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STUDIES ON THE INTERACTIONS BETWEEN *OESTRUS OVIS* (L_1) AND *HAEMONCHUS CONTORTUS* (L_3) IN EXPERIMENTALLY INFECTED GOATS

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DEBRE ZEIT, ETHIOPIA

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LIST OF ABBRIVIATIONS

AAU:	Addis Ababa University
FEC:	Faecal Egg Count
EPG:	Eggs Per Gram of Faeces
GI:	Gastrointestinal
GIT:	Gastrointestinal Tract
Hgb:	Haemoglobin
L_1:	First-stage larva
L_3:	Third-stage larva
PCV:	Packed Cell Volumes
RBC:	Red Blood Cell
WBC:	White Blood Cell

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ABSTRACT

Concurrent parasitic infection of animals is a common phenomenon in the field. The existence of one species may often positively or negatively influences the biology and development of the other. The results of previous experimental studies on sheep model under temperate conditions have shown that infection with *Oestrus ovis* adversely affected populations of either *Haemonchus contortus* or *Trichostrongylus colubriformis*. However, under tropical condition no information was available on the interactions between the parasites located in remote anatomical site mainly using goat model. Hence, an experimental study was conducted with the objective to determine the possible occurrences of interactions between *O. ovis* and *H. contortus* using specifically Ethiopian local Afar breed of goats. A total of twenty goats were allocated into four groups (O, OH, H, and C) of five animals. Groups O and OH animals, each received a total of 56 first instar larvae (L1) of *O. ovis* in five consecutive infections between days 0 and 35, and then on day 42 animals from groups OH and H were infected with a single dose of 5000 third stage larvae (L_3) of *H. contortus*. Goats from group C were kept free of any infection as non-infected controls. Faecal egg counts (FEC), blood cell counts, total serum protein levels and body weight changes were measured weekly throughout the study period. All experimental animals were humanly killed on day 98. At necropsy worm burden, female worm length and fecundity as well as *O. ovis* larval burdens in the nasal-sinus cavities of infected animals were assessed. The results showed that the presence of *H. contortus* in the abomasum of group OH had no any influence on the development of *O. ovis* in the nasal-sinus cavities. In contrast, infections with *O. ovis* before *H. contortus* was related with significant reduction ($P<0.05$) in FEC, worm burdens, fecundity and female worm length in group OH compared with mono-infected animals (group H). This was associated with in higher blood eosinophilia and packed cell volumes (PCV) which was significantly different ($P<0.05$) from *O. ovis* mono-infected animals.

Key words: Concurrent infection, *Oestrus ovis*, *Haemonchus contortus*, Interaction.

1. INTRODUCTION

Ethiopia has the largest livestock inventories in Africa, including more than 38 million cattle, 30 million small ruminants, <1 million camels, 4.5 million equines and 40 million chickens (CSA, 2004), with livestock ownership currently contributing to the livelihoods of an estimated 80 percent of the rural population. In the arid and semi-arid extensive grazing areas in the eastern, western and southern lowlands cattle, sheep, goats, and camels are managed in migratory pastoral production systems (CSA, 2004).

Domestic ruminants are frequently exposed to multiple parasitic infections throughout their life. In most cases of natural infections animals are known to harbour single or mixed parasites with various species or several different types of parasites is a common phenomenon (Clark, 2001; Cox, 2001). In the field while sharing common pasture, animals are exposed to a variety of parasites among which are gastrointestinal nematodes that cause considerable animal health problems in many parts of the world (Miller *et al*., 1998; Tembely *et al*., 1997; Waller *et al*., 2004).

In Ethiopia where farm animals are kept on pasture throughout the year and climatic conditions are favourable for development and survival of infective stages of helminth parasites, helminthosis mostly in the form of gastroenteritis is ranked as the major constraints on sheep and goat production. Parasitic gastroenteritis including haemonchosis, causes anaemia, diarrhoea, and emaciation resulting in reduced weight gains increased morality and increased production cost (Tembely *et al*., 1997). Meanwhile, 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 (Baker *et al*., 1998; Waller *et al*., 2004) in humid and sub humid tropics including Ethiopia.

Furthermore, it is known that in many countries, helminthes and myiasis agents are sympatric. One of the myiasis agent affecting sheep and goats in both tropical and temperate regions is dipteran fly, *Oestrus ovis* (Class Insecta, Order Diptera, Family Oestridae), larvae of which are found in the upper respiratory tract of small ruminants in most countries where these animals are breed. *Oestrus ovis* is an agent of naso-sinusal myiasis of sheep and goats causing nasal discharge, rhinitis, and sinusitis. There are many reports on its prevalence in temperate and tropical areas. Its prevalence rate in sheep is in: Burkina Faso 92, 40% (Belem *et al*.,

1988); Ethiopia: 88% (Tesfaye, 1993); 77.42% (Yilma and Genet, 2000); in Senegal 95% (Pangui *et al*., 1988); in France: 65% (Yilma and Dorchies, 1991; Dorchies *et al*., 2000). The prevalence of infection increases towards tropical countries (Dorchies *et al*., 2006).

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). Recently, it has been also demonstrated that mixed infection with *Oestrus ovis* larvae and some strongyle species in sheep, in spite of the site of infection and their very distant biology, has favourably enhanced inflammatory responses of the animal host to the nematode. In this regard, Dorchies *et al*. (1997), Yacob *et al*. (2000, 2002) have reported that sheep harbouring *O. ovis* larvae shade significantly lower number of nematode eggs per gram. Furthermore, female worm fecundity, the number and length of adult worms collected during autopsy were also significantly lower in the former than in the latter category. This suggests that an enhanced rejection and stunted development of parasites in previously *O. ovis* infected sheep. Dorchies *et al*. (1997); have also noted a significant difference in *H. contortus* egg output between sheep harbouring *O. ovis* larvae and those without it. A mediation through possible changes in inflammatory cells (eosinophils, mast cells and globule leucocytes) which are usually associated with the presence of both parasites have been suggested but the mechanisms responsible for these interactions remain largely undefined. Since such studies have not yet conducted in goats model but were observed in European breed of sheep and under temperate climate, due to the basic and applied interest of these first results, we decided to examine further the existence of possible interaction in Ethiopian local breed goats during experimental infections with nasal bot fly and *Haemonchus contortus*.

The present study was therefore designed in order to:

- Determine the presence of interaction between *Oestrus ovis* and *Haemonchus contortus* and assess the magnitude of interaction in artificially infected goats

- Determine changes in parasitological parameters associated with this interaction

- To examine the kinetics of blood cellular profiles and the dynamics of total protein related to these interactions

2. LITERATURE REVIEW

2.1. *Oestrus ovis*

2.1.1. Prevalence and pathological importance

Oestrus ovis (Class Insecta, Order Diptera, family *Oestridae)* is an agent of naso-sinusal myiasis of sheep and goats characterized by nasal discharge, rhinitis and sinusitis. *Oestrus ovis (O. ovis)* Linnaeus (1761), the sheep nasal bot, is a cosmopolitan myiasis producing fly whose larvae are well-recognized parasites in the nasal cavities and adjoining sinuses of sheep and goats. The first instar larvae (L_1) are usually localized in the septum and turbinate while L_2 and L_3 are present in the ethmiodal area and sinuses (Zumpt, 1965). Constant irritations produced by cuticular spines and oral hooks of the larvae, together with certain toxic substances excreted by them profoundly affect the health status of infected animals (Du Toit and Fiedler, 1956).

O. ovis can thrive in different environments (Breev *et al*., 1980; Pandey and Ouhelli, 1984) and can over winter in a diapause state, as larvae in the stages L_1 or L_2 become dormant and remain in recesses in the sinu-nasal passages of the host. They move to the frontal sinuses only in the warmer spring weather, and then complete their development (Urquhart *et al*., 1996, Horak, 1981).

There are many reports on the prevalence of *Oestrus ovis* in temperate and tropical areas. According to Dorchies *et al*. (1999) its prevalence rate in sheep is in: Equatorial Africa 48.8o%, Senegal 95%, Ethiopia 28%, Burkina Faso 92.40%, France: Arieg 65%, Aveyron 33.15%. Infestation rates of 6-52% have been recorded in Zimbabwe, 69% in India and 100% in Morocco (Wall and Shearer, 1997). It is indicated that the prevalence of *O. ovis* in central Ethiopia is 80 and 77.50% in sheep, and 77 and 74.50% in goats (Rufael, 1993; Asmelash, 1995).

Recent surveys have demonstrated the prevalence of caprine oestrosis in numerous areas all over the world, e.g. 48.3% in India (Jagannath *et al*., 1989), 4% in Argentina (Trezeguet, 1996), and 53.8% in Nigeria (Biu and Nwosu, 1999). In Mediterranean countries, the

prevalence described was 91% in Greece (Papadopoulos *et al*., 1997), 28.4% in France (Dorchies *et al*., 2000) and 14% in Morocco (Berrag *et al*., 1996).

Human beings are quite frequently infected with *O. ovis*. This has been reported in 9 publications between 1980 and 1994 where 107 cases of human ocular myiasis were described (Dorchies and Yilma, 1993). James (1947) has reported that up to 50 L_1 *Oestrus* larvae were detected in the eyes of man with conjunctivitis. It has been reported that in republic of Djibouti, human myiasis is frequent during wet season (Dorchies *et al.*, 1995).

2.1.2. Morphology and Biology

The adult sheep bot fly which has a bee-like appearance is grey fly and about 10-12mm long; with small black spots on the abdomen and a covering of short brown hairs. The head is broad with small eyes. The segment of the antennae is small. The mouthparts are reduced to small knobs and non-functional and do not feed. Thus larvae, in close contact with mucosa, play an essential role in accumulating nutrients for the free stages of the life cycle. The females are short lived, about two weeks, but during this time each can deposit up to 500 larvae (L_1) in the nasal passages of sheep and goats Adult females do not lay eggs directly on the host. Instead, fertile eggs hatch within the female fly, and she deposits up to 25 newly hatched larvae at a time in the nostrils of the host during flight (first instar larvae). Larvae pass through three stages or instars: first (L_1), second (L_2) and third (L_3) instars, of increasing size (Wall and Shearer, 1997 Urquhart *et al.*, 1996; Wall and Shearer, 1997).

The first instars larvae (L_1), are spindle-shaped, about 1.1 mm long and bear two curved oral hooks and cuticular spines when deposited by the female flies. Then L_1 actively crawl up through the nasal passages to the ethmoid, feeding on the nasal mucus and reach a size of 4.6 mm before moulting to the second instar larva (L_2). The second instar larvae are white, between 3.5 and 12 mm long, have rows of currycomb-like spines. The dorsal and ventral spines have completely disappeared (Guitton and Dorchies, 1993). In the sinus, L_2 moult to L_3 that grows within the sinuses up to 20mm in length until it is ready to leave the host. They are yellow in colour when young, changing to a light brown later, tapering interiorly with a prominent ‘step’ posteriorly. Each segment has a dark transverse band dorsally. The ventral surface of each segment bears a raw of small spines. Fully developed L_3 larvae leave the sinuses and nasal passage, drop to the ground and pupate in the soil to give further generation

of adults. The puparium are cylindrical shaped, black, only weakly wrinkled on the average 15mm long and about 5mm large (Wall and Shearer, 1997; Urquhart *et al.*, 1996).

The life cycle varies with season and country. Under favourable conditions (temperature and humidity) development of L_1 to L_3 takes place within 4 weeks (Dorchies *et al.*, 1993). When flies are active throughout the year, two or three generation are possible, but in cool or cold weather the small L_1 and L_2 become dormant and remain in the nasal passages to over winter (Urquhart *et al*., 1996).

In the temperate climate of Western Europe, adult flies are active from May–June to September-October. The length of the parasitic portion of the life cycle is quite variable lasting from 3–4 weeks to several months depending on the season and climatic conditions. In the summer, rapid larval development occurs whereas L_1 arrested development (L_1 hypobiosis) takes place in the late autumn and winter and corresponds the period of the year favourable to the adult fly activity. The hypobiotic first stage larvae represent the totality of *O. ovis* populations during the winter. From February to April, larvae resume their development and newly hatched flies are seen in May–June. In southern Mediterranean countries (Morocco, Tunisia, etc.), the hypobiosis period is shorter and in tropical and subtropical countries, adult fly activity and larval development occur all around the year (Dorchies *et al.*, 1993).

2.1.3. Pathogenesis and Symptoms

Oestrosis is a wide spread myiasis, which may severely impair the health of small ruminants. When adult fly approach sheep and goats to deposit larvae the animals panic, stamp their feet, bunch together and press their nostrils into each others' fleeces and against the ground, leading to a loss of grazing time, reduced weight gain in lambs and kids and loss of condition (wall and Shearer, 1997).

The first instar larvae, constantly irritating the mucous membranes by their oral hooks and cuticular spines, induce acute inflammation (rhinitis, sinusitis), painful congestion and serous or sero-haemorrhagic discharges (Alzieu *et al*., 1994; Yilma, 1991).

The presence of *O. ovis* larvae leads to strong inflammation (Dorchies *et al.*, 1998; Nguyen et al., 1996) in the summer (with mucus hypersecretion and nasal discharge) in response to numerous larval depositions by adult flies and in late winter (sinusitis in response to the development of larvae at the end of the hypobiotic period). In addition to these local effects, some indirect effects such as lung abscesses and interstitial pneumonia are not rare (Dorchies *et al*., 1993). Clinical symptoms of infestation may range from mild discomfort, nasal discharge, sneezing, nose rubbing or head shaking. Dead larvae in the sinuses can cause allergic and inflammatory responses, followed by secondary infection and sometimes death (Urquhart *et al*., 1996). In heavy infections larvae may occasionally enter the brain- causing disturbance characterized by ataxia, circling, head pressing, nystagmus, and blindness without apparent lesions and epistaxis (Dorchies *et al*., 1993).

Histopathological changes in the nasal tissues of infected sheep and goats include catarrh, infiltration of inflammatory cells and squamous metaplasia. The accumulation and recruitment of eosinophils and mast cells in the respiratory mucosae of infected by *O. ovis* lambs and ewes have confirmed the role of cellular inflammatory response during *O. ovis* (Abella, 1990; Dolbois, 1992). The strong mucosal congestion and inflammation are not always associated with the number of *O. ovis* larvae but rather caused by involvement of the type I hypersensitivity phenomenon (Yilma, 1992). Inflammation of the nasal cavity is immediately followed by sinusitis due to the presence of L_2 and L_3 larvae within the sinuses. Oestrosis is often aggravated by decomposition of the dead larvae within the sinuses (Wall and Shearer, 1997).

2.1.4. Diagnosis

Clinical symptoms, season of the year, geographical location of the place may help in diagnosis.

Serological diagnosis: The Haemaglutination Test (HAT) is one of the diagnostic tests but its sensitivity is less than 44%. Enzyme-Linked Immunosorbent Assay (ELISA) is more frequently used for the diagnosis of oestrosis (Yilma, 1992; Dorchies *et al.*, 1995). Its marked sensitivity enables to detect specific antibodies in the blood. Based on the same principle, Dot-ELISA test was developed by Duranton *et al*., 1995.

2.1.5. Necropcy

The final diagnosis could be achieved by post-mortem examination on sheep and goats heads to detect *O. ovis* larvae of different developmental stages in the nasal cavity and frontal sinuses (Wall and Shearer, 1997).

2.2. Gastrointestinal nematodes

2.2.1. Prevalence and pathological importance

Gastrointestinal nematodes are cosmopolitan parasites which develop within the digestive tract (abomasum and intestines) of domestic ruminants. They include a range of nematode species, which belong to the order *Strongylida* (Troncy, 1989). Among the factors seriously affecting the pre-parasitic phase of nematodes is climate, particularly of temperature and humidity. Some parasites are more adapted to temperate cool environment (*Ostertagia* spp) while others such as *Haemonchus* spp, to warm tropical environment (Rege, 1997). Nematode parasites are known to cause significant economic losses in animals inducing death of susceptible animals mainly during heavy infestation. Young animals are particularly more susceptible to this parasitism than adults. Pregnant, parturient and lactating female are more susceptible than non-reproducing females. Concurrent infections may increase host's susceptibility to nematode infections (Hoste and Cabrete, 1992; Yacob *et al*., 2001).

The major GI nematodes of small ruminants, their localization and mode of pathological effect are listed under the table below (Table 1).

Table 1: Major GI nematodes of small ruminants and their pathological effects

Site	Parasite species	Action
Abomasum	*Haemonchus contortus*	Blood sucking
	Ostertagia trifurcata	Mucosal damage
	O. circumcineta	Mucosal damage
	Trichostrongylus axei	Mucosal damage
Small Intestine	*Cooperia oncophora*	Mucosal damage
	C. curticei	" "
	C. mcmasteri	" "
	Nematodirus filicollis	" "
	N. battus	" "
	N. spathiger	" "
	T. columbriformis	" "
	T. vitrinus	" "
	Bunostomum trigonocephalum	" "
Large Intestine	*Oesophagostomum venulosum*	Mucosal damage
	Trichuris ovis	Blood sucking
	Chabertia ovina	Mucosal damage

Source: Hansen and Perry (1994).

2.2.2. Morphology and Biology

Nematodes are multicellular organisms with unsegmented with elongated body and externally covered by cuticle. The sexes are separate, with females generally larger than males. The cuticle may be modified to form inflation, expansion and papillae. The mouth opening in its simplest form is only a pore, whereas in highly specialized forms it leads to a bucal capsule of variable size (Hendrix, 1998).

The oesophagus is filariform type. In the male nematodes, the caudal end may terminate on a cuticular expansion, the copulatory bursa, with bursal lobes and rays which are useful in

identification. In the case of *Haemonchus* species asymmetrical dorsal lobe is a characteristic. Accessory male organs are sometimes important in identification, especially of the trichostrongyloids, the two most important being the spicules and gubernaculums. The spicules are chitinous organs, usually paired, which are inserted in the female genitalia opening during copulation. The gubernaculums, also chitinous, is a small structure which acts a guide for the spicules (Dunn, 1998; Urquhart *et al.*, 1996).

The life cycle is direct with a single host. Females are oviparous. The eggs passed in the external environment, under favourable conditions (temperature and humidity) hatch to first stage larvae (L_1). The L_1 develop and moult to second stage larvae (L_2), and then the L_2 in turn moult to third stage larvae (L_3), which is the infective stage. The first three larval stages are free-living constituting the pre-parasitic phases. Within GI tract of the host the L_3 moult to fourth stage larvae (L_4), which transform to a young adult stage (L_5) which eventually becomes mature and start to lay eggs (Soulsby, 1989: Urquhart *et al*., 1996).

2.2.3. Pathology and symptoms

The pathogenic effects of gastrointestinal nematode parasites depend on their localization, feeding habit, the dose of ingested larvae and immune status of the host. The fourth stage larvae of *Haemonchus* are bloodsuckers in abomasum and cause severe damage of the mucus membrane and anaemia (Urquhart *et al*., 1996). The lesions provoked by gastrointestinal nematodes may include haemorrhage, ulcerations, destruction, mucosal damage and nodules formation (Hoste and Dorchies, 2000).

In acute form of the disease, mainly observed during heavy infestations of young animals by *H. contortus*, is characterized by severe anaemia and oedema which can lead to the death of animals. The usual manifestation of the disease is a chronic form, characterized by anaemia, digestive disturbances, reduced appetite, weakness and emaciation (Hansen and Perry, 1981).

2.2.4. Diagnosis

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

Faecal culture: It is the only practical method available to identify the different species and genera of gastrointestinal nematodes present. It is indicative for degree of pathogenicity and egg production by different species of GI nematodes. The distinguishing features are the shape of the "head" (cranial extremity) of the larvae or the length of the sheath" tail", the extension of the sheath from the tip of the larval caudal extremity to the tip of the sheath (Georgi, 1985; Wyk Van and Michael, 2004).

The faecal culture enables to easily detect the infective larvae (L_3) which is the only free larval stage easily identifiable. It is based on the development of eggs into L_3 by keeping the faecal sample at 22-23^{o}c, humidity of 85-90% and optimal oxygen. After 10 to 13 days it is possible to collect the larvae by using a sedimentation apparatus of Baermann. Then the genera of the larvae could be identified (Hansen and Perry, 1994; MAFF, 1979).

Post-mortem examination: it is the method that gives the most valuable information about the worm burden. It enables to detect and count the worms, identifies the larval stages and differentiates the two sexes. For parasite counts, the gastrointestinal tract from abomasums to rectum is required. The adult and larval nematodes are carefully washed out collected, counted and identified. Most of the parasite species are identified immediately on the basis of their shape, colour, size and their localization in the GIT. Its inconvenience is that expensive to kill animals and is time consuming procedure (Hendrix, 1998).

2.3. Concurrent infections and host -parasite interactions

Interaction refers to the interdependent operations of factors to produce effect. The outcome of the disease occurrence depends on the interplay of the host, agent (parasite) and environmental factors. The interaction and occurrence of disease depends on several factors, including age, breed, physiological and immune status of the animal as well as species of the parasite involved (Thrusfield, 2005).

The structure of parasite communities, the possible existence of positive or negative interactions between populations and the analysis of the underlying mechanisms acting both at the level of supra and/or infrapopulations represent a major field of research and discussion in Parasitology and Ecology (Holmes and Price, 1986; Holmes, 1987; Comb, 1995). The mechanisms evoked for interaction at the infrapopulations level are either direct, by competition for niche or resources or indirect, through stimulation of the host immune or inflammatory responses (Halvorsen, 1976).

Combination of parasitic infections can either mutually aggravate each other′s pathogenic effect or indicate the antagonistic interaction that exists between parasites. Negative interactions have been observed in sheep between many parasites, such as *T. colubriformis* against *Ostertagia circumcincta* (Dobson *et al.*, 1992a); *T. axei* against *H. contortus* and *O. circumcincta* (Reinecke *et al.*, 1982); negative effect of the *oestrus ovis* on the outcome of gastrointestinal strongyle infections (Dorchies *et al*., 1997; Yacob *et al.,* 2002).

Coop *et al*. (1986) showed that there was no additive or synergistic effect on performance of lambs administered with concurrent infections of *T. vitrinus* and *O. circumcicta*. However, Sykes et al. (1988) found synergistic effects of mixed infections of *T. columbriformis* and *O. circucincta* on feed intake and body weight gain in lambs fed on fresh herbage. Their data showed that at the highest *T. columbriformis* infection rate *O. circumcincta* burdens increased dramatically, possibly indicating a reduction in host resistance to the latter species caused by the large populations of *T. columbriformis*.

In sheep and goats, concurrent infections with several parasites is the commonest situation in the field. Despite this general situation, relatively few studies have examined the biological, epidemiological and pathophysiologicsl consequences of such multiple infections. Moreover, most of these studies were dedicated to analysis of helminh communities from the digestive tract (Cabaret and Hoste, 1998).

Concordant data from experimental studies and field surveys indicate that interactions between distantly localized parasites such as *O. ovis* and GI nematodes do occur and that such interactions could have major consequences for the control of these parasitic diseases either through the use of chemical drugs or on the genetic selection of resistant line (Jacquiet et al., 1999).

2.3.1. Characteristics of immune response to *Oestrus ovis* and GI nematodes

In all domestic species of animals, there is variation of responses to parasitic infections. In ruminants, the level of resistance to parasitic infections is generally determined and significant differences have been observed among breeds. These variations could be inborn or acquired. Genetic variation can be exploited to improve the capacity of the animals to resist parasitic infections (Stear and Wakelin, 1998). Distinct immune responses often occur to antigens exposed at different stages of the parasites life cycle. The immune response to parasites is extremely complex with variations related to the parasite species, environmental factors and the physiological status of the host (Waston, 1986).

Recent works have indicated that a characteristic of immune response to *Oestrus* infections is manifested by an accumulation of mucosal mast cells and eosinophils in the mucosa of respiratory tract. It has been observed that in sheep subjected to single natural infection eosinophil count was 17.5 to 58.5 times greater in nasal septum, turbinate and sinus in infected group than in their control counterpart. The numbers of mast cells more or less remain the same with artificial infection (Nguyen *et al.*, 1996, Yacob *et al.,* 2002). The experimental infection of lambs with first instar larvae also induces rapid cellular changes with early blood eosinophilia (Yacob *et al.,* 2002) and an increase systemic IgG response detected by ELISA fifteen days after the L_1 *O. ovis* larvae were deposited (Frugere *et al.*, 2000). Furthermore, it was observed that establishment rate of *O. ovis* larvae after experimental infections are higher in immunosupressed than in control or immunostimulated

animals suggesting that larval populations could be affected by the local immune response (Marchenko and Marchenko, 1989).

In the study on concurrent infection of sheep with *H. contortus* and *O. ovis* (Dorchies et al.m 1997), it was postulated that the negative interactions between the two parasites could be regulated by eosinophils or other effector cells of the immune response against parasites. However, measurement to support this hypothesis was mostly lacking. An increase in mast cells and eosinophil populations in the digestive mucosae is generally described during nematode infections in small ruminants and possible role of these cells as final effectors of the host immune response against these parasites is usually evoked (Stevenson *et al*., 1994; Mille, 1996; Meeusen, 1999; Balic *et al*., 2000). Similarly, proliferation of the same cellular types in the nasal mucosae has been associated with *O. ovis* infection but their precise role remain less documented than for nematode parasitism (Dorchies *et al*., 1998; Yacob *et al*., 2004).

Helminth immunity is usually less sufficient and more transient than the immunity to microorganisms; possibly because they do not reproduce in the host as do the bacteria, viruses, and protozoa. Helminth parasites are complex organisms and they present the immune system with an extra ordinary variety of potential antigens. The primary antigens of helminthes (nematodes) may often be metabolic by-products, enzymes or other secretary products. The various stages of developing nematodes have stage specific antigens, often molting fluids, to which the host responds by various ways and special cells or specific antibody (Stiter *et al.,* 1994).

Although multiple immune responses are made to multiple antigens during parasitic infections, only certain antigens and certain responses are critical for protection of the host from parasitic disease. Different stages of the parasite (larvae versus adults) and different stages of host sensitization (primary versus secondary/multiple infections) can evoke different immune response profiles and need to be considered when interpreting results from separate studies (Balic *et al*., 1999; Meeusen and Balic, 1999).

Host immunity against most of the helminthes is acquired during natural exposure, although this immunity is partial, slow to develop and quite variable regarding when it occurs among the different host-parasite relationships. Parasite with diverse biological characteristics will cause different immune responses in their host, thus it is difficult to discuss these diversified

parasite responses as a single group, or considering that a single strategic control might apply to all members of this group. Nevertheless, information obtained from helminthes that are more amenable to study may still have a general application (Jamer and McGuire, 1996).

Acquired immunity has the potential to regulate the establishment, development, fecundity and survival of GI nematodes (Quinnell and Keymer, 1990). The different processes of the parasite life cycle are not necessarily regulated by the same immune effecter mechanisms (Stear *et al.,* 1995 b), nor are they affected simultaneously. The first manifestation of acquired immunity to GI nematodes in ruminants is usually manifested by decreased establishment of ingested larvae, decrease in egg out put and stunted growth followed by retardation and arrest in development of adult worms (Vercruysse *et al.,* 1994). Thus the expressed acquired immunity against these nematodes infecting a given host can be assessed by parasitological parameters, such as faecal egg counts, worm counts, worm length, number of eggs in the utero (fecundity) and vulval flap development (Klesius, 1988). In a number of studies, the proportion of the *Ostertagia* population that was inhibited in the L_4 stage after artificial challenge infection or a natural challenge infection was greater in immunized calves compared to previously un-infected control calves (Dorny *et al.,* 1997).

Faecal egg count is the only parasitological parameter that can be obtained regularly from the same animal during a GI parasitic nematode infection. Animals that were immunized against *C. oncophora* and *O. ostertagi* or both parasites (Nansen *et al.,* 1993) had lower faecal egg counts after a homologous artificial challenge infection (Frankena, 1987) or after a natural challenge infections compared to non-immunized animals. In addition, Michel and Sinclair (1969) observed a rise in faecal egg counts following corticosteroid treatment of calves. A reduced faecal egg out put in immune animals is the result of a reduced number of female worms and/or reduced egg production per female (Kloosterman *et al.,* 1978). Although faecal egg counts do not strictly reflect the fecundity of the parasite population, Albers (1981) and Stear *et al.* (1995) found a very good correlation between faecal egg counts and number of eggs *in vitro* of *C. oncophora* and *T. circumcincta* populations, respectively.

A reduced worm burden in immune animals is the result of decreased establishment and/or increased mortality of the worms. Michel *et al.,* 1973 reported resistance to establishment of *O. ostertagi* in calves, due to previous experience of infection. A lower number of *C. oncophora* (Hilderson *et al.,* 1995b) has been recovered from previously infected calves compared to non-immunized controls. When animals were concurrently immunized against *Ostertagia* and *Cooperia*, the numbers of *Cooperia* that were recovered after a mixed challenge infection were much lower than the numbers of *Ostertagia* (Hilderson *et al.,* 1995b; Ploeger *et al.,* 1995), illustrating that acquired immunity develops earlier and/or more strongly against *Cooperia* than against *Ostertgia* (Armour, 1989).

Acquired immunity can cause arrest in the development of established *O. ostertagi* larvae in the early L_4 stage (Eysker, 1993). In a number of studies, the proportion of the *Ostertagia* population that was inhibited in the L_4 stage after artificial challenge infection or a natural challenge infection was greater in immunized calves compared to previously un-infected control calves (Dorny *et al.,* 1997).

Stunting of *O. ostertagi* in cattle has been described to the effects of an immune response (Michel, Lancaster and Hong, 1972), while reduced length might be due to inhibited growth, a selective expulsion of large worms or shrinkage of worms during the infection (Frankena, 1987). A reduced length of adult male and/or female worms after a homologous challenge infection has frequently been observed in calves that were previously infected with *cooperia* or *ostertagia* (Albers, 1981) and lambs infected with *Haemonchus contortus* (Terefe *et al.,* 2005). Observations on the changes in morphology of GI nematodes as an index of protective immunity have largely described reduced size (stunting) of adult nematodes although the loss of vulval flap in some adult female worms have been documented (Balic *et al*., 2000).

Arrested larval development at the L4 stage (hypobiosis of larvae) of GI nematodes in the host mucosa is a common phenomenon in ruminant hosts and is associated with increased resistance of the hosts to the parasites. However, it is also associated with a number of other factors such as seasonal changes, population density of the nematode in the host and strain of the nematode (Balic *et al*., 2000).

The immunity produced by helminthes that migrate in the host appears to promote a greater immunological response than those confined to the intestine. Larvae acquired by immuned

animals may become established but are later destroyed. Immune response in ruminants to *Trichostrongyloid* infections is very complex. Somatic migrations except in the case of *Bunostomum* are not usually part of the normal developmental cycle of trichostrongyle nematodes and therefore these parasites do not have intimate contact with the host's internal immunologically responsive tissue. Both larval and adult stages however, are in contact with the epithelial surface of gastrointestinal tract (Dorny *et al.*, 1997).

Responses to gut-dueling stages are often polarized Th2 responses, characterized by eosinophilia, mastocytosis and IgE production. One effect of such a response in the gastrointestinal tract is stimulating of smooth muscles, increased gut motility, and diarrhoea (Vallance *et al*., 1998).

Researchers presently believe that mucosal inflammation may be an important initial effector mechanism of expulsion of worm burdens during self-cures and that immunoglobulins of the IgE class and mast cells play roles in the process (Wakelin, 1978). Effector mechanisms against gastrointestinal nematodes appear to involve antigen-specific T cell responses, which induce antibody response and inflammatory changes, with the release of chemical mediators ultimately leading to the expulsion of the worms (Befus, 1995).

Smith and Christie (1978) noted that globule leucocytes were more numerous in the abomasal walls of sheep resistant to infection by *H. contortus* whereas worm-free animals had few. Some recent investigations indicate that, selected animals for resistance to parasitic infections could be more susceptible to other pathogens. Accordingly, it has been proved that genetically selected sheep for their resistance to strongylids are more susceptible to *O. ovis* and other parasites of upper respiratory tract (Jacquiet *et al*., 1999; Yacob *et al*., 2001).

In general, the fact that helminthes (nematodes) excrete many different secretory and excretory antigens during their development from larva into adult makes the immune response to helminthes (nematodes) very complex and put on an obstacle in the development of successful vaccine against helminthes. However, despite all these constraints, the IgE mediated immune responses have evolved to control this parasite (Tizard, 1996).

Although conventional antibodies of the IgM, IgG and IgA isotopes are produced in response to nematodes antigens, the most significant isotope involved in resistance to nematodes is

IgE. IgE levels are usually greatly elevated in parasitized individuals, many nematode proteins preferentially stimulate IgE production and nematode antigens can also act as adjuvant specific for IgE production against other, non- nematode antigens (Tizard, 1996).

Several studies demonstrated that GI nematode infection is almost invariably accompanied by mucosal mast cell hyperplasia (Rothwell, 1989; Miller, 1996b), which may be concomitant with the immune-mediated elimination of the adult parasite following a primary infection (Miller, 1996b). Thus, intestinal mast cells have long been considered as possible effector cells at the mucosal level against GI nematodes (Askenasae, 1980; Bienenstock and Befus, 1980; Miller, 1984). However, the combination of the helminthes (Nematodes) antigens with mast-cell-bound IgE leads to mast cell degranulation and the release of vasoactive factors. These factors stimulate smooth muscle contraction and increase vascular permeability. This results in violent contraction of the intestinal musculature and an increase in the permeability of intestinal capillaries allowing an influx of fluid in to the intestinal lumen. This may lead to dislodgment and expulsion of a major portion of the worm population (Tizard, 1996).

Antibodies of other immunoglobulin isotopes also play protective role by the mechanism involved antibody mediated neutralization of the proteolytic enzymes used by larvae to penetrate tissues, blocking of the anal and oral pores of these larvae by immune complexes as antibodies combined with their excretory and secretory products and inhibition of larval development by antibodies directed against exsheathing antigens. Enzyme pathways may be blocking by antibodies acting against adult worms causing possible arrest of egg production or even interference in the development of anatomical structures. Thus female *O. ostertagi* fail to develop vulval flaps when grown in immuned calves. Similarly, spicule morphology may be altered in C*ooperia* males derived from immune hosts. The presence of larvae in tissues leads to tissue destruction and neutrophil infiltration (Tizard, 1996).

Challenge infection with *T. colubriformis* in immune sheep resulted in an increased number of IgA containing cells in the lamina propria of the small intestine (Adams *et al.,* 1980). There is a close temporal relationship between the rise in local anti-*haemonchus* IgA antibodies and the self-cure reaction. Adult sheep that were immunized against *H. contortus* showed a strong increase in IgA containing cells and/or IgA in the gastric lymph or abomasal mucosa after homologous challenge infection. Sheep that were genetically resistant to *H. contortus* had also

higher anti-*haemonchus* IgA levels in their serum and faecal extracts (Gill *et al*., 1993 a) and more IgA antibody containing cells in the abomasal mucosa (Gill *et al.*, 1994).

Cell-mediated immunity (CMI) is generally considered to be an integral part of inflammatory responses to tissue invasion. Mucosal inflammation may be an important initial effector mechanism of expulsion of worm burdens during self-cure and that immunoglobulins of the IgE class and mast cells play roles in the process (Wakelin, 1978).

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

Eosinophilia has been shown to be positively correlated with resistance to gastrointestinal nematode infections in sheep (Buddle *et al*., 1992), and recent studies have shown that eosinophils can damage and kill infective L_3 larvae of the gastrointestinal parasites, *H. contortus*, both *in vitro* (Rainbird *et al*., 1998) and *in vivo* (Balic *et al*., 1999).

Goats had substantially larger number of globule leukocytes than sheep after being challenged with *T. circumcincta* and *T. vitrinus*. However, despite less effective immune expulsion of the parasites in the goats, no significant difference in the numbers of globule leukocytes or mucosal mast cells between goats those were resistant or susceptible to *T. circumcincta* & *T. vitrinus* was observed. Further more; the effector mechanism may be different for different GI nematodes species as shown in rodents (Nawa *et al*., 1994).

3. MATERIALS AND METHODS

3.1. Study area

The experimental study took place between October and March at the compound of fattening project of the Faculty of Veterinary Medicine in Debre-Zeit 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. Debre-Zeit is located at a distance of about 45km South East of the capital, Addis Ababa. The area is located at an altitude of 1850 meters above sea level and has a total human population of 95,000. It experiences a bimodal pattern of rain fall with a long rainy season from June to October and a short rainy season from March to May and has an average annual rainfall of 800mm. The area has an average maximum and minimum temperature of 27.7 ^{o}C and 12.3 ^{o}C, respectively (CACC, 2003). Debre-zeit is the center of Ada'a Liben woreda, and , the Woreda has a total land area of about 161,056he and is divided in to three agro-ecological zones namely mid land (94%) high land (3%) and low land (3%) (Ada'a Woreda agricultural and rural development office).

3.2 Study type

Experimental study involving randomized experimental trial on the interaction between *O. ovis* and *H. contortus* in goats was conducted.

3.3. Study Period

The total project period was about 10 months; from September 2006 to June 2007. The experiment on the interaction between the two parasites was conducted for 14 weeks extending from December 2006 to March 2007.

3.4. Experimental animals and management

Twenty male local breed goats, aged ten to twelve months, weighing16-20kg and reared under traditional management condition around Metehara area, were selected and purchased from a local open market. All animals were housed in four separate boxes with raised

concrete based units and a solid partition separated by adjacent pens. Care was taken to avoid contamination of pens with nematode larvae from outside. Windows were covered by with wire meshes to prevent free fly access. Animals were allowed to feed locally dried hay with sufficient quantities of concentrate feed and water *ad libitum* throughout the adaptation and experimental period.

To ensure that the goats were nematode free, faecal samples from all animals were examined for the presence of nematode eggs. Laboratory testes comprised standard faecal analysis by floatation, sedimentation and faecal culture techniques (MAFF, 1986).

All animals were dewormed with Ivermectin at 200 µg /kg (FARQUIMICA,Cerrilos-Santiago-Chile), Praziquantel 15mg/kg (APF,Ethiopia) and Triclabendazol 250mg/25kg (Fasinex 250,EAP,Etiopia) body weight to clear any parasite that may present. The anthelmintic doses were calculated individually according to body weights and were administered orally.

3.5. Experimental design

At the end of the adaptation period animals were weighed, ear tagged for easy identification. A randomized complete block design was used and goats were randomly allocated into blocks based on body weight and age (10 to 12 months old). Within blocks experimental animals were randomly allocated into one of the four experimental groups. A first group of five animals (group C) remained uninfected as controls.The three additional groups of five goats were infected either exclusively with *O. ovis* (group O) or wth *H. contortus* (group H), or with both parasites (group OH). At the beginning of the experiment (Day 0), animals from group O and OH were infected with *O. ovis* first instar larvae (L_1). A total of $56L_1$ was given to each goat on five occasions as followes: $13L_1$ on day 0;$12L_1$ on day 7; $10L_1$ on day 14; $14L_1$ on day 21 and finally $17L_1$ on day 35. The larvae were freshly harvested from heads collected from slaughter houses. The artificial infections were performed according to the procedure described by Yilma and Dorchies (1993). On day 42 of the experiment, each animal from group H and OH received a single dose of 5000 third stage larvae (L_3) of *H. contortus*.

The infective larvae of *Haemonchus contortus* (L_3) were obtained from cultured eggs, collected from adult *Haemonchus* female worms from naturally infected goats, slaughtered at

Debre Zeit ELFORA export abattoir. The eggs were cultured on helminthologically sterile horse faeces to the third larval stages in jars at room temperature (22-24^{o}C) for 10-12 days. Larvae (L_3) harvested using Baerman technique from faecal cultures were stored in small volumes of water in aerated, flat-bottomed flasks at 4^{o}C until used (MAFF, 1979).

Table 2: Experimental design of scheduled infections

Group	Infection days with *O. ovis* L_1 and *H. contortus* L_3							
	D0	D7	D14	D21	D28	D35	D42	D98
O	$13L_1$	12 L_1	10 L_1	14 L_1	-	17 L_1		Necropsy
OH	$13L_1$	12 L_1	10 L_1	14 L_1	-	17 L_1	5000_{L3}	
H	-	-	-	-	-	-	5000_{L3}	
C	-	-	-	-	-	-	-	

Blood samples were taken weekly from each individual goat in order to determine packed cell volume (PCV), total red blood cell (RBC) counts, total white blood cell counts (WBC) and differential WBC counts as well as to measure serum level protein. Faecal samples were taken twice weekly from day 56 (*H. contortus* infection) to the end of experiment to measure nematode egg excretion. The body weight of each animal was recorded weekly. All the animals were euthanised on day 98 after the start of experiment. At necropsy, the abomasum of each animal was taken and processed for worm counts with organ contents and washings. The number of worms for each goat was counted on a 10% aliquot and the sex and stage of development of worms were determined. Lastly, the number of eggs in utero was measured in 20 female worms per goat in order to evaluate any effect on worm fecundity. In addition, the heads of the goats were split opened and the number and stages of *O. ovis* were determined according to the method previously described by Yilma (1992).

3.6. Parasitological examination

3.6.1. Faecal egg output

Faecal samples were collected from the rectum of individual animals from groups OH (n=5) and H (n=5) twice a week starting from day 56 (*H. contortus* infection) until the end of the experiment (D98) to measure nematode egg excretion. The collected faeces were placed in airtight plastic sterile bottles and stored at 4^{o}C. Faecal egg counts (FECs) were performed using a modified McMaster technique with a sensitivity of 50 eggs per gram of faeces after counting eggs on both chambers using 33% saturated zinc sulphate solution as a floatation medium. (MAFF, 1979).

3.6.2. Worm identification and counts

At necropsy, heads and abomasums were removed and processed for parasite counts to determine the larval and worm burden. The abomasums were legated at both ends, separated and opened along the greater curvature, the contents were removed by successive washings with tap water and collected in a plastic bucket and passed through a 150μm sieve to eliminate coarse materials. Further more, the whole abomasum was digested in pepsin-hydrochloric acid solution at 37^{o}C by incubating it overnight to collect the tissue dwelling stages. The contents, washings and digested materials, were preserved in 10% buffered formalin. These materials were put into separate containers and then adjusted to 2 litres volumes. The population of worms in the abomasums of each animal was determined from a 10% aliquot using the classical counting procedure indicated by MAFF (1979). Each suspension was stirred vigorously and a sample of about 200ml obtained by plugging a 50ml beaker into the suspension. An alcoholic solution of iodine (5%) was added to colour the material in the samples. Before collecting the worms, sodium thiosulphate solution was added to the samples until dark colour disappeared. This procedure gave the worms a brown colour, which enabled easy collection and counting. Worms were differentiated according to sex and counted using a stereomicroscope. The number of adult- and immature- worms, the length of adult worms, the sex ratios, and the number of eggs per female were determined according to the methods described by Kloosterman *et al*. (1978). Twenty adult female worms were randomly picked from each sample for total length measurement and egg counts in utero. For

this purpose, individual female worms were allowed to disintegrate using sodium hypochloride 4% solution and all eggs liberated from the uterus of each female worm were counted.

The heads of goats from groups, infected with *O. ovis* (O and OH) were separated from the body, opened sagitally using a hand saw and examined carefully for *O. ovis* larvae in the nasal cavities according to previously described methods by Yilma and Dorchies (1991). The larvae found in the nasal and sinus cavities were collected, identified to their stages of development and counted under a stereomicroscope according to Zumpt (1965).

3.7. Haematological examination

Blood samples from all animals were collected weekly via jugular venipuncture at weekly interval from the start of experiment (D0) until the end of the trial (D98). Whole blood samples were collected from each animal into ethylene diamine tetraacetic acid (EDTA) as anticoagulant, coated 4ml vacutainer glass tubes (K3 EDTA 7.2mg, Italy) and serum separator vacutainer tubes (plain) of 7ml (BD Vacutainer, U.K.). Blood in serum separator tubes was allowed to clot for about 30 minutes and the tubes centrifuged within one hour to separate serum from the cells. Sera were stored at –20^{o}C until analysis and used for total blood protein levels determination.

3.7.1. 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 Wright`s stain solution, washed with distilled water and dried on the air. Thin smears were microscopically examined under oil immersion magnification (X100) and counting and classifying of 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). For the purpose of this study, the proportional percentage of lymphocytes, neutrophils and eosinophil count were only considered.

3.7.2. Total white and red blood cells, haemoglobin concentration and Packed Cell Volumes

Blood samples in EDTA tubes were gently mixed using an automated blood mixer (KJMR-IV) and were used for the determination of RBC, WBC numbers, PCV, hemoglobin (Hgb) levels and differential leukocyte counts. The concentrations of RBC, WBC, Hgb and PCV were determined using an Automated Haematology Analyzer (Poch-iV Diff, Kobe, Japan).

3.7.3. Total plasma protein

For determination of total serum proteins concentration the Biuret method was employed (Weicshselbaun, 1946) using an electronic spectrophotometer (reference).

3.8. Body weight measurement

The animals were weighed weekly for the whole duration of the experiment using a dairy scale spring balances (Hansen, Model 603, U.S.A.).The body weight of each animal was recorded starting from one week before the start of experimental infection.

3.9. Statistical Analyses

The kinetics of egg excretion, blood eosinophil counts, packed cell volumes and total serum protein level were compared between the four experimental groups using analysis of variance (ANOVA) with repeated values (using SPSS software programme). The Pearson correlation test was used for assessing the correlation between parameters. Comparisons of the number of *O. ovis* larvae, and *H. contortus* worm populations at necropsy and the number of eggs in utero per female were performed using analysis of variance (ANOVA).

4. RESULTS

4.1. Parasitological data

4.1.1. Egg excretion of *Haemonchus contortus*

No egg excretion was observed in groups C and O throughout the experimental period. In groups OH and H, the egg excretion patterns were similar starting from three weeks after the first *H. contortus* infection, increasing regularly from day 67 i.e 25 days after *Haemonchus* infection up to day 98 with significant differences between the two groups ($p<0.001$). Meanwhile, the egg excretion in group OH was significantly reduced ($p<0.05$) compared to group H (Fig.1).

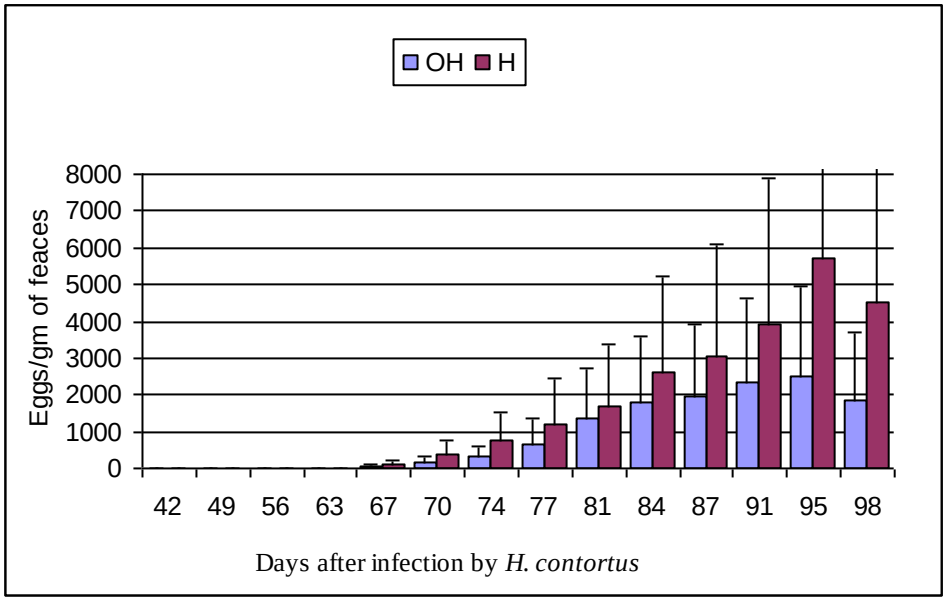

Fig. 1. Nematode egg excretion from *H. contortus* in goats infected with *H. contortus* or mixed with *O. ovis* and *H. contortus*

4.1.2. Worm burden and fecundity

The total mean number of *H. contortus* recovered at necropsy from abomasums of goats from groups H and OH were respectively 2758.4 (±220.51) and 724.8 (±90.01), the difference between both groups being significant ($P<0.001$). In addition a highly significant difference was also observed in worm fecundity between groups H and OH ($P<0.001$). The worms

recovered were mainly adult worms and only very few immature stages (especially fifth larval stages) were observed in both groups. The majority of adult worms obtained from group OH animals were stunted and the uteri of the females contained fewer eggs compared to group H. The mean female to male *H. contortus* sex ratios for group OH and H were 1.3 and 0.9, respectively, with no significant differences between the two groups (P>0.082). Animals that had previously received *O. ovis* (group OH) had significantly fewer eggs per female (P<0.05) than those from group H. The mean number of eggs in utero per female was 859.6 (± 158.9) in group H and 329 (±74.4) in group OH respectively. Significant differences (P<0.05) in the establishment rates of worms were observed between *H. contortus* infected groups. Percent establishment of infection was 55.17% ± 8.79 in group H and 14.49% ±7.2 in goats from group OH. Female length, calculated as the mean for 20 females from each parasitized animal, ranged from 15.1 to 23mm for group OH and 26.15 to 27.4mm for group H animals. The mean body length of adult female worms was 18.88 (± 2.95) and 26.83 (±0.48) for animals from groups OH and H, respectively with significant difference (P<0.05) between groups with length in group OH being significantly lower (Table 1). Female worm length was strongly associated with in utero egg counts (r=0.83, P<0.003) and with FEC (r=0.86, P<0.001). However, worm burden was moderately associated with FEC (r=0.589, P=0.73) but this correlation was not significant. In utero egg counts was moderately associated with blood eosinophils (r=0.59, P=0.069) but was not significant.

Table 3: Total worm burden, sex composition, sex ratio, female length and number of eggs in utero of adult female *Haemonchus contortus* populations

	Group OH	Group H
Male worms	305.4	1447.2
Female worms	419.4	1311.2
Sex ratio	1.3	0.92
Fecundity (eggs/female)	329.02	859.6
Female length (mm)	18.88	26.83
Establishment rate (%)	14.5	55.17

4.1.3. *Oestrus ovis* population

No *O. ovis* larvae were found in groups C and H animals. No significant difference (P>0.05) was observed for the mean number of *O. ovis* larvae recovered from the nasal-sinus cavities of groups O (10.8±2.1) and OH (8.0±3.8) which represented 19.29% and 14.29% establishment rate, respectively (Table 4). No L_1 larva was recovered from any of infected by *O. ovis* groups. The larval proportion of L_2 and L_3 was 59.26 and 40.74% for groups O and 60 and 40% for group OH, respectively showing no significant difference in the composition of populations with the different stages between both groups (P>0.05). The mean larval burdens in infected animals were 6.4 (± 2.1) and 4.8 (±2.6) for L2 and 4.4 (±2.9) and 3.2 (±2.3) for L_3 in groups O and OH, respectively.

Table 4. Total number and establishment rate of *O. ovis* larvae recovered from animals infected with L_1 *O. ovis* (groups O and OH) at necropsy

Groups	Larval dose/animal	Mean no. of *O. ovis* larvae recovered				Establishment rate (%)
		L1	L2	L3	Total	
O	56 L1	0	6.4 (59.26%)	4.4 (40.74%)	10.8	19.28
OH	56 L1	0	4.8 (60%)	3.2 (40%)	8.0	14.28

4.2. Haematological data

4.2.1. Blood eosinophil counts

The number of blood eosinophils in the control group (C) was at a physiological level throughout the experiment. Eosinophil counts were monitored and compared with the values for a control group of uninfected goats. Eosinophil counts between infected groups and the control group was highly significant (P<0.001). Blood eosinophilia was very prominent phenomenon in animals infected with *O. ovis* and *H. contortus* and peak values were attained on D14 for group O, D49 and D56 for OH and D56 for group H (Fig.2). The dynamics of eosinophil counts showed similar pattern in group O and OH, with peak on D14, two weeks

after the first infection with *O. ovis* larvae which was followed by a drop in group O animals starting from D42. One week after infection with the nematodes (D49), higher number of blood eosinophils were observed in group OH than in group H (P<0.01). Towards the end of the experiment, a gradual fall in eosinophilia was seen in all three infected groups. Eosinophilia was moderately associated with worm burden, female length and fecundity (r=0.56, P=0.09; r=0.61, P=0.063 and r=0.59, P=0.069, respectively) but was not significant. However, the correlation matrix revealed a significant positive correlation between eosinophil and FEC (r=0.68, P=0.031).

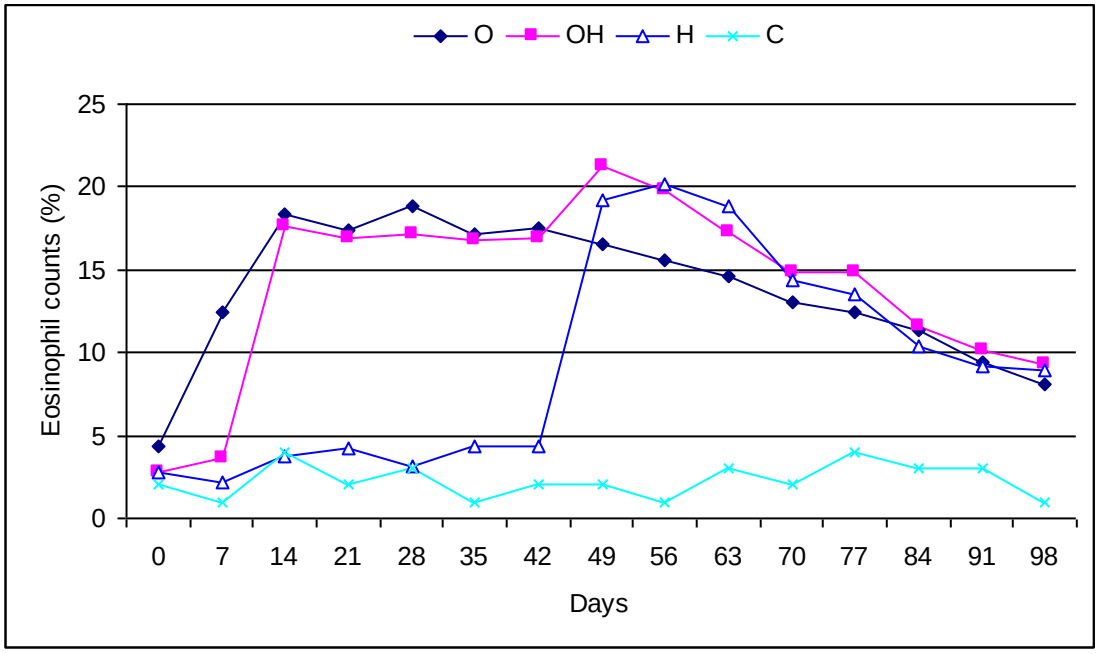

Fig. 2. Evolution of mean blood eosiniphils in the four experimental groups

4.2.2. Packed cell volumes (PCV)

The PCV pattern of all groups is shown in Fig.3. The animals infected with *O. ovis* alone (O) and uninfected controls (C) retained physiological PCV values (28% and 29%, respectively). Animals infected with *O. ovis* (L_1) and *H. contortus* (L_3) larvae showed significant reduction (P<0.01) in PCV when compared with uninfected controls. The animals of group H were severely anaemic at the termination of the experiment with a mean PCV of (15.82%). Only a slight decrease in PCV value was observed in group OH animals (23.86%).The overall decrease in PCV was significantly greater in animals of group H (P<0.01) than in those in group OH.

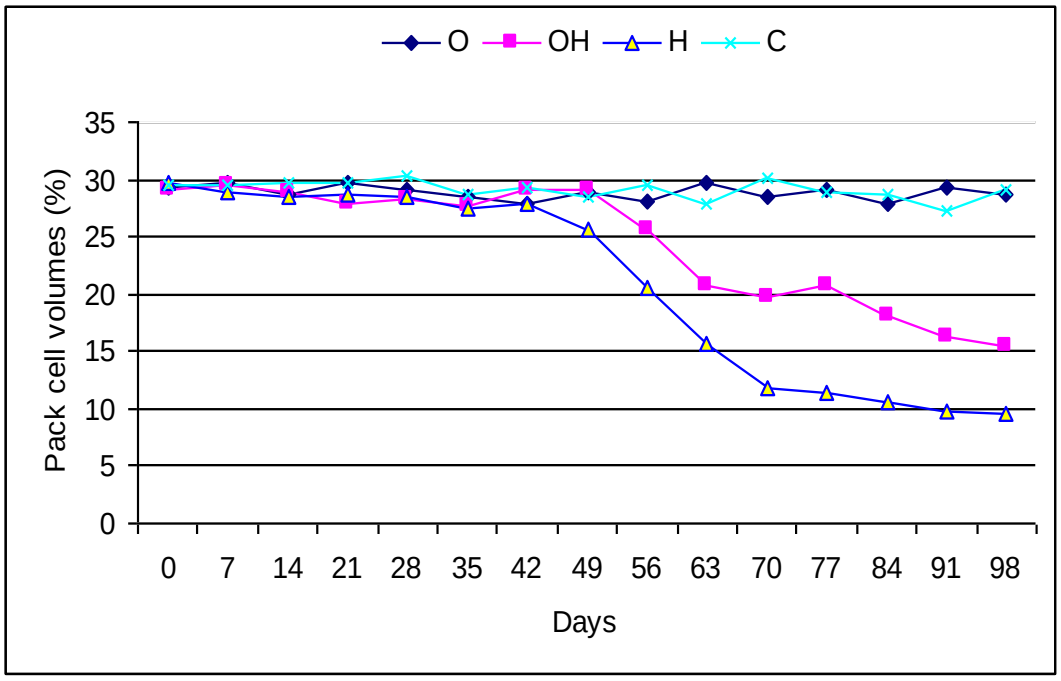

Fig. 3: Patterns of mean packed cell volumes (PCV) in the four experimental groups

4.2.3 Red blood cell, White blood cell counts and Haemoglobin concentration

The total red blood cells and Hgb concentrations were reduced significantly ($p<0.05$) in goats infected with *H. contortus* L_3 larvae (groups OH and H), being lower in group H. However, there were no significant differences between groups for total WBC number and differential WBC counts concerning neutrophls, lymphocytes and monocytes.

4.2.4. Serum protein levels

The total protein concentration was relatively lower in the three infected groups compared with control value (Fig. 4). Mono-infected with Haemonchus animals (H) had significantly lower values ($P<0.001$) compared with the rest three experimental groups (C, O and OH). Mean while, there was no significant correlations with worm burden for all animals infected with nematodes ($r = 0.28$, $P = 0.44$).

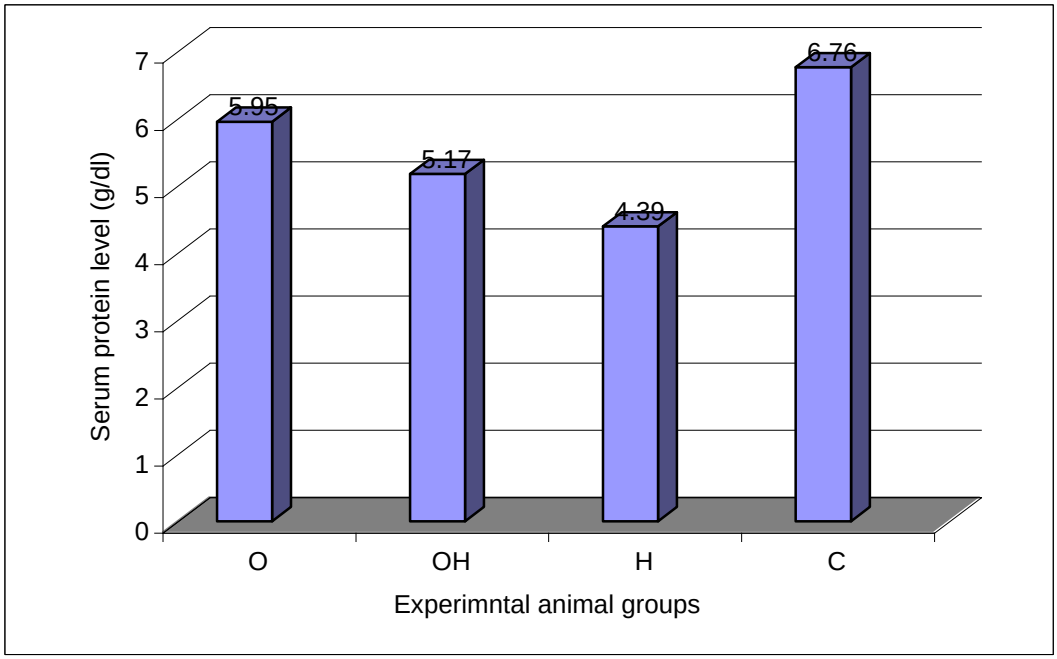

Fig. 4. The mean serum protein levels in the four experimental groups

5. DISCUSSION

This study was conducted with the objective to asses whether interaction exist between *Oestrus ovis* and *Haemonchus contortus* in artificially infected Ethiopian local breed goats and to determine the magnitude of interaction as well as explore the role of parasitological, haematological and some pathophysiological parameters in response to these interactions.

There were no marked differences in the number and development of *O. ovis* larvae collected in groups O and OH animals. Hence, it is assumed that the presence of *H. contortus* in the abomasum of animals in group OH had no notable influence on the development of *O. ovis* in the nasal-sinus cavities. In contrast, the presence of *O. ovis* was related to significantly reduction ($P<0.05$) in nematode egg excretion, female worm fecundity populations and as well as worm burden. These results were similar to the results obtained from previous experiments on interaction between O. *ovis* and gastro-intestinal nematodes using a different model i.e the intestinal worm, *T. columbriformis* (Yacob *et al*., 2002, 2004) and abomasal nematode, *H. contortus* (Terefe *et al*., 2005). *Oestrus ovis* larval proportional development, assessed by the presence of L_2 and L_3, in both groups of animals indicate the existence of a favourable season for the development of the larval instars.

In this study, the significantly lower faecal eggs and in utero egg counts recorded in animals from group OH as compared to group H indicates a previous *O. ovis* infection had led to a limited fecundity of *H. contortus*. There was significant difference in the total worm burden and female worm length between the two groups. Differences in the establishment rates of worms were also observed between the two *H. contortus* infected groups (OH and H). Our data provided evidence that the parasite development and the female fecundity were depressed in previously infected animals with *O. ovis* L_1 larvae (group OH) compared to *H. contortus* mono-infected animals (group H). This delay in parasite development has been shown after repeated exposure of lambs to *H. contortus* (Schalling *et al*., 1995; Dorchies *et al*., 1997) or *T. circumcincta* (Stear *et al*., 1995, Yacob *et al*., 2004). These alterations of parasite traits (development of worm and female fecundity), which might be related to the earliness of effector mobilization, rather than to the final level of the responses as established in repeated infection of lambs with *H. contortus* (Lacroux *et al*., 2003). The strong positive association found between female nematode worm length and faecal egg count or between worm length and in utero egg counts was in agreement with Claerebout *et al*. (1998) and

Gruner *et al*. (2003). Hence, the difference in the number of eggs might be directly related to the reduced worm burden and length of female *H contortus* in group OH. Ractliffe and Lejambre (1971) have demonstrated for different species of trichostrongyles from sheep and horses that the number of eggs depends on the worm growth (reflected on the length of worms). Stear *et al* (1995) demonstrated that the worm length was the main factor influencing eggs production and the number of eggs in utero for the *T. circumcincta* species. The lengths of female worms were significantly affected in the presence of *O. ovis* larvae in group OH. These differences might be due to longer worms found in group H. The negative effects of the presence of *O. ovis* on the biology of *H. contortus* populations observed in this study in group OH animals were manifested by a significant reduction in nematode egg excretion, fecundity, female worm length and worm population. This is in agreement with the results from a previous experiment on interaction between *O. ovis* and *T. columbriformis* (Yacob *et al.*, 2002) and *O. ovis* and *H. contortus* (Terefe *et al*., 2005). Similarly, Dorchies et al. (1997) reported differences in faecal egg counts while studying the effect of the presence of *O. ovis* larvae on the pathogenicity of *H. contortus* in sheep. However, they did not find notable difference in worm burden. Results from both studies thus strongly suggest that *O. ovis* has an antagonistic effect on worm biology of *H. contortus*. In contrast, in both studies, the presence of nematodes did not correlate with any change in the biology of *O. ovis* larvae. This suggests that when nematode infections occurred after the establishment of nasal bot fly larvae, the interactions were not reciprocal.

In the study on concurrent infection with *H. contortus* and *O. ovis*, it was postulated that eosinophils or other effector cells of the immune response against parasites could regulate the negative interactions between *O. ovis* and the nematodes. Although our results did not provide any data on the various mucosal cellular reactions participating in such interactions, an increase in mast cell, globule leukocytes and eosinophil populations in the digestive mucosae is generally described during nematode infection in small ruminants and a possible role of these cells as final effectors of the host immune response against trichostrongyles is usually evoked (Stevenson *et al*., 1994; Miller, 1996; Meeusen, 1999; Balic *et al*. 2000). Similarly, a proliferation of the same cellular types in the nasal mucosae has been associated with *O. ovis* infection but their precise role remains less documented than for nematode parasites (Nguyen *et al*., 1996, 1999; Dorchies *et al*., 1998; Yacob *et al*., 2001).

In our study, eosinophil counts were moderately associated with worm population, female worm length and fecundity although it remains non-significant. However, eosinophilia was significantly correlated with the establishment rate of the larvae of *H. contortus* and *O. ovis* in infected animals. Eosinophils are considered to be important elements in the response against helminth infections and are frequently associated with the expression of resistance to the parasites (Balic *et al*., 2000; Dawkins *et al.,* 1989; Pfeffer *et al*., 1996). In this study, there was an increased mobilization of circulating blood eosinophils against *O. ovis* larvae. This was in agreement with Yacob *et al*. (2004) and Terefe *et al*. (2005). Furthermore, animals infected with *H. contortus* alone showed considerable degree of blood eosinophlia as compared to the non-infected animals. Eosinophis mobilized against specific parasites were frequently found to cause immobility and death of larvae of homologous or heterologous parasites often in association with antibodies and/or other factors (Emery *et al*., 1993; Kazura and Grove, 1978; Rainbird *et al*., 1998; Rotman *et al*., 1996).

In our study, activated circulating eosinphils might have influenced the development of *H. contortus* in the abomasums. This may be through migration into the abomasum and acting directly on the worms in association with antibodies and other inflammatory cells or by releasing various toxic protein/factors (Wardlaw, 1996) into the blood circulation, which could be in contact with haematophagous stage of *H. contortus*. Holmes and Price (1986) evoked to main mechanisms to explain interactions between parasite populations at the infrapopulations level, either directly, by competition for ecological niches or resources; or indirectly, through stimulation of the host response. Because *O. ovis* and *H. contortus* occupy remote anatomical sites, we suggest that mechanism explaining the negative interactions could be based on some immune/inflammatory effectors, affecting the two parasite species and connected through the mucosal immune system.

The haemotocrit (PCV) is an essential parameter, which may be used beside faecal egg counts to describe resistance against nematode parasites in sheep in situations where the dominant nematode species sucks blood (Amarante *et al.,* 2004). The PCV differences between groups as well as between the two nematode infected groups were significant ($P<0.05$), group OH being less anaemic than group H. This might be attributed to the difference in the size and numbers of the parasites, assuming that longer and large number of parasites feed more blood than the shorter and smaller number ones. Hence, animals concurrently harbouring larvae of

O. ovis seem to tolerate the pathogenic effects of haemonchosis by reducing parasite length and numbers thereby minimizing the quantity of blood loss caused by the parasites. We have also observed that PCV was negatively correlated with female worm length and FEC in support of the findings of Gauly and Erhardt (2002).

The experimentally infected animals with *H. contortus* showed reduced serum protein concentrations being significantly lower ($P<0.05$) in group H. These changes might be attributed to blood loss and impairment of appetite, digestion and absorption. This was in agreement with Arzoun *et al* (1984).

Our results illustrate the basic value of this model of mixed infection with *O. ovis* and abomasal nematode to further study the interactions between parasites and explore the related mechanisms. Besides, in the field, mixed infections of goats with nasal bot flies and trichostrongyles are common in tropical regions. Therefore, the current model of study has also applied implications, for instance to understand what are the consequences of these interactions on the epidemiology of both parasite infections, or to examine specific treatment against one parasite on the dynamics of infection with the second group.

6. CONCLUSION AND RECOMMENDATION

The structure of parasite communities, the possible existence of positive or negative interactions between populations and the analysis of the underlying mechanisms acting both at the level of supra and/or infrapopulations represent a major field of research and discussions in Parasitology and ecology. In goat concurrent infections with several parasites is the commonest situation in the field. The dipteran flies *O. ovis* and the nematode *H. contortus* are often sympatric. Despite this general situation, relatively few studies have examined the biological, epidemiological and pathophysiological consequences of such multiple infections.

In this study it was demonstrated that repeated infections of goats with *O. ovis* larvae had a prominent influence on the growth and egg laying capacity of a subsequent *H. contortus* infection. This influence might be through the enhanced recruitment of activated inflammatory cells and/or their products towards the gut mucosa that finally created unfavourable environment to the nematode population, which resulted in reduction of worm development, length, fecundity and faecal egg outputs. Infection of the digestive tract with nematodes did not modify the biology of *Oestrus ovis*. In contrast, the negative effects of the presence of *Oestrus ovis* was manifested by significant reduction in nematode egg excretion, fecundity, worm length and adult worm population.

Minor changes and differences between mono-infected or groups infected with both parasites were detected in circulating eosinophils while these cells significantly increased in both groups rapidly after infection indicating the possible role of these cells during parasitism as well as such interactions. Infection with *Haemonchus contortus* resulted in significant reduction of red blood cell and hemoglobin concentrations as well as while serum protein level that might be due to blood loss and impairment of digestion and absorption.

Explanations of the exact mechanism that could explain the observed negative interactions between these two distantly localized parasite populations remain largely unidentified. However, a mediation could be through possible changes in inflammatory cells (eosinophils, mast cells and globules leucocytes).

The current study provide a unique model to examine interactions between parasites which do not share the same anatomical sites, hence investigation should further be conducted to

understand the mechanism and the role of host immune or inflammatory response within the mucosal immune system

Although the present study was conducted on limited number of goats per group and also did not provide any data on the various mucosal cellular reaction components participating in such interactions we suggest detailed studies using more animals per group restricting the analyse of the effector mechanisms to indirect effects through host immune or inflammatory response within mucosal immune system.

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

- Studies of these concurrent infections between *Oestrus ovis* and nematodes offer a unique model to examine interactions between parasites which do not share the same anatomical sites, hence investigation should further be conducted to understand the mechanism and the role of host immune or inflammatory response within the mucosal immune system

- These biological, epidemiological and pathophysiological consequences illustrated during this experiment should be further compared with concordant data from field observations

- This model of study should be rationalized and conducted by using animals with known genetic origin as well as of the same sex and age group.

- Any possible existence of interactions between parasites of the same or distant group should be further explored

- Since studies on such interactions could have major consequences on the epidemiology and control of these parasitism further studies should be conducted after natural challenge of animals

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8. ANNEX 1: PARASITOLOGICAL TECHNIQUES

A. Procedures for recovery of nematodes from the abomasum

1. Separate the abomasum from the intestine for wash.
2. Open the stomach in bowl and collect the contents.
3. Wash the stomach wall thoroughly under stream of water from the tap and rub the mucous membrane carefully with the fingers to remove any adhering to it.
4. Pour the contents of the bowl a little at a time to a wire mesh screen with an aperture of 0.15 mm and then wash with a stream of water from a rubber tube attached to a tap until no more coloured matter or feed particle pass through.
5. Flush the content on the sieve with a jet of water from the tap.
6. Fill the content of the bucket to 4 litters.
7. Agitate the whole content vigorously and take an aliquot of 200 ml by using a beaker and place in a glass Petri dish and examine under a stereomicroscope.
8. Adding few drops of iodine solution and allowing standing for 35 minutes can facilitate examination. Adding sodium thiosulphate leaving the parasites stained can also make decolourization of the stained debris.
9. Count the number of each species and multiply by a factor to arrive at the total parasite burden (in this case by 20 assuming total volume of 4 litters).

B. Procedures for nematode faecal egg counts using McMaster egg counting technique

1. Weigh 3 gram of faeces.
2. Break up thoroughly in 42ml of water in a plastic container.
3. Pour through a fine mesh sieve (aperture 250microns).
4. Collect the filtrate, agitate and fill a test tube, preferably 15ml and flat bottomed.
5. Centrifuge at 2000 rpm for 2 minutes.
6. Pour off supernatant, agitate sediment and fill the test tube to the previous level With flotation solution.
7. Invert the test tube 6 times and remove fluid with pipette to fill both chambers of McMaster slide.
8. Examine one chamber and multiply number of eggs under one etched area by 100 or two chambers and multiply by 50 to arrive at the number of eggs per gram of

Faeces (EPG).

Mathematics of McMaster chamber

3grams of faeces dissolved----------42 ml water

Total volume ----------42+3 = 45ml

Therefore:

1gm------ (3gm=45ml) ----45/3=15ml

The volume under (1g) faeces etched area is 0.15ml

Therefore the number of eggs is multiplied by 100

If two chambers are examined, multiply by 50.

C. Procedure of faecal Culture

1. Take a small amount of faeces up to 10 gram from the rectum of an animal.
2. Grind the faeces into pieces (pelleted faeces) using pistle and mortar
3. If the faeces are dry, add small amount of water to moisten it. If the faeces are wet (diarrheic) add sterile faeces or animal charcoal to stabilize its moisture content.
4. Culture the material using glass culture dishes and place it in the incubator at 27°c for 7 days.
5. Recover the larvae using Baerman technique

D. Procedure of Baerman technique for larval collection

1. Take 10 gram of cultured faeces
2. Wrap the faeces using gauze and place into the Baerman apparatus funnel to which a sieve is placed
3. Cover the faeces using lukewarm water (40-45°c)
4. Allow to stand overnight
5. The clipper on one end of the rubber tube is then released and the liquid collected in a test tube
6. Centrifuge the collected material for 2minutes at 2000rpm
7. Discard the supernatant and examine the sediment for moving larvae

ANNEX 2: HAEMATOLOGICAL EXAMINATION

A. Thin blood smears preparation for a white blood cell differential count

1. Place a glass microscopic slide on a level surface.
2. Place a small drop of thoroughly mixed whole blood sample near one end of the slide.
3. Spread the blood in an even film by means of another slide.
4. Allow the blood films on slides to air dry and then stain it.

B. Staining blood films on slides by Wright's staining method

1. Place the slide on the staining rack.
2. Add 4 to 10 drops of stain with a medicine dropper.
3. Allow the concentrated stain to act for 1 minute (Fixation period).
4. Add an equal number of drops of buffer or neutral water and mix thoroughly by blowing until a metallic film forms on the surface of the stain-buffer mixture.
5. Let the diluted stain act for 2 minutes (Staining period).
6. Float off the metallic film with tape water (quickly flooding water over the slide).
7. Examine the slide with the 100X (oil-immersion) for differential white blood cell counts.

9. CURRICULUM VITAE.

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1963- 1966: Bahar Dar Pedagogical collage, Certificate of teaching

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A. Basaznew Bogale (2007): Study on the interactions between *Oestrus ovis* (L_1) and *Haemonchus contortus* (L_3) in experimentally infected goats. MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia, pp, 1-56.

10. SIGNED DECLARATION SHEET

This thesis is my original work and has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name: Basaznew Bogale

Signature: _____

Date of submission _____

This thesis has been submitted for examination with my approval as University Advisor.

Dr. Yacob Hailu _____
(DVM, MVSc, PhD, Asst. Professor)