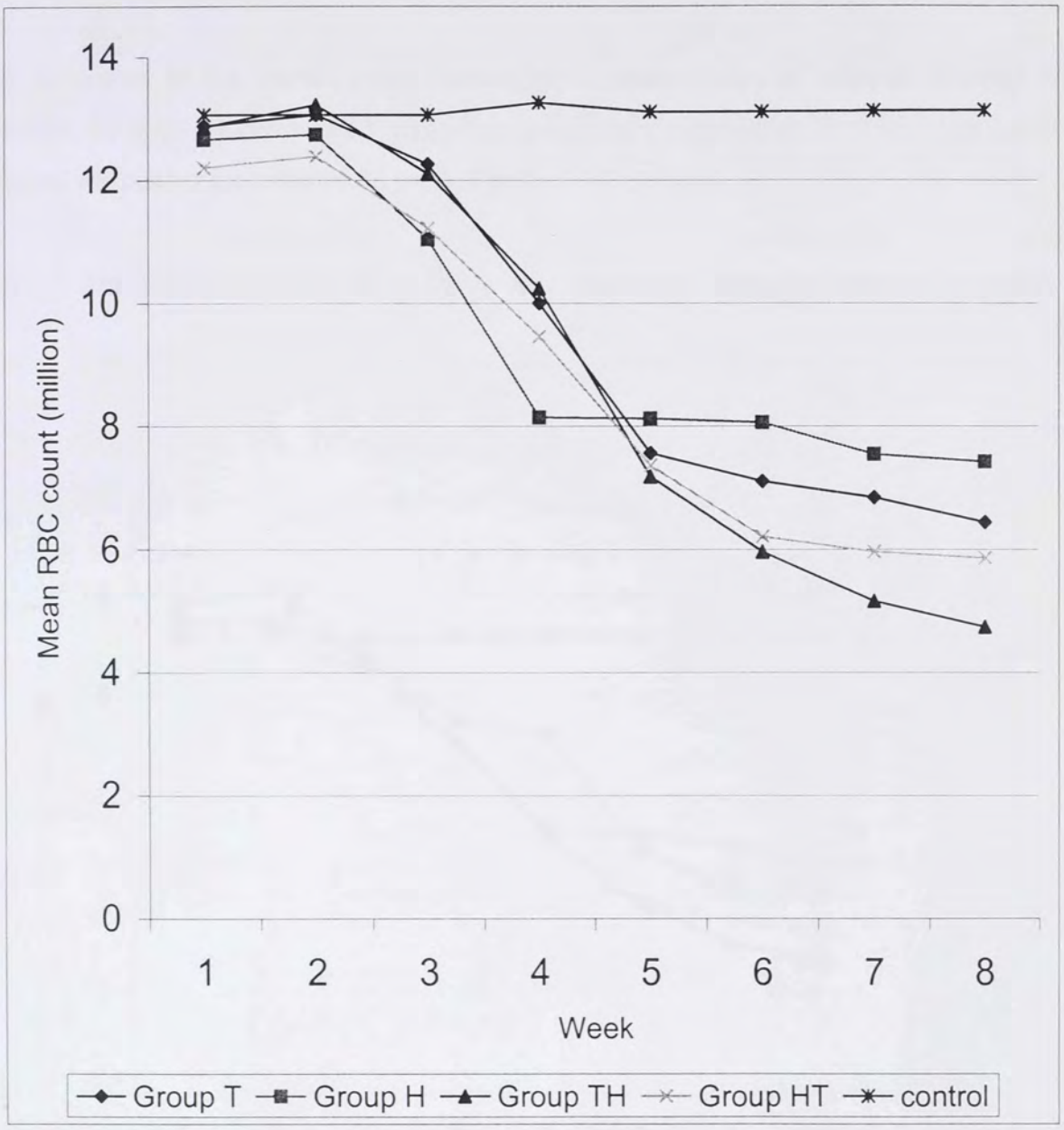
Table 4: The PCV values and the parasitaemia of different treatment groups in relation with time.

Weeks post infection	Parasitaemia Trypanosome/ml			PCV (%)		
	Group T	Group TH	Group HT	Group T	Group TH	Group HT
2	$2.4x10^2$	$2.2 x10^2$	$2.2 x10^2$	27.6	26.8	25.4
3	$771x10^3$	$771x10^3$	$771x10^3$	22.9	21.6	20.82
4	$463 x10^3$	$463 x10^3$	$463 x10^3$	17	16.72	16.3
5	$678 x10^3$	$478 x10^3$	$475 x10^3$	15.46	11.96	13.63
6	$479 x10^3$	$463 x10^3$	$463 x10^3$	15.02	11.44	12.95

4. 2. 2. RBC counts

Infection with parasite caused decline in mean RBC count in both single and mixed infected groups versus the control group ($13.03x10^6/\mu l$), which remained similar during the observation period. It was observed that RBC count was significantly influenced ($P<0.05$) by time in all treatment groups. Significant changes in the over all mean RBC count ($8.653 \times 10^6/ \mu l$) was observed at week 4 post infection. The overall mean change of erythrocyte count of animals infected with mixed infections ($8.65 \times 10^6/ \mu l$) was more marked as compared to single parasite infection ($9.5 \times 10^6/ \mu l$) and this difference was statistically significant ($P<0.05$) (Fig 6).

Figure 6: The pattern of mean RBC count among the treatment groups in relation with time.



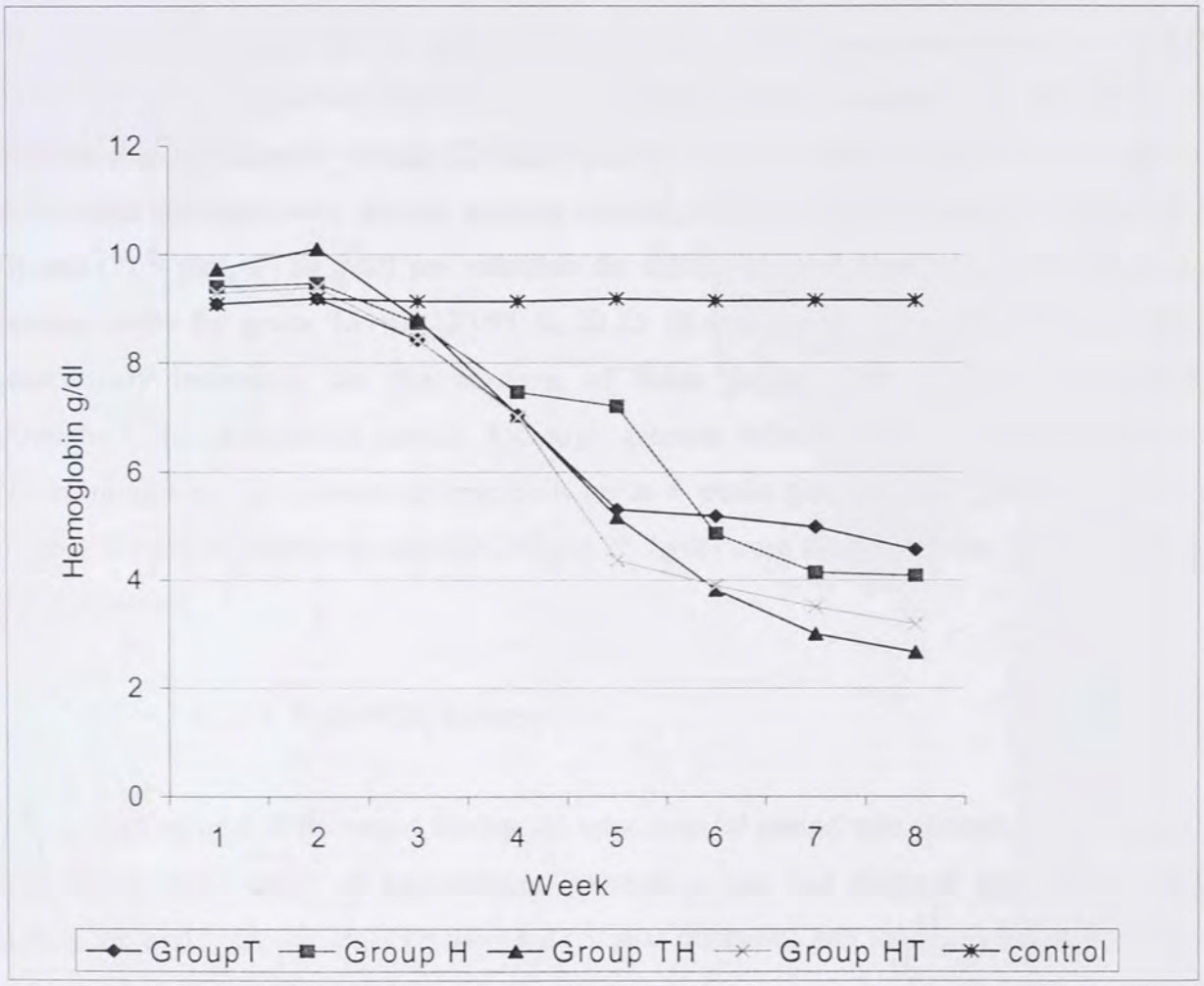
4. 2. 3. Haemoglobin

In all infected animals, either single infection (*T. congolense* and *H. contortus*) or mixed with the presence of both parasites irrespective of the order of infection, decline in mean haemoglobin

concentration was observed throughout the whole observation period compared to the higher mean levels of haemoglobin maintained in the control group (9.2 g/dl) and the difference was statistically significance ($P<0.05$).

The difference in the overall mean haemoglobin concentration of animals infected with the presence of both parasites (5.23 g/dl) was statistically significant ($P<0.05$) than those single infected with either parasites (6.83 g/dl) (Fig 7).

Figure7: The mean levels of haemoglobin among treatments during the observation period.



4. 2. 4. MCV and MCHC

Post infection all treatment groups have showed a common pattern of MCV and MCHC response during trypanosome and haemonchus infection, except Group H animals, which have showed the relative increase in the mean MCV value at weeks 6 and 7 post infection and the relative drop of MCHC values at similar weeks post infection. The result of the present study indicate that the MCV and MCHC values of all treatments remained under normal range for MCV and MCHC values for goats (Coles, 1986) similarly no difference was noted as compared to the control group ($P>0.05$).

The mean MCV and MCHC values of animals infected with *T. congolense* alone, was 21.4 fl and 31.02 g/dl pre infection and 23.5fl and 30.7 g/dl post infection, respectively. The above results indicate that the anaemia during the acute phase of trypanosome infection of this group was normocytic normochromic. Similar patterns of mean MCV and MCHC values of (22.46 fl, 23.22 fl) and (32.7 g/dl, 33.88 g/dl) pre infection for Group TH and Group HT were maintained at normal levels for goats, having (23.98 fl, 22.25 fl) and (31.02 g/dl, 32.3g/dl) post infection, respectively indicating that the anaemia of these groups was normocytic normochromic throughout the observation period. Although animals infected with *H. contortus* alone have shown normocytic and normochromic cells up to 4 weeks post infection (23.4fl and 29.3g/dl), macrocytic and hypochromic cells (26.3fl and 27.3g/dl) were observed there after until the end of the experiment.

4. 2. 5. Total WBC Count

The pattern of total WBC count during the experimental period was plotted in the figure8. The mean total WBC count of trypanosome-infected groups had dropped significantly ($p<0.05$) during the first peak of parasitaemia (14 days post infection) and remained below control levels (13.35×10^3/ml) throughout infection.

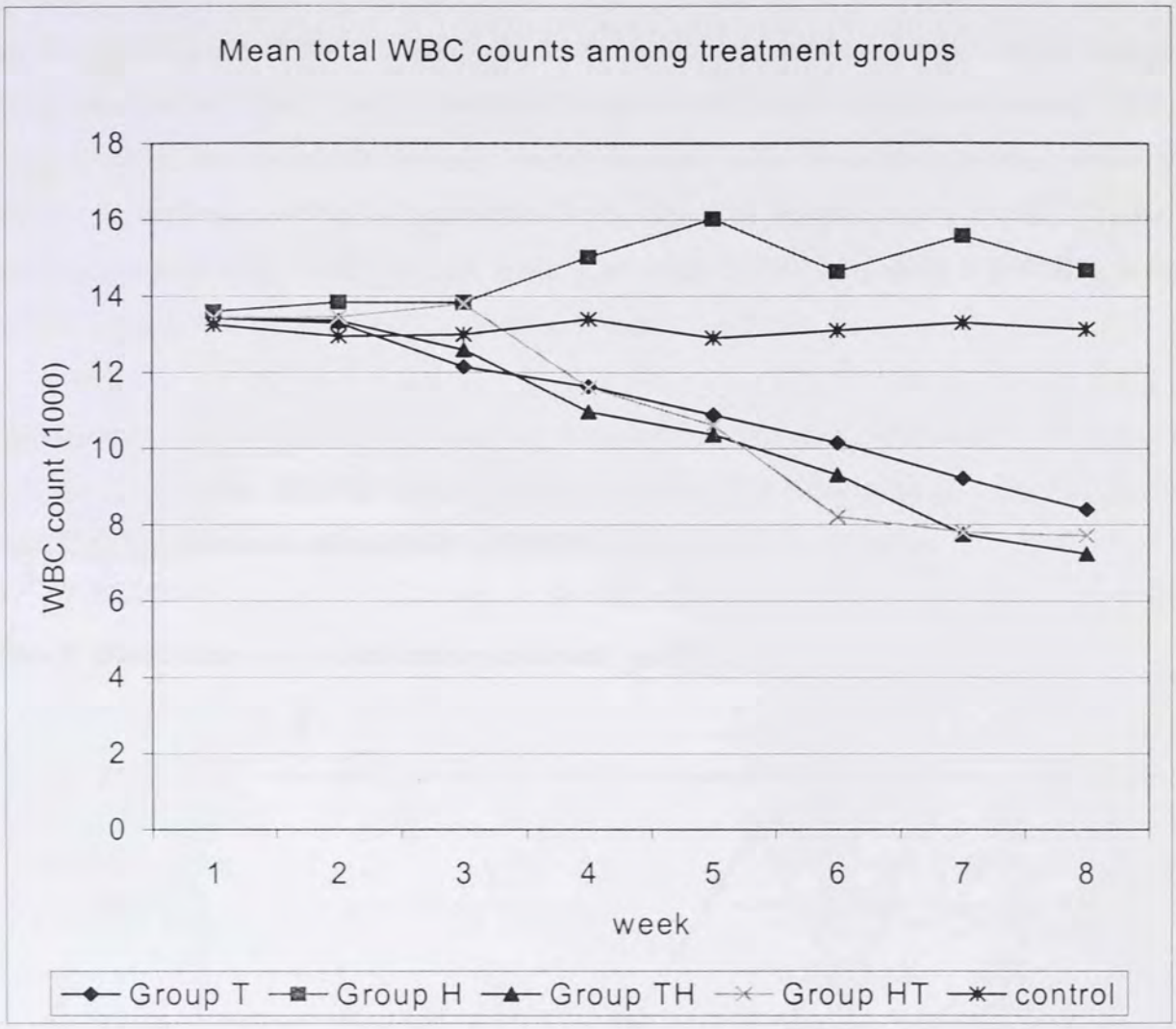
Total WBC counts of the three treatment groups, TH, HT, and T have shown progressive decline following challenge infection from 13.36×10^3/ml, 13.44×10^3/ml, 13.3×10^3/ml pre infection

levels to 7.24 $x10^3$, 7.68 $x10^3$, 8.22 $x10^3$ post infection, respectively. Although preceding *T. congolense* infection followed by subsequent *H. contortus* infection (10.62 $x10^3$/ml) resulted in lower mean total WBC counts, the differences between the three groups (11.13/ml) were not statistically significant ($P>0.05$).

The above observation indicates that *T. congolense* either in single or mixed infections resulted a pronounced reduction of total WBC. Total WBC count of the control group (13.35 $x10^3$/ml) remained higher than that of the other treatment groups and the difference was statistically significant ($P<0.05$).

Mean total WBC counts of group H was slightly higher (14.65 $x10^3$) than the control group (13.35 $x10^3$/ml) and the difference were statistically significant ($P<0.05$) indicating active involvement of host immune response against the nematode that resulted in higher total WBC counts over the controls (Fig. 8).

Figure 8. Mean total WBC counts among treatment groups along with time.



4. 2. 6. Differential leukocyte Counts

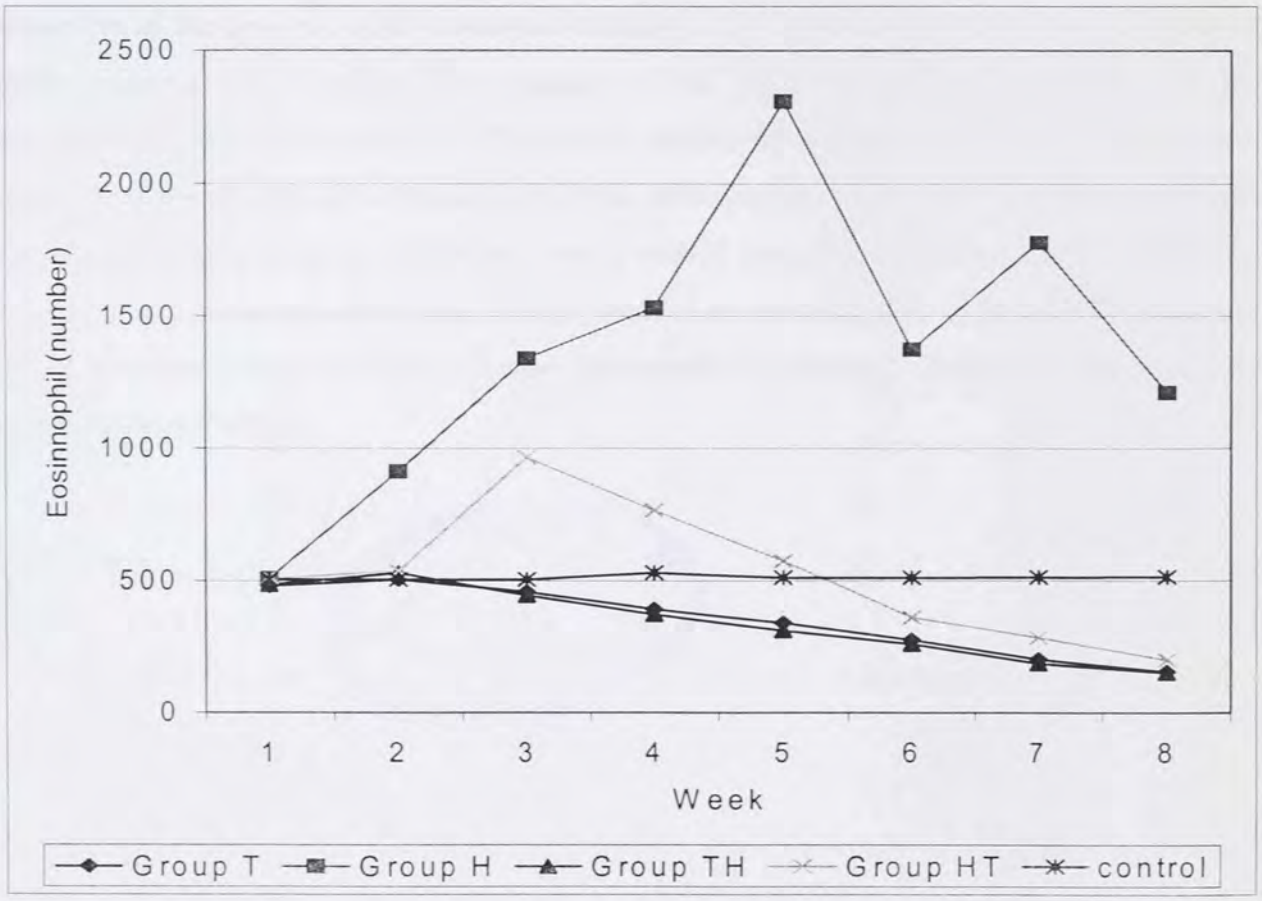
4. 2. 6. 1. Eosinophils

Eosinophil counts were found to progressively decline throughout the whole observation period, in goats infected either with the trypanosome alone (351.15) or mixed infections with preceding trypanosome and subsequent nematode infections (342). The mean counts of blood eosinophils in uninfected parasite free goats (controls) did not changed significantly (504.4) over the period of the experiment (Fig. 9).

The mean eosinophils number of both groups T (351.2) and TH (342.3) were found to be lower than the control group (504.4) in which the mean eosinophils number remains similar during the experimental period. There were no statistically significant ($P>0.05$) differences among treatment groups when eosinophil counts at week 1were compared with those on succeeding weeks after infection. In contrast eosinophils enumerated in the blood of animals infected with *H. contortus* alone were significantly different from week 1 at week 2 ($P<0.05$), week 3 ($p<0.05$), week 4 ($p<0.05$) week 5, ($p<0.05$) week 6, ($p<0.05$) and week 7 ($p<0.05$).

Trypanosome infection alone or preceding trypanosome infection followed by *H. contortus*, resulted in progressive decrease of eosinophils, indicating that subsequent *H. contortus* infection followed by trypanosome infection did not affect the number of eosinophils.

Figure 9: Blood eosinophil count among treatment groups.



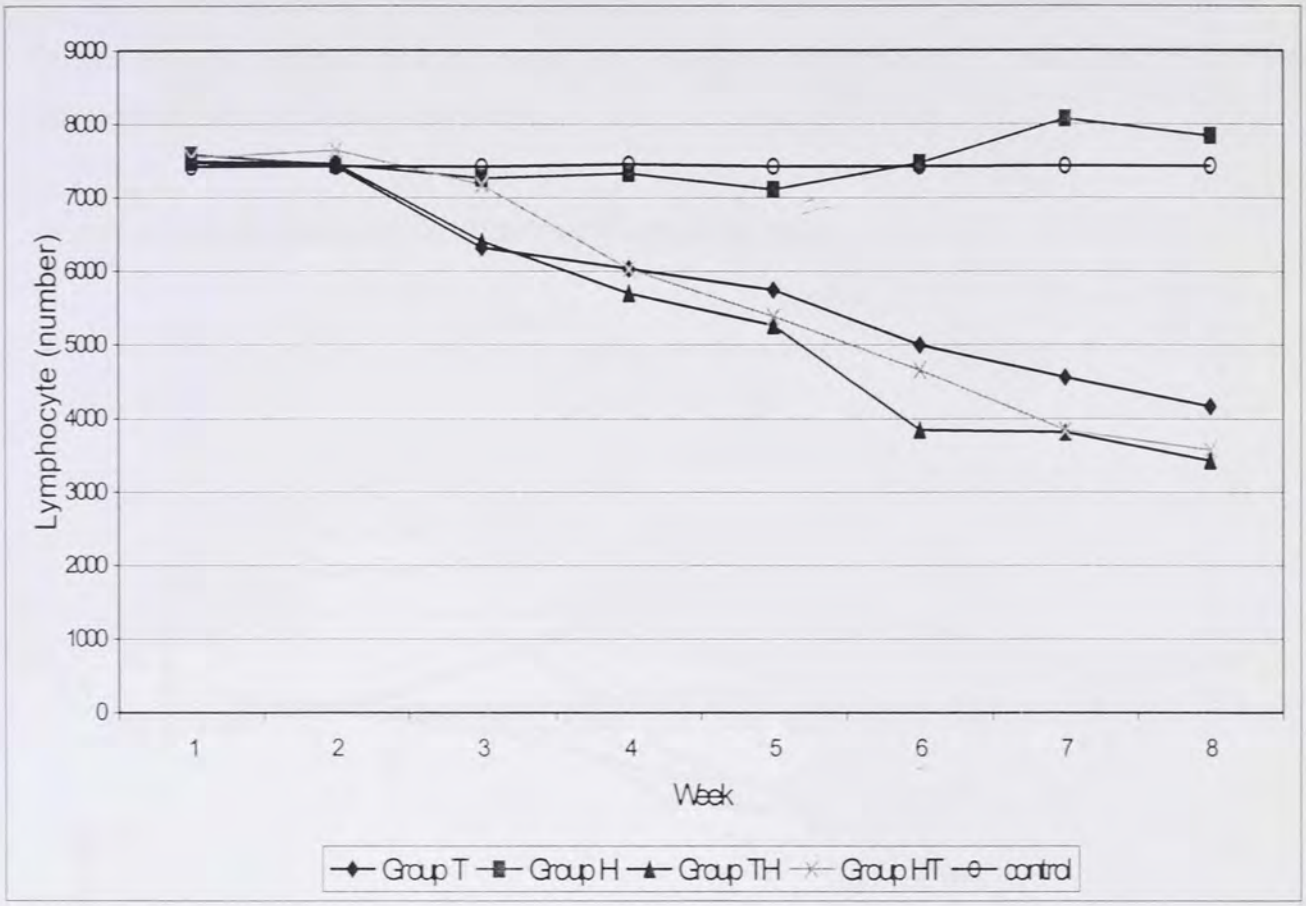
4. 2. 6. 2. Lymphocytes

In all treatment groups, following one week of challenge infection, mean lymphocyte number have been found to decline gradually until the end of experimental period at week 7 post infection except for group H animals. The mean gradual and progressive decline of lymphocyte number of groups T, TH, and HT was from (6760, 6916, and 7315) pre infection to (4083, 3576, and 3798.75) at week 7-post infection, respectively (Fig. 10).

The above result indicate that infection of *T. congolense* preceding one week *H. contortus* resulted lower mean lymphocyte count (5547.6) until the end of the experiment than either *T. congolense* subsequent infection following one week prior to infection of *H. contortus* (5770.9) or group of animals infected only with *T.congolense* (5837.6). Mixed infection with *T. congolense* induced higher lymphocytopenia than *T. congolense* single infection and the difference was statistically significant ($P<0.05$).

Irrespective of the presence of *H. contortus* infection, *T. congolense* infection resulted a greater lymphocytopenia and therefore, the presence of *H. contortus* did not contribute for the stimulation of lymphocyte response. The mean number of lymphocyte counts of the control group (7370) remained unaffected throughout the experimental period and this was higher than all treatment groups except for the group infected with *H. contortus* alone in which the difference was statistically significant ($P<0.05$). The differences in the decline of mean lymphocyte count induced by mixed infections (5659.25) were statistically significant ($P<0.05$) than that caused by single infections (7493.2).

Figure 10. Mean lymphocyte count among treatment groups.



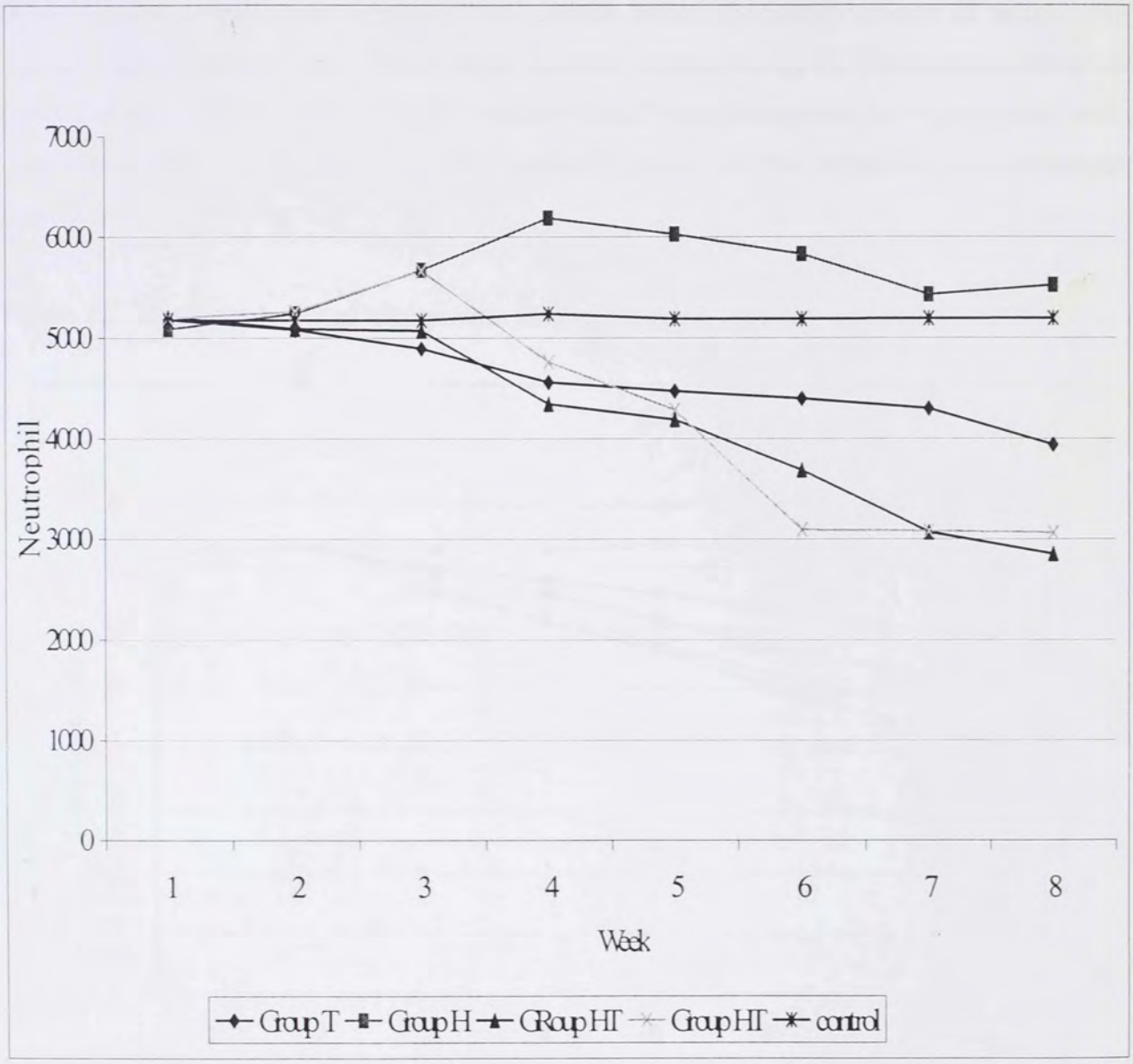
4. 2. 6. 3. Neutrophil

There was a slight neutrophilia in animals infected only with *H. contortus* during the first two weeks post infection and has recorded from 5233 pre infection level and to 6163.8 at week 3. Similarly animals in Group HT have shown a mean slight neutrophilia from 5254.4 pre infection levels to 5660.2 at week 2 post infections. However, animals infected either with *T.congolense* alone or a primary *T.congolense* followed by subsequent infection of *H.contortus* have shown gradual fall of mean neutrophils number from 5170 and 5185 pre infection levels to mean value of 2925 and 2847 neutrophils at week 7 post infection, respectively (Figure 11).

The overall mean neutropenia was significantly ($P<0.05$) greater in animals with mixed (4291) infection than with single infections (5111.25). The mean number of neutrophils of the control

group (5202) remained unaffected throughout the experimental period and this number was higher than all treatment groups except in *H. contortus* single infected groups and the difference was statistically significant ($P<0.05$).

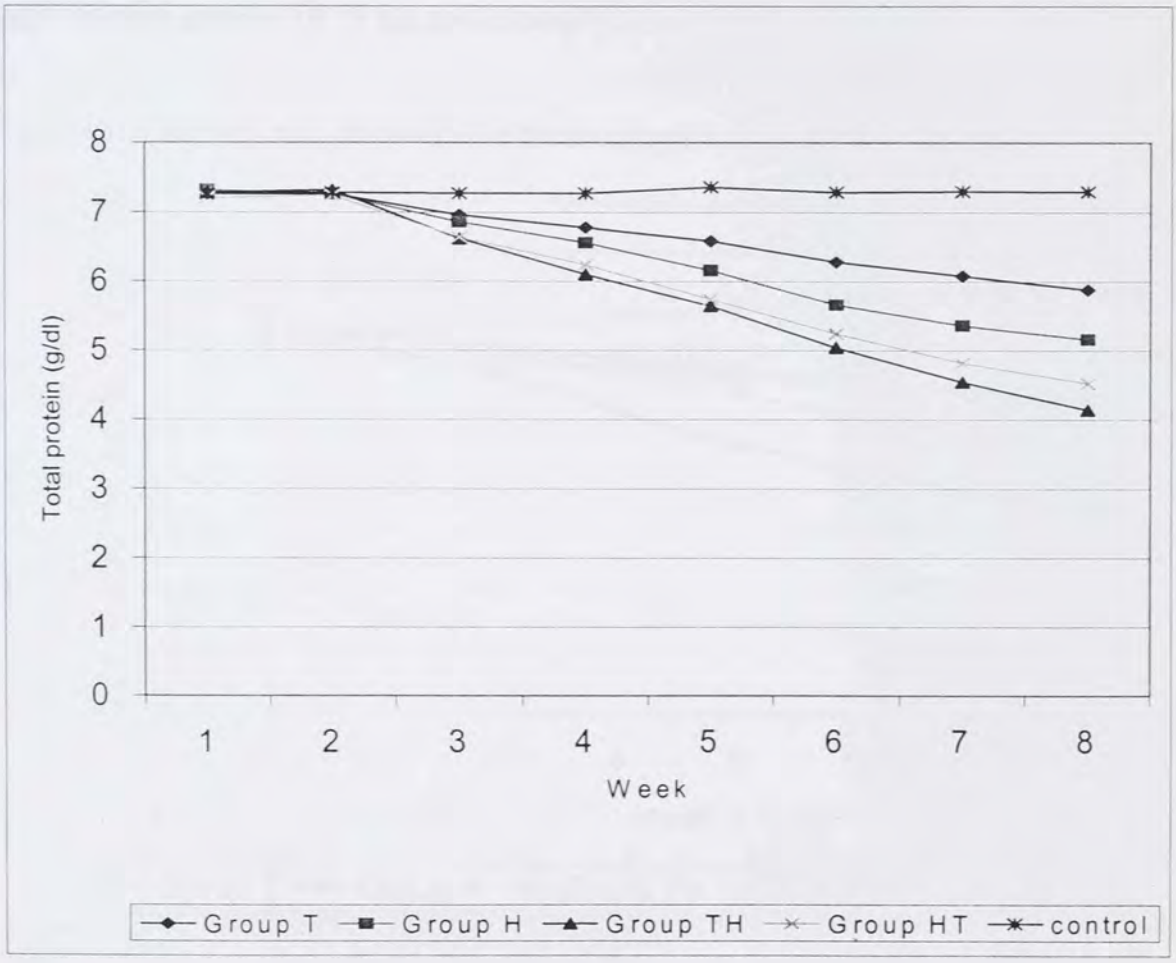
Figure 11. Mean neutrophil count among treatment groups.



4. 2. 6. 4. Total protein

Animals in all treatment groups showed a statistically significant ($P<0.05$) gradual decline in the mean total protein levels than the control group (7.3 g/dl). Although the decline in the mean level of total protein was slightly greater (6.64 g/dl) for trypanosome single infection than infection induced by *H. contortus* alone (6.29 g/dl), and the different was not statistically significant ($P>0.05$). The overall mean levels of total protein were significantly greater in animals with mixed (5.2g/dl) infection than that of single infected animals (6.45g/dl). The mean levels of total protein of the control group (7.3 g/dl) remained unaffected throughout the experimental period and this number was higher than all treatment groups and the difference was statistically significant ($P<0.05$) (Fig. 12).

Figure 12. The mean levels of total protein among treatment groups.

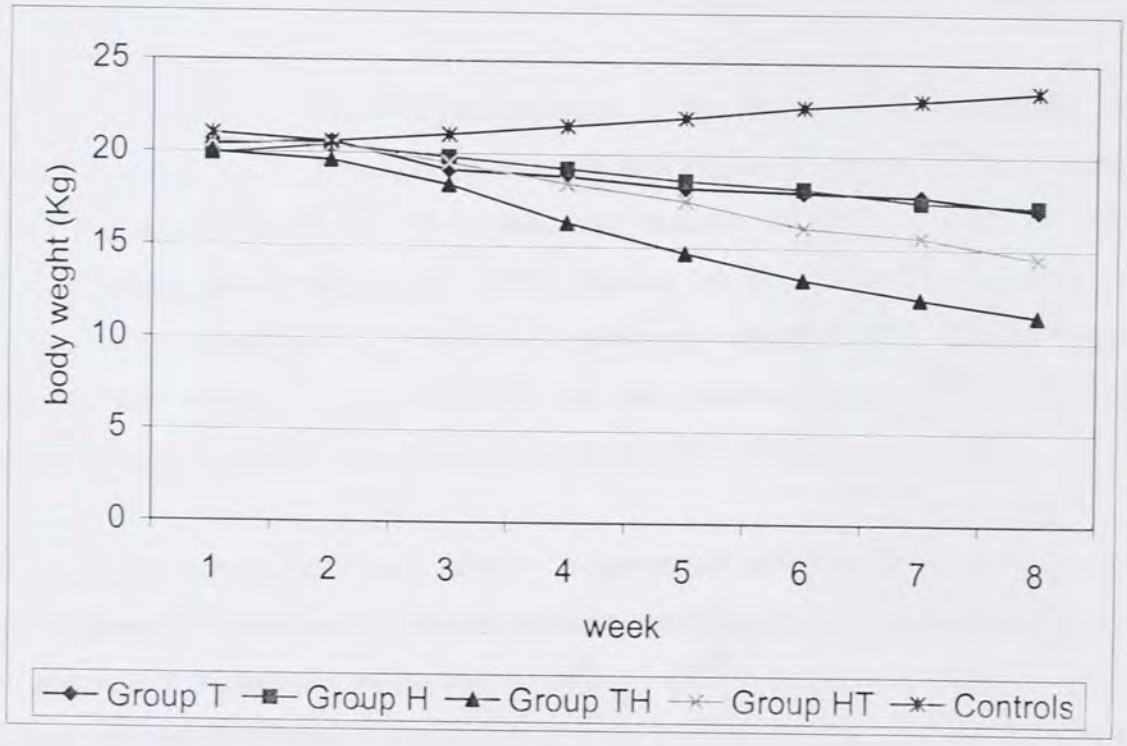


4.2.6.5. Body weight changes

Control group of animals have shown a progressive gain of body weight with mean body weight of (21.9 Kg) throughout the experiment compared to the treatment groups that have shown gradual loss of body weight and the difference was statistically significant ($P<0.05$).

The difference in mean body weight gain of the control group of animals post infection and pre infection was 3.5 kg. The gradual loss of body weight observed in Group T and Group H were virtually indistinguishable. *H. contortus* and *T. congolense* infections alone induced loss in body weight of 3 kg and 3.3kg at the end of the experiment, respectively. Progressive but sharp decline in mean body weight was observed in groups exposed to both parasites than either of single parasites. The mean body weight change of a primary *T. congolense* infection and animals in group HT was 8 kg and 4.97 kg at the end of the experiment, respectively. The mean body weight loss induced by mixed infected groups (16.79 kg) was significantly higher than that induced by single infected groups (18.78 kg) ($p< 0.05$) (Fig. 13).

Figure 13. The body weight changes of treatment groups in relation with time.



5. DISCUSSIONS

The present study was performed with an attempt to observe the effect of concurrent experimental *T. congolense* and *H. contortus* infection in goats and accordingly the findings of the current study indicated that the time course of *H. contortus* infection in goats was significantly altered by concurrent *T. congolense* infection.

In animals infected with *H. contortus* alone the usual prepatent period was observed and this ranged from 21 to 25 days. In Group HT where, *T. congolense* superimposed one week later on a primary *H. contortus* infection all members started shading eggs at the same date, 21 days post infection and the prepatent period remained unaffected. While in Group TH, preceding *T. congolense* infection followed by *H. contortus* one week later, the prepatent period was significantly reduced from the usual 21 days post infection to 17 post infection days. The percentage of animals began to discharge eggs at day 17 was 25%. However all the remaining animals of this treatment group have been shown to discharge eggs at day 19 post infection. Reduction of the prepatent period of *H. contortus* in a primary *T. congolense* infection has been reported to occur as the result of the effect of primary *T. congolense* infection in animals (Griffin *et al.*, 1981; Sharma *et al.*, 2000; in goats, Kaufman *et al.*, 1992 in cattle; Goosens *et al.*, 1997; in sheep).

One of the hallmarks of African trypanosomosis is its capacity to down regulate the host immunological response to a variety of antigens and pathogens including the nematodes of laboratory animals (Fakae *et al.*, 1994; Onha and Wakelin 1999; Chiejina *et al.*, 2003) and domestic livestock (Kaufmann *et al.*, 1992; Gossen *et al.*, 1997; Sharma *et al.*, 2000). Trypanosomosis is classically associated with profound suppression of immune response in affected animals, which show reduced cellular and antibody responses usually becoming susceptible to concurrent infection (Onha and Wakelin 1999; Chiejina *et al.*, 2003).

As a result of this immunosuppressive effect of trypanosome infection, the affected animals show reduced response to a number of helminth parasites resulting in increase fecundity, adult worm burden and failure of immune mediated expulsion of adult worms (Onha, 1992). A primary *T. congolense* infection facilitated the growth of *H. contortus* worms and led them to early maturity

which might be due to marked suppression of antiworm IgA and IgE by *T. congolense* (Griffin *et al.*, 1981; Chiejina *et al.*, 2003) which otherwise normally is produced sufficiently by the host in response to parasites of alimentary tract (Ogilivie and Parrolt, 1974). This was responsible for nematode rejection by it's immediate hypersensitivity effect and aggravated the damaging effect of *H. contortus*.

In the current study higher EPG was observed one week after patency (4^{th} week post infection) in mixed infected groups of animals than single infected groups of animals and this was in agreement with the reports of Kaufmann *et al* (1992) and Sharma *et al* (2000). However, the present result on EPG output disagree with Griffin *et al* (1981) who observed persistently lower fecal egg out put in goats with mixed infection and suggested that the severe anaemia might contribute for the lower faecal egg counts in mixed infections.

A significantly higher egg out put one week after patency (week 4 post infection) of *H. contortus* in Group TH may be attributed to large number of worms being established and the early maturity.

Increasing trends in the mean EPG out put of all treatment groups was observed throughout the infection period except for the slight decline in the mean EPG out put registered at the last week of the experimental period. Although there was an increasing trend in the mean EPG output of all treatment groups a relatively higher rate of egg out put after week 5 was registered in groups infected only with *H. contortus* than groups infected by both parasites. The increase in the pattern of egg output with the increase in the percentage of animals shedding eggs and with maturation of existing worm population stared to shed eggs from day 25 post infection onwards may contribute for the relatively increased egg out put in Group H animals as compared to groups HT and TH across time. This relative increase observed during this time may also be due to lower rate of eggs produced from the presence of large number of worms in groups infected with presence of both parasites because of intense competition within abomasum leading to reduced fecundity (Ratclief and Le Jambree, 1971).

Similar trends of progression in trypanosome parasitaemia were observed in all trypanosome-infected groups of goats (Groups H, TH and HT). *T. congolense* parasitaemia follows a time course across all treatments. Our finding indicate that *H. contortus* infection either preceded or superimposed with *T. congolense* infection did not affect the progression of trypanosome parasitaemia. similar findings were reported by Kaufmann *et al.* (1992). Parasitaemia became detectable in all trypanosome infected goats 1 week (7 days) post infection and there was no difference for the pre patent period among treatments. reports indicated that *T. congolense* infected goats and sheep become parasitaemic within 7 days post infection (Ogunsami and Taiwo, 2001) and trypanosomes occurred in the peripheral blood of cattle infected with *T. congolense* 6-7 days post infection (Kaufman *et al.*, 1992). In this study a peak level of 771x 10^3/ μl, 771 x10^3/ μl, and 769 x10^3/ μl mean trypanosomes were observed in groups T, TH and HT at days 14 post infection respectively. After interadermal needle challenge maximum parasitaemia of were observed between 10-16 days post *T. congolense* infection in cattle (Kaufman *et al.*, 1992).

In animals infected with blood stream forms of trypanosomes peak parasitaemia can occur within a few days of post inoculation. In cattle subjected to tsetse transmitted trypanosome infections, parasites can not be detected in the blood until second and third weeks before considerable multiplication taking place in the skin prior to dissemination to blood stream (Murray and Dexter, 1988).

The present study indicates that the progressive decline in PCV level, RBC counts and Hb levels were more marked in mixed infected groups of goats than the single infected groups. Infected goats had significantly lower PCV levels, lower mean RBC counts and lower mean Hb levels than their respective control groups over the whole observation periods.

Although the decline on mean PCV levels, mean RBC count and mean Hb concentrations induced by a primarily *T. congolense* infections (mean PCV, RBC and Hb levels of 20.1%, 8.93x10^6/ μl, 6.14g/dl, respectively) in group TH were slightly higher than that induced by prior infection of *H. contortus* in groups HT, (mean PCV, RBC and Hb levels of 20.31%, 8.96x10^6, 6.28 g/dl, respectively) the different was not significant ($P>0.05$).

Similarly *T. congolense* infection alone induced a greater PCV decline, lower RBC counts and lower Hb levels (mean PCV, mean RBC counts and Hb levels of 21.6%, $9.52x10^6$ and 6.72g/dl, respectively) than groups infected only with *H. contortus*. These marked decline of PCV levels, mean RBC counts and Hb levels reported in mixed infected groups supporting the findings of Griffin *et al.*, 1981 and Kaufmann *et al.*, 1992.

The progressive depression of hematological (PCV, RBC and Hb) traits during trypanosome infection has been described in different breeds of cattle, sheep and goats (Griffin, 1979; Goosaans *et al.*, 1998; Murray and Dexter, 1998). Similarly anaemia caused by Haemonchus infections is the result of chronic hemorrhage with secondary iron deficiency (Dargie and Allonby, 1975). The marked depression of PCV levels, RBC count and Hb levels observed in a primary *T. congolense* infection over the other treatments may be attributed to the immuno suppressive nature of trypanosome infections in affected animals where they become increasingly susceptible to concurrent infections (Onha, 1992) and the added effect of *H. contortus* with increased pathogenicity removing more blood from affected animals already having lowered body resistance (Kaufmann *et al.*, 1992).

All infected animals were found to be anaemic at the end of experiment. In animals infected with *T. congolense* alone the course of *T. congolense* was essentially acute with anaemia as the main feature of the disease. The acute phase of trypanosome infections was characterized by progressive anaemia accompanied by parasitaemia and the initial fall in PCV values RBC and Hb is associated with the first wave of parasitaemia (Murray and Dexter, 1988).

The present study indicates that the sharp fall in PCV and RBC and Hb values in trypanosome infected groups coincide with the intensity of trypanosomes in the blood of infected animals. In acute phase of trypanosome infection the presence of live trypanosome is necessary for the development of anaemia and the observed fall in the hematological values coincide with patency of infection (Murray and Dexter, 1988).

The development of anaemia in trypanosomosis has been described as multifactorial (Jenkins *et al.*, 1980). In cattle infected with *T .congolense* increased red blood cell breakdown commences with the development of parasitaemia (Holmes, 1976) and would appear to be most rapid during the next 2 to 4 weeks. Though the precise mechanism of development of anaemia was not investigated in the present study, however, hypothesis on this include the involvement of specific Immunoglobulin against *T. congolense*, which form complexes with antigen and complement on the surface of red blood cells leading to their sequestration and destruction in the reticuloendothelial system. Other possible factors include the role of 'non-specific hemolytic factors', which might facilitate the destruction of normal red blood cells by macrophages (Murray and Dexter, 1988).

Although the course of trypanosome parasitaemia was similar in both mixed and single infected groups, the values of hematological traits indicate lower PCV levels, RBC counts and Hb levels in the presence of both parasites than infection induced by *T. congolense* alone. In association with the first parasitaemic peak, the PCV, RBC and Hb of all treatment groups deteriorated rapidly, but these were more pronounced in mixed than single infected groups (infected only with *T. congolense*) due to the added damaging effect of *H. contortus*. During this early acute stage, there was therefore, a reasonable close relationship between the onset and severity of anaemia and appearance, duration and level of parasitaemia.

The relationship between trypanosome parasitaemia and the development of anaemia was described by Dargie *et al*, 1979. The current study indicates that the presence of *H. contortus* did not affect *T. congolense* parasitaemia but affect PCV levels, RBC counts and Hb concentration of mixed infected groups which induced a greater loss of hematological parameters (PCV, RBC, Hb).

The significant depression of PCV, RBC, and Hb in mixed infection despite the presence of *T. congolense* infection may be attributed to the added effect of *H. contortus* with its blood sucking nature where a single mature adult parasite removes about 0.05 ml of blood per day (Urquhart *et al.*, 1996). This is in agreement with Kaufmann *et al.*, 1992; Griffin *et al.*, 1981 who reported greater PCV fall, lower RBC count and lower Hb levels in mixed infection than the fall recorded

by the presence a single parasite alone. Despite the marked fall in PCV, lowered RBC count and lowered Hb level in association with the parasitaemic peaks and removal of blood by *H. contortus,* the levels of the above hematological traits in all trypanosome infected groups fell progressively through out the whole observation periods.

The results of the mean PCV, mean RBC count and mean Hb concentration of groups infected only with the nematode (*H. contortus*) alone; indicated that there was a dramatic fall of PCV, RBC and Hb during the first three weeks post infection as a result of blood loss induced by developing but immature young *H. contortus*. Although at this stage the blood loss in absolute volume was not as large as large when the parasites were mature, the haemopoeitic system of the host was not fully mobilized to produce red cells in quantities sufficient to meet the needs of the animals (Dargie *et al.*, 1979). As infection progresses from week 4 onwards post infection, the animals continue to loss a maximum blood as a result of increased removal of blood by mature adult parasites and the associated hemorrhage when the actively feeding parasites frequently change their feeding sites resulting leakage in the abomasal mucosa. The slight 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 (Dargie *et al.*, 1979).

The PCV levels, RBC counts, Hb levels continue to drop markedly at about the end of infection, despite the mobilization of the haemopoeitic system and serum iron levels because the capacity of animals to reabsorb haemoglobin for further RBC synthesis was limited during this period indicating a dyshaemopoiesis as a result of iron deficiency.

The course of anaemia in groups infected with *H. contortus* only was in agreement with that was described by Dargie, *et al.* (1979) for anaemia induced by abomasal nematode parasite *H. contortus*. Griffin *et al.* (1981) and Kaufmann *et al.* (1992) reported similar findings where the trend of anaemia in *H. contortus* infection follows rapid fall in the first 3 weeks post infection followed by no further drop in haematological parameters for a week, then after a marked drop in the haematological parameters were observed until the end of this experiment at acute phase of infection.

The current study indicate that the order of infection in mixed trypanosome and nematode infections were very important for the outcomes of mixed infections in which a primary infections with *T. congolense* induced a greater loss in mean levels of haematological traits than that caused by subsequent *T. congolense* infection following a preceding *H. contortus* infection. This finding is in agreement with the results of Kaufmann *et al.* (1992); Griffin *et al.* (1997) and Sharma *et al.* (2000).

Although the order of infection were not included in their study, Shilima *et al.* (2005) on donkeys of South Ethiopia, reported enhanced effects of trypanosome infections resulting in depression of mean PCV level by 2.3% in the absence of internal parasites and by 4.1% in the presence of internal parasites.

Similarly the current study revealed that the over all mean PCV depression to be 9% by mixed and 6.96% that of single infections. These findings indicate that mixed infections result in severe anaemia than the anaemia induced by either parasite alone.

Similarly Zewdie *et al.* (2000) suggested that concurrent trypanosome and Fasciola infection is the most harmful combination of parasitosis resulting in an increased pathogenicity when helminthosis is superimposed on trypanosome infection. Animals infected with trypanosome have been frequently infected with strongyles and the egg counts of trypanosome-infected cattle have been found to be higher over those where no trypanosomes were detected in the blood (Dwinger *et al.*, 1994).

Normocytic anaemias have normal MCV and MCHC and are detected only by a decreased number of erythrocytes, a low packed cell volume and a reduction in total haemoglobin (Coles, 1986). Analysis of mean MCV and mean MCHC values revealed no significant difference between all treatments and control groups ($P<0.05$). The over all mean values of MCV and MCHC between mixed infected (22.64fl and 29.41g/dl) respectively and single infected (23.63fl and 31.1g/dl) groups were not statistically significant ($P>0.05$). The present findings indicate that the type of anaemia in groups of animals infected only with *T. congolense* alone was normocytic normochromic through out the whole observation period. The anaemia due to

trypanosome infection in acute phase was reported to be normocytic normochromic although normocytic hypochromic cells do occur in chronic cases (Murray and Dexter, 1988).

Normocytic normochromic anaemia during the acute phase of *T. congolense* infection was reported in trypanotolerant breeds of WAD goats (Goosens *et al.*, 1998). Normocytic normochromic anaemia was also observed in East African goats infected with *T. congolense* or macrocytic normochromic anaemia in different breeds of goats (Whitelaw *et al.*, 1985). Trypanosusceptible zebu cattle and Holstein-Friesian calves infected with *T. congolense* developed a normocytic, normochromic anaemia (Valli *et al.*, 1978).

Normocytic anaemia has normal MCV, MCHC and MCH and is detected only by a decreased numbers of erythrocytes, a low packed cell volume and a reduction in total haemoglobin. Such anaemia occurs when there is depression of erythrogenesis (Coles, 1986). Normocytic, normochromic anaemia observed in the trypanosome infected goats during acute phase is non-regenerative anaemia, which is often caused by failure of erythropoiesis (Morris and Dunn, 1992). The type of anaemia in groups infected with either a primary or subsequent mixed infection of *T. congolense* and *H. contortus* was similarly found to be normocytic normochromic through out the whole observation period. These results were in agreement with the findings of Kaufmann *et al.*, 1992 in cattle.

The type of anaemia in animals singly infected with *H. contortus* alone was normocytic normochromic up to 4 weeks post infection and there after it becomes macrocytic hypochromic until the end of the experiment. An increase in the numbers of reticulocytes in the peripheral circulation usually results in macrocytosis. Macrocytosis is an indication of good bone marrow response (Coles, 1986). Anaemia caused by haemonchus infection is the result of heamorrhage with secondary iron deficiency (Dargie and Allonby, 1975).

The present study indicated that following the first 3-4 weeks post infection, the haemopoeitic system was mobilized to produce RBC from bone marrow to compensate for the lost blood resulted from the blood sucking activity of *H. contortus*. After weeks 4 post infection, although there was bone marrow response as evidenced by macrocytosis, the capacity of animals infected

with *H. contortus* to reabsorb haemoglobin for further synthesis of RBC was limited. Red cell activities fall sharply since much of the iron from the degraded cells was not available for haemoglobine synthesis indicating dyshaemopoeisis (Dargie *et al.*, 1979). Kaufmann *et al.*, 1991 indicated that the anaemia in *H. contortus* mono infected animals was prognostically favorable because the removal of the parasite burden by chemotherapy resulted in a rapid restoration of normal blood parameters.

The present study revealed the progressive decline of total WBC counts, eosinophil, lymphocyte and neutrophil counts in all trypanosome infected animals throughout the observation period as compared to the non infected control group that maintained higher counts until the end of the experiment. The decline of the above counts was more pronounced in mixed infections as compared to single infections and the different was significant ($P<0.05$). The leucopoenia was mainly due to the decline of lymphocyte count. Kaufmann *et al.*, 1992, reported similar observations revealing pronounced decline in mixed infections. A significant decrease of the PCV, eosinophils, lymphocytes and neutrophils in the peripheral blood during the acute phase of trypanosome infections by the intensification of the immunological response in the bone marrow was reported (Anosa *et al.*, 1997). According to Anosa *et al.*, 1997, during the early stage of this phase there was slight proliferation of granulocytic elements and proliferation and activation of macrophages with destruction of mature and maturing erythroid and granulocytic cells. The ability of animals to respond to bacterial infections and other hetrologous antigens seem to be seriously compromised because of the down regulation of granulopoiesis and lymphopoiesis with the resultant panleucopenia, this is the reason why trypanosome infected animals succumb readily to secondary bacterial and other infections (Anosa *et al.*, 1997).

Animals that received *H. contortus* alone mounted considerable degree of blood eosinophilia as compared to the non infected animals and this was in agreement with similar work conducted in cattle that reveled higher eosinophil in animals infected only with *H. contortus* (Kaufmann *et al.*, 1992)

A progressive decline and gain in mean body weight of all treatments and control groups was observed through out the study period, respectively. The difference in the mean change of body

weight between mixed (16.79 kg) and single infections (18.78 kg) was significant ($P<0.05$). a primary *T. congolense* infection (15.74 kg) resulted in higher weight loss over a preceding *H. contortus* infection (17.83 kg) and the different was significant($P<0.05$). Single infected animals either with *T. congolense* or *H. contortus* have shown progressive but indistinguishable loss in body weight and the difference was not statistically significant ($P>0.05$). The body weight loss of trypanosome infected animals is due to the wasting nature of trypanosome infection and this has been explained by the decline in dry matter intake and catabolism of body reserve to meet the increased requirement for maintenance (Verstegen *et al.*, 1991).

The significant body weight loss observed in mixed infections in this study was in agreement with Kaufmann *et al.*, 1992 who observed greater body weight loss in N'Dama cattle as a result of combined infections, while the body weight change resulting from primary *T. congolense* infection was greater than that induced by prior infection of *H. contortus* with superimposed infection of *T. congolense*. N'Dama calves infected only with *T. congolense* alone gained weight while those infected with *H. contortus* alone slightly lost weight (Kaufmann *et al.*, 1992). Due to their trypanotolerant nature the N'Dama were able to control parasitaemia and develop less severe anaemia (Murray and Dexter, 1988).

The majority traits provide evidence of the negative effect of trypanosomosis on live weight nevertheless there is marked variation in live weight response to infection in N'Dama cattle. Although trypanosome mono infection were tolerated by N'Dama cattle with out significant clinical signs, the ability of animals to show a normal response to preceding or subsequent *H. contortus* infection appear to be markedly impeded (Kaufmann *et al.*, 1992). Similarly *H. contortus* infection resulted in body weigh loss (Urquhart *et al.*, 1996). Haemonchosis causes anaemia, diarrhea, and emaciation resulting in reduced weight gains increased morality and increased production cost (Mulugeta *et al.*, 1989).

All infected animals showed a significant ($P<0.05$) progressive decline in mean total protein level than non-infected controls that remained similar throughout the observation period. *T. congolense* infection alone resulted in a slightly greater decline in mean levels of total proteins (6.64 g/dl)

over animals infected with *H. contortus* alone (6.29 g/dl) where the difference was not statistically significant ($P<0.05$).

A significant difference in the mean levels of total protein was observed between mixed infected groups (5.9 g/dl) and animals infected only with a single parasite (6.46g/dl), indicating that mixed infection had a significant influence over plasma protein levels. This result is in agreement with Goossens *et al.* (1997) who reported a decreased plasma protein levels in mixed infections of *T. congolense* and *H. contortus* compared to single infections The major changes induced by trypanosomes infection were a decline in total protein level in both young and adult ewes together with a decline in albumin concentration in ewe lambs (Osaer *et al.*, 2000).

Reduction in feed intake is common in parasitised animals and this is a feature in trypanosomosis. However this is less apparent in animals, which are provided with high protein diets, since there are benefits from both the quality and quantity of the dietary intake resulting in significantly reduced improvement from the disease (Verstegen *et al.*, 1991).

Although animals used in the current study were provided with equal quality and quantity of ration, it was reported that supplemented animals had a higher total protein than those receiving only basal diet (Osaer *et al.*, 2000; Faye *et al.*, 2002). According to this report providing supplemental diet can help trypanosome infected animals to maintain total protein levels.

6. CONCLUSIONS AND RECOMMENDATIONS

The findings indicate that the time course of *H. contortus* in goats is significantly altered by concurrent *T. congolense* infection with increased pathogenicity when helminthosis is superimposed on trypanosome infections. Although concurrent trypanosome and helminth infections mutually aggravate each other's pathogenic effect, the pathogenic effects of concurrent infections due to *T. congolense* and *H. contortus* were predominantly influenced by *T. congolense* infections.

Experimentally induced infections by a combination of *T. congolense* and *H. contortus* infections have a negative overall effect on health of animals by affecting haematological (PCV, RBC, Hb) and parasitological parameters of the affected animals. The current study indicate that the order of infection in mixed trypanosome and nematode infections was very important for the outcomes of mixed infections in which a primary infections with *T. congolense* induced a greater loss in mean levels of hematological traits than that caused by subsequent *T. congolense* infection following a preceding *H. contortus* infection. Animals become more susceptible to *H. contortus* infection that may result from reduced body resistance as a result of concurrent infection of *T. congolense*. Significant reduction of the prepatent period was observed in concurrent primary *T. congolense* infection. Pronounced excretion of eggs per gram of faeces and higher worm burden suggest the significantly enhanced establishment rate of *H. contortus* 3rd stage larva. A markedly increased egg excretion by animals with mixed infections might play an essential environmental role as it will increase the environmental contamination with infective parasite larvae and thus risk of helminth infection. Marked fall of hematological traits such as PCV, RBC, Hb and WBC was observed as a consequence of combined infection over single infection where the difference were statistically significant ($P<0.05$). All infected animals were found to be anaemic at the end of experiment.

The negative effect of mixed infections by *T. congolense* and *H. contortus* on live weight and total protein was more marked over single infection by either parasite. The difference in the mean change of body weight and total protein level between mixed infection and single infections was significant ($P<0.05$). Concurrent infections have shown to induce a marked lymphocytopenia,

neutropenia and lower eosinophil count. High mortality rate and high risk of succumbing of animals was observed in mixed infections than the single ones.

Generally the present study indicate that the interaction existing in experimentally induced concurrent *T. congolense* and *H. contortus* infections significantly altered a number of haematological, parasitological and clinical parameters and the interaction between two or more parasites is inevitable situation, which may lead to a significant biological change in the body tissue, and fluids, which could not be, attributed in presence of single infections alone. Therefore based on the above conclusions the following recommendations are forwarded.

The short prepatent period of *H. contortus* with increased pathogenicity, resulting in marked egg excretion and environmental contamination in mixed infections with a primary *T. congolense* infection and high risk of nematode infection by animals necessitate the need to devise strategic nematode and that of trypanosome control in areas where both anaemia causing pathogens are prevalent.

The effects of concurrent infections, with the consideration of different species of animals, supplemental diet and other factors of the environment that might have interactive effects with mixed infections both in the field and in the laboratory have to be conducted to devise an effective, sound and strategic control of these two anaemia causing diseases.

Generally in endemic areas, whenever, control strategies are made by concerned bodies, the interactions and pathogenic effects of these two highly pathogenic anaemia-causing diseases has to be considered.

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8. ANNEXES

Annex 1: Differential parasitic counting of the abomasum

- The abomasum was ligated at both ends and removed from omasum and duodenum then it was opened along the greater curvature and its content was thoroughly washed into a graduated bucket under a slow jet of water.

- The mucus membrane being carefully rubbed with fingers to remove any worms adhering to it. The contents and washings were made to a total of 4 litters, and then vigorously stirred until all food material, mucous and water were thoroughly mixed. Then a total of 200ml contents were transferred to measuring cylinder in five steps of 40 ml per step while stirring the mixture.

- Then a sub sample of 20 ml were transferred to a small Graduated baker to which 2-3 ml sodium thiosulfate solution was also added to decolorize debris.

- 3-4ml of the sub sample placed in a petridish having parallel lines marked on it 5ml apart Diluted with water and the worms counted under a stereomicroscope. Samples were examined for the presence or absence of the parasite, identified and counted.

- The total number of worms counted in the 20ml sub sample was then multiplied by 100 to get the total number of worms present in the abomasum (MAFF, 1977).

Annex 2: Culture and Recovery of infective larvae of *Haemonchus contortus* from goats.

- The contents of the abomasum were poured a little at a time onto a sieve mesh screen with an aperture of 0.15mm.

- Then washed with the steam of until no food mater passes through. Then the sieve is inverted over a tray and by means of stream water the worms and the food materials

collected on the sieve are washed onto it. Then sufficient number of strain female worms were picked by forceps and collected into a small clean beaker as outlined by (MAFF, 1977).

- Then the collected female worms were Tinley sliced by scalpel blade in 0.8%Nacl solution to release their eggs. The egg suspension was spreaded over finely broken up horse faeces that was 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 infective larvae (MAFF, 1977; Hansen and Perry, 1994).

- Then the mixture was transferred to petridish and placed in an incubator running at 27°C and left there for 7-10 days to get 3rd infective stage.

- The cultured petridish were stirred each day to avoid the growth of fungi and aerate the lower layers of the culture and water was added to culture every 1-2 days. Sufficient number of infective larvae was obtained after several culturing of the eggs from freshly collected female worms (Hansen and Perry, 1994; Bouman, 1995).

- To harvest infective larvae the culture were removed from the incubator and the faeces were tipped out from petridish into a 300ml wide mouthed jars. Then lid of the culture petridish and its cover were 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 petridish was inverted over the mouth of the jar and this were turned upside down so that the inverted jar stands in the petridish and allowed to stand overnight. The next day the fluid in the petridish containing many L_3will be pipetted into a conical centrifuge and concentrated by centrifugation (MAFF, 1977; Hansen and Perry, 1994).

- The larvae was passaged once in the goats before transit in that this isolate of *H. contortus* was served as initial infective larval source and maintained in apparently nematode free goats for production of subsequent source of infective larvae for

experimental infection. The L_3 used for experimental infections will be obtained through faecal culture at 27 °C for 7-10 days Baerminization of the faeces as above, purification by sedimentation and recovery of the clean larvae. The larvae harvested were stored in tap water at +4°C until use (MAFF, 1977; Hansen and Perry, 1994)

Annex 3: Modified McMaster egg Counting Technique

- 3g of feces was weighed and broken up thoroughly in 42ml of tape water. The mixture was then poured through a pour mesh sieve of 250µm aperture.

- The filter was then collected, agitated and filled into 15 ml centrifuge tubes and centrifuged at 2000rpm for two minutes.
- The supernatant was poured of; the sediment was agitated very well and replaced by saturated salt solution as floatation fluid to the previous level.

- The suspension was then poured and inverted six times so as to have very good homogeneous distribution of the eggs in the mixture.

- Then using the Pasteur pipette the fluid was removed to fill both chambers of the McMaster slide without interruption to avoid the formation of bubbles in the compartment. After 5 minutes the eggs float to the cover slide and transferred under the microscope and all the eggs in the ruled areas of the Mc Master were counted.

- The number of eggs at both chambers of the Mc Master are counted and multiplied by 50 for determination of the total number of eggs per gram of faeces (MAFF,1977; Kaufmann, 1996).

Annex 4: Thin blood smear preparations:

- Clean slides with 70% ethanol and tissue paper were used to remove grease.

- After mixing blood well and a small drop was put onto the slide with the capillary tube.

- The spreader (another slide) was placed at an angle f 30°C and it was drawed back ward to make contact with the blood.

- The blood was allowed to run to each end of the spreader and the blood was spread along the slide in a rapid, steady motion. The thumb and for finger was used to guide the motion to ensure that the smear is straight.

- The slide quickly dried by waving it in the air and the slide was labeled with the relevant data using the pencil.

- The smear was fixed in methanol for 5 minutes and then dried in the stain for 30 minutes.

- A 1 in 10 dilution of Giemsa was stain prepared for 30 minutes.

- The slide was rinsed in running tape water of pH 7.2 or in the phosphate buffer until the smear assumes a bluish pink colour and then the slide was drained and dried in an upright position.

Annex 5: The buffy coat/Phase contrast or Dark Ground Technique

- The blood in the capillary tube with one end sealed with cristaseal was centrifuged for 5 minutes.

- The PCV determined in the Haematocrit reader.

- Capillary tube was cut 1mm below the buffy coat to include the top layer of the red cells

- The contents of the capillary tube was expressed on to a clean slide and covered with a 22x22mm cover slip.

- Approximately 200 microscope fields of the preparation using phase contrast or Dark field Microscopy was examined. Then the trypanosome spices were identified and the parasitaemia was estimated according to Murray *et al.*, 1977.

Annex 6: The buffy coat/Phase contrast or Dark Ground Technique

Score	Trypanosome/ field x 250	Trypanosome concentration
6+	Swarming >100	$>5x10^4$/ml
5+	>10	$>5x10^5$/ml
4+	1-10	10^4/ml
3+	1/21	10^3
2+	1-10/ preparation	$103-10^4$/ml
1+	1/ preparation	$102-10^3$/ml

Source: (Murray *et al.*, 1977).

9. CURRICULUM VITAE

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10. SIGNED DECLARATION SHEET

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

Name: Abebayehu Tadesse

Signature _____

Date of Submission_____

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

Dr. Hagos Ashenafi_____

Study On Congurrent t.Congilense & H. Contortus Experimental Infection In Goats:Interaction & Pathogenic Effects

Abebayehu Tadesse