Thesis Ref. No._____

ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTUER,

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

EXPERIMENTAL *TRICHOSTRONGYLUS COLUBRIFORMIS* INFECTION PROFILE IN SHEEP AND GOATS AND *IN VITRO* NEMATOCIDAL EFFECT OF *ARTHROBOTRYS OLIGOSPORA* AGAINST L3 OF THE PARASITE

MVSc THESIS

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JUNE 2020

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MVSc THESIS

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Parasitology

BY

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As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by Tamirat Kaba Tona, entitled "**Experimental T*richostrongylus colubriformis* infection profile in sheep and goats and *in vitro* nematocidal effect of *Arthrobotrys oligospora* against L3 of the parasite**" and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Veterinary Parasitology.

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STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an MVSc degree at Addis Ababa University, College of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Name: Tamirat Kaba Tona Signature:

Date of submission: 8 June 2020

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LIST OF ABBREVATION

AAU	Addis Ababa University
AH	Anthelmintic
CVMA	College of Veterinary Medicine and Agriculture
dpi	days post infection
epg	eggs per gram of feces
EDTA	Ethylene Diamine Tetra-Acetic Acid
ESGPIP	Ethiopia Sheep and Goat Productivity Improvement Program
FEC	Fecal Egg Count
GINs	Gastrointestinal nematodes
PDA	Potato Dextrose Agar
PCV	Packed cell volume
SE	Standard error

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ACKNOLWDGMENTS

First for most, I thank the almighty God for his kindness to be everywhere I live in order to help me at each stage of my life; without his assistance, nothing would have happened.

I am highly indebted to my research advisors Dr. Getachew Terefe and Dr Gezahegne Mamo for their unreserved support, intellectual guidance and encouragements from conception to the end of this research work.

I would like to extend my gratitude to my employer, Arba Minch University for financial support to realize my Masters study and research work.

This research was funded by the graduate programs' research support of Addis Ababa University and the BiOCON thematic research project being funded by the Office of the Vice President for Research and Technology Transfer of the University.

Lastly, but not least, I thank all my academic instructors at CVMA, AAU who have thought me not only academic matters, but also professional experience and social affairs.

ABSTRACT

Trichostrongylus colubriformis is one of the major gastro intestinal nematodes (GINs), which cause severe morbidity and mortality in small ruminants worldwide. This comparative study on the infection profile of sheep and goats with an intestinal worm *Trichostrongylus colubriformis* was aimed to assess the performance of the parasite and the responses of the hosts. It also evaluated the effect of a local isolate of nematophagus fungus species, *Arthrobotrys oligospora* on the L3 of the parasite *in vitro*. A total of 14 sheep and 14 goats were employed in such a way that half of them were drenched with 10000 L3/animal and the remaining halves were uninfected controls. The infection profile and impacts of infection on the animals were assessed by measuring faecal egg count, packed cell volume and body weight gain for up to 8 weeks. After 8 weeks of infection, experimental animals were killed in humane manner, worms were recovered from the intestines, counted, and worm burdens were determined. In addition, nematophagous fungus (*Arthrobotrys oligospora)* was used for *in vitro* trial against L3 after incubating the fungus and the worms in potato dextrose agar supplemented with 0.05% chloramphenicol. Accordingly, faecal egg count at 18, 45, 52 and 56 days post infection was significantly higher in goats than in sheep, and so is worm burden at the end of the study ($P<0.05$). Packed cell volume was significantly reduced in both sheep and goats ($P<0.05$). However, it came back to normal earlier in sheep while it remained significantly lower than the value of control in infected goats. Goats experienced significantly lower weight gain compared with sheep. *In vitro* trial revealed, *Arthrobotrys oligospora* reduced survival of 84% of infective larvae after 10 days incubation. In conclusion, sheep performed better than goats to the impacts of experimental infection by *T. colubriformis* based on assessed parameters. Efficacy of the local strain of *A. oligospora* is a promising step for future biological control options against nematodes. Therefore, husbandry management of small ruminants particularly goats, in which infection results in severe production loss, in the areas where *T. colubriformis* prevails should use local epidemiological knowledge and focus on minimizing the exposure to infective stage in the field. A wide scale *in vitro* trail and further *in vivo* studies about efficacy of *Arthrobotrys oligospora* against *T. colubriformis* and other GINs is also recommended.

Key words:*, Arthrobotrys oligospora, Experimental infection, , Goat, Sheep, T. colubriformis*

1. INTRODUCTION

Trichostrongylus colubriformis (T. colubriformis), in addition to other gastro intestinal nematodes (GINs), appears to pose significant economic impacts in small ruminants' production worldwide, in particular tropical and subtropical regions. It is probably the most important parasite second to *Haemonchus contortus (H. contortus)* in small ruminants. In Ethiopia, GINs are amongst the primary causes of morbidity and mortality in small ruminants (Bekele *et al.,* 1992b). According to several studies (Bekele *et al.,* 1992a; Haile *et al.,* 2018; Mohammed *et al.,* 2016), *T. colubriformis* is the major parasite among many GINs that infect small ruminants in the country. The economic impact of this parasite is associated with poor performance with respect to growth rate, body weight gain, milk yield and wool production. The impact of this parasite and other GINs in sheep have been reviewed by Mavrot *et al.* (2015).

Assessing the impacts of different GINs in small ruminants is crucial to prioritizing the type of parasite for which control strategies are targeted. In field conditions however, impact assessment for monospecific infection is quite challenging, as mixed infection often takes place (Tan *et al.,* 2014). Consequently, monospecific infection profiles by several GINs are often assessed through experimental trials. Experimental studies (Barker, 1973; Kyriazakis *et al.,* 1996) show that *T. colubriformis* significantly reduce economically important production traits, such as body weight, which might be associated with loss of protein and in appetence during infection (Kimambo *et al.,* 1988). It is not clear whether *T. colubriformis* induces similar effect in both sheep and goats in experimental trials. Since the burden of infection and level of host immunological response dictate the effect, comparative study on parasitological profiles, such as faecal egg count and worm burden would help identifying relatively better resistant or resilient species of small ruminants. In natural conditions, goats appear to have relatively lower worm burdens compared to sheep, presumably due to their feeding variations (goats browse on leaves of trees and shrubs on which parasite cannot attach, whereas sheep prefer to graze on pasture grass on which infective 3rd instar larva (L3) often present). It could also be possible that the two species of hosts differ in their inherent susceptibility to the infection. Such

variation can better be assessed by exposing both sheep and goats to common contaminated pasture grass, where both species co-graze or by controlled experimental infection in order to determine whether the variation in infection is attributable to the inherent species difference.

The control strategies of this parasite are not quite different from the strategies against other GINs. The use of chemotherapy as a control method is still widely practiced in many farming communities. Various anthelmintic (AH) chemotherapies have been shown to be successful in eliminating most species of nematodes, including *T. colubriformis* during their early periods of utilisation, and some still remain effective in different parts of the world (Terefe *et al.,* 2007). However, resistance is becoming widespread because of the relatively few chemically dissimilar groups of AH available for the last many decades and their misuse (Aiello *et al.,* 2005). Resistance to common AH by many GINs, including *T. colubriformis* have been documented worldwide (Zvinorova *et al.,* 2016a), which makes AH control strategy difficult in the future. Although not identified to species level, the genus *Trichostrongylus* (Sissay *et al.,* 2006; Kumsa and Abebe, 2009; Seyoum *et al.,* 2017) has been reported to resist to common AH compounds circulating in Ethiopia.

As a solution against increasing emergence of AH resistance, the use of non-chemical alternative methods over AH usage has been recommended (Maqbool *et al.,* 2016). Of several alternatives, the application of nematophagous fungi, as a biological control method has been shown to have promising results in both experimental and field conditions in different countries (Larsen, 2004). Researches show that several types of nematophagous fungi appear to exist worldwide, and are found in natural environment at different agricultural ecology, including fecal material of domestic animals. Most of identified nematophagous fungi in different countries that have proven predatory activity against free-living stages of nematodes are those that belong to genus *Arthrobotrys and Daddingtonia* (Cai *et al.,* 2017a; Falbo *et al.,* 2013; Longo Ribeiro Vilela *et al.,* 2016; Ojeda-Robertos *et al.,* 2019). Among many strains under genus *Arthrobotrys,* the efficacy of *Arthrobotrys oligospora* against the free-living stage of nematodes, including *T. colubriformis* have been documented in many countries (Ghahfarokhi *et al.,* 2004; Cai *et al.,* 2017b; Alfaro Gutiérrez *et al.*, 2011; Ojeda-Robertos *et al.,* 2019).

Nevertheless, efficacy may vary depending on the region where the strain are isolated; suggesting, the local isolates should be used for efficacy study.

Therefore, the general objective of this study was to compare monospecific experimental infection profiles of sheep and goats with *T. colubriformis* and to determine *in vitro* efficacy of local isolates of the soli fungus, *Arthrobotrys oligospora* against the infective stage of the parasite.

Specific objectives:

- To compare parasitological profiles between sheep and goats experimentally infected with *T. colubriformis*
- To compare the effects of experimental infection of *T. colubriformis* on live weight and packed cell volumes of infected animals
- To assess *in vitro* efficacy of *Arthrobotrys oligospora* against L3 stage of *T. colubriforms*.

2. LITERATURE REVIEW

2.1.Biology

Like other GINs, *T. colubriformis,* infect the alimentary tract (small intestine) of small ruminants (Taylor *et al.,* 2016), hence the biology of this parasite shares common pattern with the biology of other related GINs (Figure 1). Infection occurs by ingestion of the L3. On its way to small intestine, L3 losses its protective cover (cuticle) at stomach. After this step, L3 reaches at small intestine to penetrate mucosa for further development to L4. Finally L4 stage burst out of the mucosa to appear in the lumen of the most first segment of intestine, where they become sexually dimorphic adults. To sustain their life, adult female lay eggs, which are shaded through the fecal pellets of small ruminants to the environment for further hatching under optimum environmental conditions.

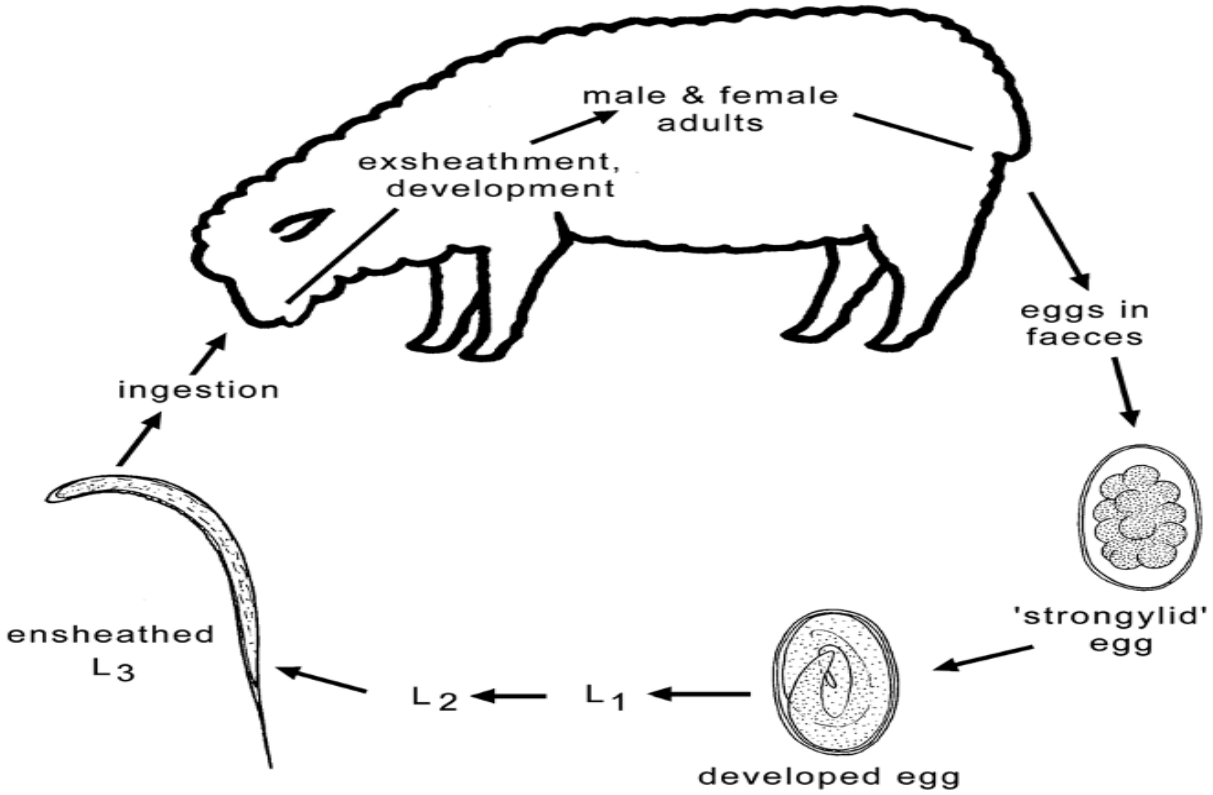

Figure 1: Biology of GINs parasites. Adapted from (Roeber *et al.,* 2013)

2.2.Epidemiology

2.2.1. *Distribution and magnitude*

T. colubriformis occurs worldwide, where small ruminants are raised, but it is more important in tropical and sub –tropical countries. A frequent diagnostic rate of this parasite in tropical regions, including Ethiopia, suggest the economic importance of this parasite in small ruminant farming communities. The prevalence of this parasite varies depending on the geography as reviewed by Mavrot *et al*. (2015). In Ethiopia, study has shown that this nematode parasite affects the small ruminants' production sector in South, West, East and North-West of the country, with a pooled prevalence ranging from 20 to 87.2% (Asmare *et al.,* 2016).

2.2.2. *Environmental and seasonal factors*

T. colubriformis performs better at slightly warmer and relatively humid environment. However, it can also adapt well during prolonged winter or dry period in temperate and tropical countries respectively. As with other GINs, infection with *T. colubriformis* often considered as seasonal problem in small ruminants flocks, because many environmental abiotic and biotic components, including temperature, rainfall, plants, bacteria and humidity that determine survival of parasites on the natural environment fluctuate based on the season of the years (Singh *et al.,* 2017). As reported by scholars of Ethiopia, hot and rainy season appears to favor survival of several GINs, including *T. colubriformis* whereas prolonged dry season inhabit their survival (Sissay *et al.,* 2007; Mohammed *et al.,* 2016; Haile *et al.,* 2018). This seasonal variation most likely attribute to the differences in temperature and relative humidity between wet and dry seasons. It has also been shown that the infection magnitude between different agro-ecology of the Ethiopia does not vary significantly, indicating similar impacts of this parasites across the country (Asmare *et al.,* 2016).

2.2.3. *Parasite factors*

The parasite factors, such as survival, infectivity and ability to induce pathology in the host are crucial determinants that affect infection magnitude. The type of infection weather it is mono or mixed clearly determine the magnitude of infection. In small ruminants, clinical disease become worst in mixed infection of *T. colubriformis* and other GINs. However, it appears that the sole infection by *T. colubriformis* can induce pathology in small ruminants in both experimental and natural infections (Wagland *et al.,* 1996; Alessandro *et al.,* 2007). Indeed, the infectivity and severity of infection of *T. colubriformis* depends on the amount of infective dose. Furthermore, yet it is unclear whether sheep derived strain can induce significant infection in goats and vice versa; as some reports, such as study by Rahman *et al.* (1990) have examined that low infectivity of the parasites in goats when sheep derived strain was used.

2.2.4. *Host factors*

T. colubriformis parasitism in small ruminants is affected by host related factors, which include species, age, sex and breed. As many reports reveal, sheep appear to contract higher infection compared to goats under extensive production. This variation may be attributed to the different feeding behavior of sheep and goats (Bansal *et al.,* 2015). Even though they are kept together in the same contaminated pasture, where leaves of trees and shrubs are available, goats often have low infection burden possibly due to low intake of L3. On other hand, many researches demonstrate the possibilities of goats to be infected as high as sheep when they co-graze with sheep in natural contaminated pasture, where plants are not available for browsing suggesting goats are protected by their feeding habit. However, providing equal chance of exposure (preferably experimental infection or the same feeding conditions) between sheep and goats would help determining whether the infection variation is due to inherit species difference.

With respect to age, young are more vulnerable than adults to the parasites probably because, young have under developed immunity relative to adults (Zahangir Islam *et al.,* 2017). Contrary to this, some studies have shown that infection magnitude is significantly higher in older than in younger animals possibly, because of the level of exposure variations. Many scholars have

investigated sex wise infection rate, and it seems that reports are not always consistence. According to the reports (Singh *et al.,* 2017; Zahangir Islam *et al.,* 2017), the infection rates are significantly higher in female than in male. This variation happens due to hormonal change during pregnancy and lactation in female, which compromise the immunity in female. On the other hand, Zvinorova *et al*. (2016b) reported that infection rate was higher in male than in female goats for unknown reason. Of the biological host related factors, breed is one of the determinants of *T. colubriformis* infection in small ruminants. Breed variation is often associates with individual's genetic difference (Onzima *et al.,* 2017).

2.3. Pathogenesis, Clinical Signs and Significance of *T. colubriformis*

Once unsheathed L3 reach at the first segment of small intestine, they penetrate the mucosa to undergo further development (L4). After a couple of days, the L4 stage burst out of the mucosa to appear in the lumen of intestine. This developmental pathway creates severe mucosal damage and sometimes hemorrhage; consequently, loss of protein and other nutrients can occur. The parasite causes a dark diarrhea and hypoproteinemia, hence bottle jaw with poor appetite and weight loss are common manifestation. Milder infections are associated with soft stools and poor growth rates. Affected lambs and kids may have soiled fecal material on the perineal area and tail (evidence of diarrhea) (Urqahart *et al.,* 1996). The significances of this parasite are therefore, poor performance in terms of production traits, such as body weight gain.

2.4. Indicators of Infection

2.4.1. *Parasitological parameters*

Manifestations of GINs infections often assessed through determination of parasitological (fecal egg count (FEC), worm burden), hematological (Packed cell volume (PCV), Red blood cell count, leucocyte count, hemoglobin determination), clinical (Body weight, Body condition) and biochemical parameters (serum protein, albumin, globulin, enzymes, immunoglobulin). One of parasitological quantitative measurement used to identify infection burden is FEC. Although FEC is not specific for individuals' parasite species, it indirectly measures the burden

of worm infections of the host. It also helps evaluating susceptibility/resistance status of the hosts without the need of killing animals. Many studies have identified that FEC correlates positively with worm burden, though this does not always holds true (Onzima *et al.,* 2017), as several factors, such as parasite sex ratio, patency, host immunity, genetic basis of breed of host, host physiological conditions etc. affect its measurement. The principle behind this parameter is that, infection and worm establishment rates are high in susceptible hosts, resulting in a significant number of adult worms in the predilection site that shed huge number of eggs through fecal pellets depending on the number of female worms.

Unlike FEC, which is an indirect measure of infection burden, worm count is a direct quantitative parameter that measure the true burden of infection, as it regards counting of both sexes of adult and immature worm. It corrects the bias associated with FEC, which disregards the presence of adult males and immature worms. Nevertheless, it is not always practical and economical to kill animals to evaluate infection burden with this technique. In order to count *T. colubriformis* alone, one has to differentiate it from other *Trichostrongylus* species and genera found in the small intestine of sheep and goats. Differential count of adult *T. colubriformis* in mixed infections requires referring micromorphometery studies of the parasite with respect to its size (Alessandro *et al.,* 2007) and cuticular modifications (bursa, spicules, gubernaculum, excretory notch, tail area etc.). The presence of excretory notch at the esophageal region is a distinct generic feature of this parasite (Figure 2). Sex identification is based on the morphology of tail area. In female, the tail is tapering and pointing with eggs visible in the uterus, where as in male; the tail region has swelling of cuticular modification (bursa) along with spicules. In addition, the female has double ovejectors and no valval flap. Species differentiation is mainly on the characteristics of male spicules, which is broad, small boat shape and slightly unequal in *T. colubriformis* in contrast to *T. vitrinus* for which, spicules are leaf shape, equal in length and pointed at the tip (Taylor *et al.,* 2016).

Figure 2: Spicules of *T. colubriformis* and excretory notch of genus *Trichostrongylus*

Source: (Shahbazi *et al.,* 2012; Taylor *et al.,* 2016).

The third quantitative parasitological trait is worm establishment rate, which estimates the number of infective larva that are successfully developing into successive immature and adult stage at the predilection site of animal tissues. Its magnitude indirectly tells us the hosts' resistance capacity to the infection caused by GINs. The difference in worm establishment rate for many GINs may be associated with variations of host related factors, such as genetic and immunity. Studies reveal that the worm establishment rate of experimental infection of *T. colubriformis* rarely exceeds 50% (Table 1). In fact, many factors, including infection frequency and infection duration appear to influence the establishment rate of the worm.

Table 1: Literatures on worm establishment rate of single dose experimental infection with *T. colubriformis* in small ruminants

Animal species	**Average WER (%)**	**dpi**	**References**
Goat	23.8	21	(Rahman *et al*., 1990)
	72.3	21	(Moreno-Gonzalo *et al.,* 2014)
Sheep	39	21	(Roy *et al.*, 2004)

WER= worm establishment rate, dpi=dpi

Finally, in addition to quantitative parameters mentioned above, the worm population structure, such as worm sex and development stages and phenotypic traits, including worm length and female fecundity are taken in to consideration, as hosts' inherent genetic factor and immunity affect them. Phenotypic traits of worms are highly related to each other's, and they indicate worms' reproductive potential (life cycle). It has been shown that a significant correlation exists between worm length and fecundity for *T. colubriformis* infection in small ruminants (Chiejina *et al.,* 1974). Any host related factors that affect the worms' length can also affect the fecundity of female worms. Moreover, a worm population characteristic that affect the epidemiology of GINs in animals is the number of inhabiting male and female worm population. Female to male sex ratio always positively correlates with FEC of infected animals. Empirically, higher female to male sex ratio in animals' tissue suggest the more pasture contamination by the animals.

2.4.2. *Clinical parameters*

Various clinical parameters like production traits, such as body condition, body weight, milk and carcass yield and vital clinical parameters, including body temperature, pulse and respiratory rate can be assessed as GINs infection indicators in small ruminants. One of the production traits of animal that is often affected by *T. colubriformis* infection is live body weight. Reduction of live weight in animal is presumably due to the pathogenic effect of the worm, as they pose pathology of intestine from, where micronutrients are absorbed to the system. In general, the worms disrupt the absorptive capacity of the intestine and thereby decrease the availability of nutrients in the tissue of animals. In addition, reduced appetite caused by parasitic infection appears to involve in retarding the weight gain. Many experimental studies have reported the occurrence of significant variations in live weight gain among infected and uninfected groups of small ruminants (Kimambo *et al.,* 1988; Kyriazakis *et al.,* 1996; Beriajaya and Copeman, 2006), despite other claims that no significant differences exist (Chiejina *et al.,* 1974).

2.4.3. *Haematological parameters*

Several workers have determined haematological indicators, such as total red and white blood cell count, differential leucocyte count and packed cell volume (PCV) in small ruminants experimentally infected with *T. colubriformis* (Beriajaya and Copeman, 2006; Cardia *et al*., 2011). Along with other haematological parameters, different works have reported the threshold PCV value in healthy small ruminants' species (Table 2). The physiological PCV threshold value of healthy individual small ruminants depends on the types of breed, sex, nutritional status, age and other conditions. Researches show that hematophagous GINs, such as *H. contortus* cause significant reduction of PCV value in small ruminants (Beriajaya and Copeman, 2006; Rouatbi *et al.,* 2016). On other hand, despite other GINs, such as *T. colubriformis* are not blood feeders, their infection sometimes pose significant reduction in PCV level in susceptible sheep and goats (Beriajaya and Copeman, 2006; Cardia *et al*., 2011). The pathophysiology about PCV reduction in case of infection with non-hematophagous GINs, including *T. colubriformis* remains unanswered, though some scholars associate it with hemorrhage caused by mucosal penetration by immature worms.

Table 2: Selected Literature on PCV of adult healthy sheep and goat breeds in some countries

Species/breed	**PCV (Mean ±SD)**	**Country**	**Reference**
Sheep			
Menz	32.06±0.34	Ethiopia	Tibbo *et al*. (2004a)
Tukur	30.46±0.61		
Wello	30.53±0.48		
Ars-Bale	27.69±2.75		Tadie *et al*. (2018)
Dhumba	33.7±6.1	Bangladesh	Islam *et al*. (2018)
Nepalese Baw	35.92±1.59	Nepal	Amatya Gorkhali *et al*. (2017)
Goat			
Arsi-Bale	25.53±0.36	Ethiopia	Tibbo *et al.* (2004b)
Central Highland	26.28±0.35		

Long Eared Somali	23.89±0.37		
Omani breed	38.29±4.06	Oman	Al-Bulushi *et al*. (2017)

SD =standard deviation

2.5. Prevention and Control

2.5.1. *Anthelmintic (AH) usage*

The use of AH is the most extensively employed method to suppress GINs parasitism. The most common AH drugs used to control GINs in many countries are the old ones that belong to class Benzimidazole (e.g. Albendazole), Imidazothiazole (e.g. Levamisole) and Macrocyclic lactones (e.g. Ivermectin). However, resistance to these drugs is becoming widespread because of the relatively few chemically dissimilar groups of AH available for the last many decades and their misuse (Aiello *et al.,* 2005). As a solution, the new advancement in veterinary AH reported in some European countries, such as Switzerland (Epe and Kaminsky, 2013), which advocates the uses of new AH drugs that have new mode of action is a promising step to combat AH resistance.

But nowadays, instead of relaying only on the use of chemicals to control parasitism, integrated parasite control mechanisms have been suggested in different agro-ecology (Maqbool *et al*., 2016). Integrated parasite control approach is the use of multiple methods including, AH treatment, pasture management, improving animal management (feeding and watering, housing, sanitation, veterinary service), breeding for resistance trait, biological methods and others.

2.5.2. *Use of nematophagous fungi*

The use of non-chemical control methods in livestock farming are increasingly getting due attention to lessen the escalating problem of AH resistance in livestock farming. A biological control (the use of nematode trapping fungi) is among many non-chemical control methods of GINs. Most of the fungi are harmless either to host or environment, and are free living, but they

trap and destroy when they encounter larva stages of GINs (Faedo and Krecek, 2002). The environmental conditions that favor the performance of free-living stages of parasites do also favor the nematocidal effect of the fungi, suggesting the local strains of fungi species are important in reducing the number of L3 on pasture. According to available evidences, several isolates of fungi have nematocidal effect against L3 of GINs, including *T. colubriformis* of small ruminants (Table 3).

Identifying the natural sources of these fungi is of paramount importance for mycological studies. Researches show that nematophagus fungi can be isolated, identified and characterized from various natural sources, including pasture and woodland soil, cultivated land, vegetation, livestock fecal material, dung compost etc. from which laboratory samples/subsamples can be obtained. However, it is not yet known, whether their ecological distribution coincides with the occurrence of GINs. The methods of laboratory technique to harvest the fungi have been described elsewhere (Ghahfarokhi *et al.,* 2004; Gray, 1984).

Table 3: Local isolates of nematophagous fungi in different countries

Country	Species	Effective against	Natural Source	Authors
China	*Arthrobotrys sinense and Arthrobotrys oligospora*	*H. contortus* *T. colubriformis*	Pasture barn, woodland soils and sheep & cattle feces	(Cai *et al.,* 2017b; Ya-Juan *et al.,* 2018)
Brazil	*Arthrobotrys musiformis*	*Haemonchus, Trichostrongylus Cooperia Strongloides*	Pasture soil	(Graminha *et al.,* 2005)
Iran	*A. oligospora*	*H. contortus*	Pasture soil	(Ghahfarokhi *et al.,* 2004)
France	*D. flagrans*	*T. colubriformis Teladorsagia. circumcincta*	Preserved strain	(Chartier and Pors, 2003)
South Africa	*D. flagrans* *A. oligospora*	*H. contortus*	Soil, faces and compost sample	(Durand *et al*., 2005)

The mechanisms of action of nematophagous fungi depends up on the type of strain. Some nematophagous fungi, such as *A. oligospora* involve in production of trapping net, which are sticky in 3-dimensional forms (figure 4a) while others strains, such as *A. conoides*, *Monacrosporium thaumasium and A. dactyloides* form active ring that surround nematode larva's' body (figure 4b). The production of these nets depends on the movement activity of nematode larva and the abiotic components of microclimate, such as temperature, moisture and PH (Xue *et al.,* 2018a). It has been indicated that larval activity, growth and movement in the fecal pellet/pats enhance the production of trapping nets by fungi as shown in Figure 4. Capturing is followed by penetration of cuticle, which results in infection of larva. Hyphae grows out of infection site and cover all over the body of larva.

a) b)

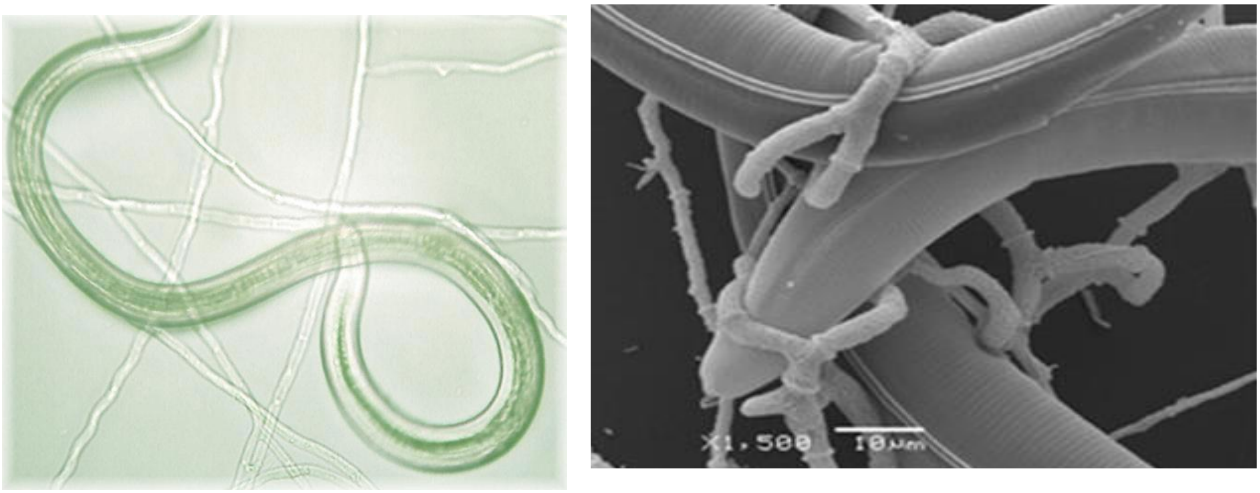

Figure 3: Mechanisms of trapping activities of nematophagous fungi against nematode larva

Source: (Alfaro Gutiérrez *et al.*, 2011; Falbo *et al*., 2013)

The spores/chlamydospores of nematophagous fungi can be given to the animals as feed supplement if the fungi tolerate and are viable after being passed through GIT of animals or can be applied directly on pasture otherwise. These application methods of fungi have been suggested after elucidating the presence of isolates, which have different nematocidal effect in two subsequent trials (in *vitro* laboratory culture and after passing fungi through GIT of animals) (Graminha *et al.,* 2005). Some strains are effective *in vitro* laboratory culture in reducing nematode larva, but lacks their efficacy when they are passed through GIT of animals

due to their inability to withstand the harsh gastro intestinal environment of animals. On the other hand, few isolates such as *A. oligospora* tolerate the passage though GIT of animals, as they possess very thick conidia that resist the mechanical and chemical digestion of the gut (Chartier and Pors, 2003). Also knowing the time at which adequate proportion of fungi spores present in the fecal pellets/pats after passage through GIT would provide a deep insight for the efficacy study. According to Cai *et al*. (2017a), adequate amount of fungi can be found in the fecal pellets/pats between 24 and 72 h after the dose.

Evaluation of the nematophagous fungi efficacy involves measuring percentage of larva reduction, which relies on the recovery and quantification of L3 among fungi treated and untreated groups. The principle of this evaluation is that a significant reduction of nematode larva can be achieved by the application of nematophagous fungi either *in vitro* culture or after passing it through GIT of animals. Several *in vitro* research works show that the efficacy of some nematophagous fungi could be as low as 50%, while efficacy for others' could be as high as 100% (Table 4) suggesting some of them are as high effective as AH drugs. Nonetheless, it appears that the type of strain, dose of conidia, temperature and humidity affect the evaluation of the efficacy (da Silveira *et al.,* 2017).

Table 4: Selected literatures on the in vitro efficacy of different nematophagous fungi against infective stage of GINs

Fungal species (strain)	**Type of L3**	**Efficacy (%)**	**References**
Arthrobotrys conoides	*H. contortus* and *T. colubriformis* (mixed)	80-97.4	Xue *et al.* (2018a)
Arthrobotrys robusta	Mixed	98.29-99.81	Cai *et al*. (2017), Falbo *et al*. 2013
Arthrobotrys musiformis	Mixed	60.72-99.89	
Arthrobotrys oligospora	Mixed	50.12-99.99	Cai *et al*. (2017b)
Arthrobotrys musiformis	*H. contortus*	100	Ojeda-Robertos *et al.* (2019)
Arthrobotrys oligospora	*H. contortus*	55.5-65.9%	
Arthrobotrys oligospora	*H. contortus*	90.3%	Alfaro Gutiérrez *et al.* (2011)
Monacrosporium thaumasium	*H. contortus*	62.3%	

3. MATERIALS AND METHODS

3.1. Experimental Site and Period

This study was conducted in the period between October 2019 to April 2020 at the premises of College of Veterinary Medicine and Agriculture (CVMA), Addis Ababa University (AAU), Bishoftu, Ethiopia. The altitude of the site is about 1880 meter above sea level. The average, temperature and rainfall respectively of the site during study period were 20.6°C and 77.4mm (World Weather Online, 2020). The experiment was conducted in fly-proof animal holding facility, which has concrete floor and equipped with feeding and watering troughs. The house comprised animal holding pens with good air movement.

3.2.Experimental Animals and Sample Size

Local Arsi-Bale sheep and goats were purchased from Asella open market (East Arsi Zone of Oromia Regional State, Ethiopia) in June 2019 and managed indoors for 6 months before the start of the experiment. These breeds are extensively studied (Tibbo *et al*., 2004a; Tibbo *et al*., 2004b; Tadie *et al*., 2018) and are widely distributed breeds in various regions of Ethiopia (ESGPIP, 2008). Their mean age at the time of purchase ranged between 12 and 18 months. The sample size required for this study was obtained from the hypothesis that the difference in average body weight gain between infected and uninfected lambs was 2.87kg (from previous study) (Idika *et al.,* 2012). For this calculation, 2-sided directions, P-value at 5% and a study power of 80% were considered by assuming the ratio of animal number in the groups was equal. R-program (R Core Team, 2018) was used to compute sample size calculation using the function **n.for.2means()** in **epiDisplay** Package. According to the output, six animals per group were required (six infected and six uninfected sheep). Similarly, sample size for goats was calculated based on the hypothesis that difference in average weight gain between infected and uninfected goats was 3.5kg (Beriajaya and Copeman, 2006). According to the software output, 6 goats per group were supposed to participate in the study. However, given attrition rate, the sample size was increased to seven in both sheep and goats per treatment group (14 sheep and 14 goats, in a total 28 small ruminants were included).

3.3.Management of Experimental Animals

At the experimental site, two subsequent acclimatization periods were provided. Upon arrival, the experimental animals were treated with Tetramisole (H-Tetra 600mg, Hebei, China) and Triclabendazole (Fasionox®, East African Pharmaceuticals, Ethiopia) orally at the dose rate of 15 and 7.5 mg/kg body weight respectively. Diazinon (Vetazinon 60%, Adamitulu Pesticide Processing Plant, Ziway, Ethiopia) was sprayed on the body of animals. Then, they were managed in semi-intensive system for two weeks through partly grazing together on pasture and feeding on wheat bran and grass hay in a confined animal facility. During the second acclimatization periods (five and a half months), all animals received second treatments and sprays as above and kept indoors until the start of the experiment. This is to make sure that, the animals are completely cleared of any helminth infections and provide ample time for healing processes against any previous infection. During this period, animals were tested for fecal egg/larva by standard parasitological methods (Annex 1, 2 & 3) and changes in PCV to ensure normality. There were no nematode or fluke egg in any fecal samples nor signs of ectoparasite infestations. However, some animals have shown coccidia oocysts in their feces. These animals were treated with Amprolium (Joprox®, Amman, Jordan) in drinking water and no fecal oocysts was detected at the start of the experiment. Both before and during the experimental infection, the feed stuffs provided for animals included concentrate ration (wheat bran) and grass hay. The experimental pens were cleaned twice every day to minimize any possible cross contamination. They were also allowed to exercise outdoors on a fenced concrete floor (Annex 10).

3.4. Larvae Preparation

Intestines of sheep and goats were generously provided by Hashim export abattoir (Bishoftu, Ethiopia) for recovering adult female *T. colubriformis* parasites. The slaughtered animals were reported to have been originated from different areas of the Southern lowlands of Ethiopia. Adult female *T. colubriformis* worms were recovered based on its morphological features (Taylor *et al.,* 2016) and according to the method briefed by Wagland *et al*. (1996). Briefly, soon after evisceration, the two ends (abomaso-duodenal junction and at 20cm of duodenum)

were ligated and separated from the rest of the compartments as this site is preferential area for the parasite. The samples were placed in plastic container in an icebox, and transported to Veterinary Parasitology Laboratory, CVMA-AAU for worm identification and recovery (Annex 6). Eggs of *T. colubriformis* were harvested from female worms' uterus following the same procedures depicted by Getachew *et al*. (2015) for the recovery of eggs of *H. contortus*. Briefly, adult female worms were, picked up from petri dishes using fine hooked needle, whilst being observed under stereomicroscope and light microscope. Then, the worms were rinsed in normal saline, pooled and gently crushed in small size pistil and mortar to release eggs from uteri. The collected materials were pooled and used for culturing within few minutes of recovery. Fecal material for egg culturing was obtained from cattle that were tested negative for any worm egg and no history of anthelmintic treatment within last two months prior to fecal collection. The fecal materials collected directly from the rectum were further heat treated at 70^{o}C for 2 hours in water bath to kill any undetected helminth eggs (Nwosu *et al.,* 2006). The prepared egg suspensions were then mixed with the fecal material and the content moisturized. The material was incubated at room temperature for 12 days, and this was then followed by harvesting L3 using modified Baermann technique (Annex 4 & 13). After recovery, the volume of suspension was adjusted and then larvae in one ml of suspension were quantified to determine the number of larva in the final volume of suspension. Then the suspension was stored at room temperature for one to two week followed by infection of one donor sheep for parasite amplification in order to acquire adequate number of larvae to infect 14 animals. After the donor animals were detected positive for parasite eggs, as much volume of fecal material as possible has been collected over one week to produce larvae by culturing for 10-12 days as described above. The larvae were stored at room temperature for one to two week before they are given to experimental sheep and goats.

3.5. Experimental Animal Grouping and Infection

Experimental animals were blocked based on species, and randomly assigned to one of the two treatment groups (infected vs uninfected) after being adjusted for their body weight in each block. Accordingly, twenty-eight small ruminants were categorized into four treatment groups of seven animals each (IS, IG, US and UG). Groups IS and IG comprised of sheep and goats

that received single dose of 10,000 L3/animal whereas US and UG contained uninfected control sheep and goats respectively The groups were managed separately in different pens, but they were provided with the same feed and water. The two infected groups received L3 larvae through oral route on the same day (D-0).

3.6. Ante Mortem Data Collection

3.6.1. Fecal egg count (FEC)

Fecal samples were taken directly from the rectum bi-weekly starting from day zero (D-0) and extending for eight weeks using plastic gloves and processed for egg counting according to the method described previously. McMaster egg counting technique with a sensitivity of 50 eggs per gram (EPG) of feces (Anne and Gary, 2012) was employed (Annex 5).

3.6.2. Body weight gain

The body weight of the animals in each group was measured on D-0 of experimental infection using hanging spring balance (Annex 12) and then continued every week until end of the experiment.

3.6.3. Packed cell volume (PCV)

PCV values of animals in each group were measured on day zero and every week up to the end of experiment by hematocrit method as per the procedures of Weiss and Wardrop (2010). Briefly, blood samples were taken from the jugular vein using vacutainer tubes (Hunan Liuyang Medical Instrument Factory, Hunan China) coated with ethylene diamine tetra-acetic acid (EDTA). Soon after collection, blood was filled three/fourth volume of the microhaematocrit tubes coated with EDTA. One end of the tube was sealed with capillary tube sealing wax (Versieg Hirschmann®, Barcelona, Spain) and then, the tubes were centrifuged using micro hematocrit centrifuge (Haematospin® 1300, Hawksley, England) at 10,000rpm for 5 minutes, and PCV was read using microhaematocrit reader (Hawksley, England).

3.7. Postmortem Parasitological Parameters

3.7.1. Postmortem and worm recovery

At the end of experiment, animals in each group were humanly killed using intravenous injection of barbiturate followed by slaughtering for post mortem parasitological examinations. The small intestine was ligated at one end and all the contents removed and the lumen rinsed by flushing with tape water. All washings were sieved through 150µm pore sieve to recover the coarse material left on the sieve for worm analysis (Annex 7, 8 & 9). Worm burden, Sex ratio, in utero egg count of worm and Worm establishment rate (Moreno-Gonzalo *et al.,* 2014) were the parameters evaluated between infected groups (IS and IG) according to the methods explained by Chiejina and Sewell (1974) and Fantu *et al*. (2012).

3.7.2. Adult worm count and measurement

The coarse material (debris along with worms) retained on the seive were transferred to plastic containaier and preserved in 70% ethanol. The volume of contents and ethanol was standardized and made up 1L final volume in all samples. After mixing throughly the contents, 10% of the total volume of preseved samples were obtained in to flat bottom flasks. In order to count worms, 4ml of mixture was transferred at a time to glass Petri dishes that had grid marks to enhance counting. The worms in 10% intestinal wash were counted as male and female using stereomicroscope. To get the worm burden, 10 multiplied the total number counted in 10% aliquot. Furthermore, female to male ratio was obtained from this count.

3.7.3. In utero egg count

For these procedures, 30 matured female worms were randomly picked up from preserved samples. The worms were, then immersed in lacto phenol solution for 5 minutes for clearing their cuticle. Then, individual worms was mounted on glass microscopic slide with small drop of saline. Eggs in their uteri were counted using 10X objective.

3.8. *In vitro* efficacy of Nematophagous Fungus

3.8.1. Fungal species used for biological control trial

The nematophagus fungus, *Arthrobotrys oligospora* already isolated from soil and generously provided by Dr Maradona Birhanu (MSc fellow in the Department of Microbiology, Immunology and Vet. Public Health of the College of Veterinary Medicine and Agriculture, Addis Ababa University) was used in this study. The fungal species was isolated on Potato Dextrose Agar (PDA) supplemented with 0.05% chloramphenicol and identified based on the morphology of trapping devices, conidiophores and conidia as per the method described by da Silva *et al.* (2013). Fungal specimens were kept at 4°C, protected from light until further use.

3.8.2. In vitro nematocidal efficacy of Arthrobotrys oligospora

In vitro efficacy was done according to the procedures given by Falbo *et al*. (2013) with few modification. For this procedure, 12 plates that contained fresh PDA supplemented with 0.05% chloramphenicol were prepared in 5 cm glass Petri dishes and stored in refigerator at 4℃ until used. In six of the plates, 2 months old pure *A. oligospora* was transferred along with 2x2mm size agar block. The rest of the plates were used as control, in which no fungus was inoculated. Then, the margins of the plates were sealed with plastic tape to prevent cross contamination or contamination by other environmental fungi. They were incubated at room temperature for 10 days in the dark. After 10 days of incubation period, an equal volume suspension (100µL) containing 400 washed and cleaned L3 of *T. colubriformis* was added on the surface of the 12 plates (six fungus treated and six control plates). The plates were incubated at room temperature for additional 10 days. After these days, the predatory activity of the fungus was observed under a compound microscope using magnifications of 10 and 40X by removing trapped L3 from the plate and transferring it to clean microscopic slide or by observing the trapped L3 in the plate under stereo-microscope. This was followed by recovering L3 from the plates by standard Baermann technique and counting their number using stereomicroscope. Before counting, the volume of recovered fluid was adjusted to 1ml, and then the number of

larva in 1ml was counted. The % reduction in larval count was determined as per the formula of Xue *et al*. (2018b) as indicated below.

$$\%\text{efficacy} = 1 - \left\{\frac{mean\ larva\ in\ fungus\ treated}{mean\ larva\ in\ control}\right\} * 100$$

3.9.Ethical Considerations

All the protocols were conducted as per the guidelines and principles of using animals for experimental purposes. Approval has been obtained from Animal Research Ethics Committee of, the College of Veterinary Medicine and Agriculture of the Addis Ababa University (Ref No VM/ERC/17/01/12/2020).

3.10. Data Analysis

Information generated from the study were recorded in hard copy format before being prepared in electronic Microsoft excel sheet. One animal in uninfected sheep group was severely suffering from illness, hence removed from the study at 14 days follow up period. As a result, statistical analysis included 27 experimental animals. The FEC, PCV and body weight gain between the groups were analyzed using repeated measure ANOVA in linear mixed regression model. Tukey's Post Hoc test was used for pair wise comparisons between the groups. The model included the main effects (post infection time and treatment group), their interaction term and random error (individual animal identity; due to repeated measurement of the same animal). The formula of the model used was as follows:

$$Y_{ijk} = \mu + G_i + T_j + G_iT_j + \varepsilon_{ijk} \quad \text{.....1)}$$

Where Y_{ijk} =measurement of outcome variables, such as FEC, PCV and body weight of the $_K^{th}$ individual within $_i^{th}$ treatment group at $_j^{th}$ time. μ is overall mean, G_i is the effect of treatment group $_i$, T_j is the effect of time $_j$, G_iT_j is the effect of interaction of group $_i$ and time $_j$, ε_{ijk} is random error.

In addition, post mortem parasitological parameters, including worm burden, female to male sex ratio, worm establishment rate and in utero egg count were presented in descriptive statistics. The comparisons of these parameters between infected sheep and goats (IG & IS) were analyzed using one-way ANOVA from the model 2 indicated below, which did not include main fixed effect "post infection time".

$$Y_{ij} = \mu + G_i + \varepsilon_j \quad \text{.....2)}$$

Where Y_{ij} =measurement of post mortem outcome variables (worm burden, worm establishment rate, female to male sex ratio, fecundity, worm length and number of immature worm) of the j^{th} individual within i^{th} treatment group. μ is overall mean, G_i is the effect of i^{th} treatment group and εj is random individual error. *In vitro* reduction rate of *A. oligospora* between treated and control plates were analyzed using independent t-test.

Before analysis, normal distribution of the residual errors for every measurement was assessed by graphically (Histogram and Q-Q plot) and official normality tests (Shapiro-Wilk Test) (Ghasemi and Zahediasl, 2012). In all cases, non-normal data were transformed according to the recommendations given by McDonald (2014) however, the results were reported in back transformed mean values. Results of normal data were expressed as arithmetic mean (±standard error). For all analysis, 5% marginal error, and 95% confidence level were considered. The R program (R Core Team, 2018) was used to analyze the data.

4. RESULTS

4.1.Ante Mortem Data

4.1.1. Fecal egg count (FEC)

All non-infected sheep and goats remained negative for fecal eggs until the end of the experiment. Preliminary analysis showed that the residual error of FEC was not normally distributed, which did not allow using parametric test. Hence, data were transformed to log10 (FEC+100) scale in order to stabilize variance for parametric analysis. However, the results were presented in back transformed mean epg values for ease interpretation. Accordingly, a two-way repeated measure ANOVA indicates statistically significant ($P<0.05$) effect of treatment groups, post infection time and their interaction (group*time) on log10 (FEC+100). Back transformed FEC remained at zero value (0 epg) in uninfected control groups throughout experimental period. The mean back transformed FEC was significantly higher ($P<0.05$) in infected goat (110 epg) than in infected sheep (0 epg) at 18 dpi. After 18 dpi, the average count steadily climbed in both infected sheep and infected goat without significant variation ($P>0.05$) until it hit the peak by 38 dpi. Mean egg count significantly ($P<0.05$) declined thereafter, to reach its lowest value below 750 epg in infected sheep by end of study, while the count in infected goat remained roughly constant for further additional week before it dropped to 1433 epg by end of study. In fact, mean egg counts of the last two weeks in infected goat remained significantly higher ($P<0.05$) than the egg counts in infected sheep (Figure 4).

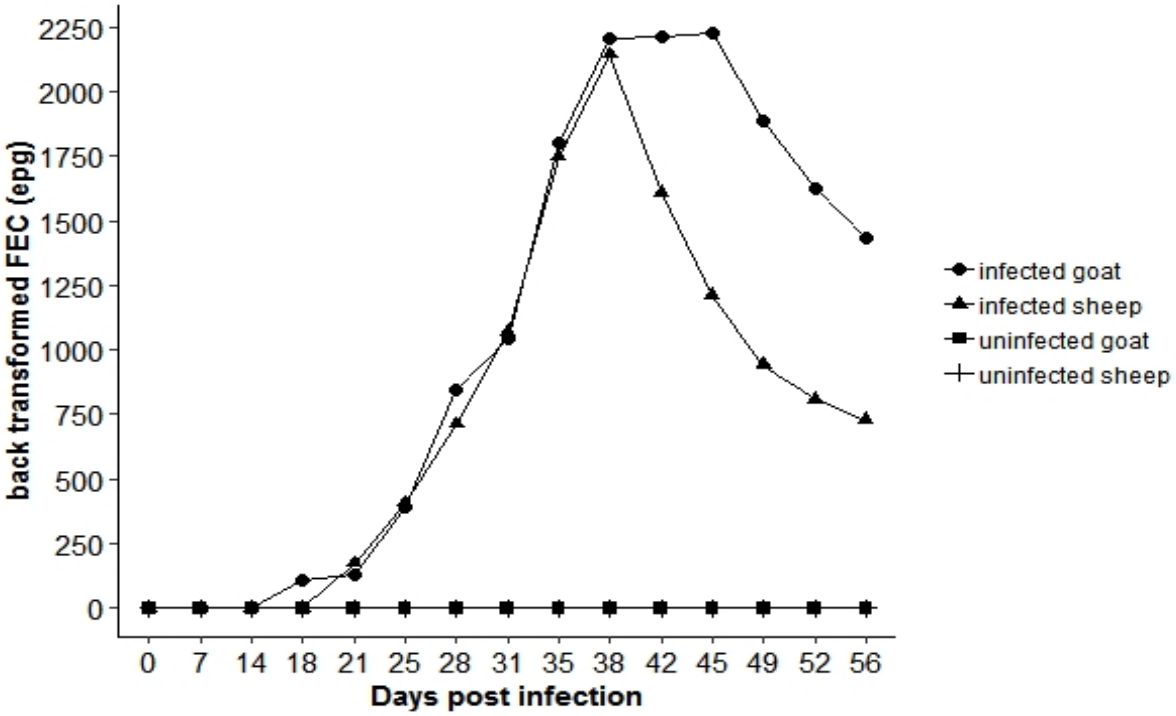

Figure 4: The mean back transformed log10 (FEC+100) fecal egg count of single experimental infection with 10000 L3 of T. colubriformis in sheep and goats over the period of 8 weeks

4.1.2. Body weight gain

Repeated measure factorial ANOVA reveals significant effect of *T. colubriformis* infection on live body weight ($P<0.05$). Initially, mean body weights of all treatment groups were not significantly different ($P>0.05$) with 27.9±1.43 Kg and 27.8±1.55 Kg for infected and uninfected groups of sheep respectively and 26.1±1.43 Kg and 25.5±1.43 Kg for infected and uninfected groups of goats respectively. Tukey's Post Hock contrasts show the mean live weight of both uninfected goat and uninfected sheep consistently increased throughout the study period. Mean body weight reached 31.9±1.77 Kg in uninfected sheep by end of study showing a statistically significant gain (+4.1 Kg) compared to the initial weight ($P<0.05$). A similar situation was noticed for uninfected goats with mean weight gain of about 3kg. A progressive body weight gain has also been observed in infected group of sheep whereby final body weight at the end of the study was higher than the measurement on day zero (+1.8Kg) whereas in infected goats, it gradually declined below the initial value from day 28 to day 49 post infection and almost returned to the baseline level at the end of the study. Overall, infected

sheep achieved significant weight gain by end of study, while infected goat did not experience weight gain at all (Figure 5).

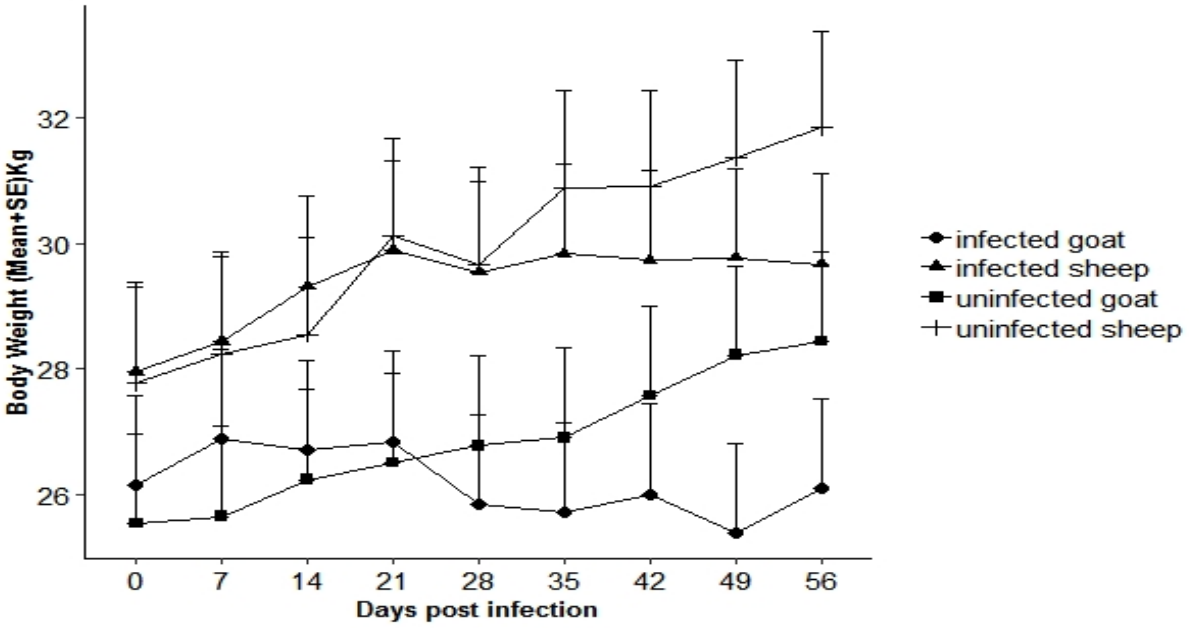

Figure 5*:* Comparisons of mean body weight of small ruminants infected with single dose of 10000 L3 of *T. colubriformis* and uninfected control small ruminants over 8 weeks period. Error bars represent the standard error of the mean

4.1.3. Packed Cell Volume (PCV)

Mean PCV of all treatment groups was not significantly different (P>0.05) at day zero; the initial values being 27.43, 30.29, 28. 85 and 30.5% respectively for infected goat, control goat, infected sheep and control sheep. There was no significant change in mean PCV in both control groups throughout the experimental period. On the other hand, from day 7 onwards a gradual reduction in PCV was observed in both infected sheep and goat groups with significant difference between the initial values above and final 21.86 and 25.29% (P=0.0060 and P=0.0003) in infected goat and sheep respectively 56 dpi (Figure 6). At the end of the experiment, mean PCV value for infected goat was significantly lower than that of infect sheep (P=0.014).

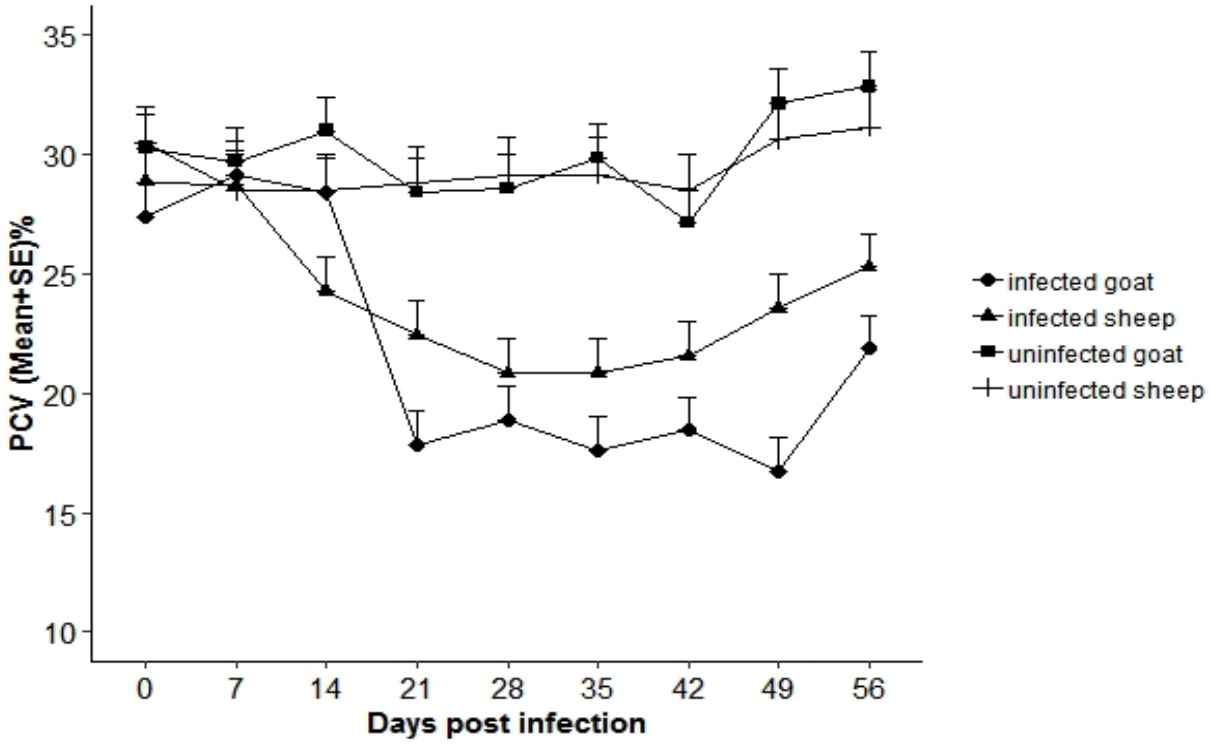

Figure 6: Mean PCV of small ruminants infected with single dose of 10000 L3 of *T. colubriformis* and uninfected controls over 8 weeks period. Error bars represent the standard error of the mean

4.2. Post Mortem Parasitological Parameters

Post mortem parasitological analysis showed that both worm burden and establishment rate of infective larva were significantly higher in infected goat than in infected sheep ($P<0.05$). However, female to male worm sex ratio and in utero egg of female worm were not significantly varied ($P>0.05$) between the groups (Table 5).

Table 5: Mean ± SE (95% CI) comparisons of post mortem parasitological indicators among sheep and goat experimentally infected with a single dose of 10000 L3 of *T. colubriformis*

Parameters	**Infected Goat**	**Infected Sheep**	**P-value**
Worm burden	5016±48 (3962-6069)[a]	3447±483 (2392-4499)[b]	0.0406
Establishment rate (%)	50.2±4.8 (39.6-60.7)[a]	34.5±4.83 (23.9-45.0)[b]	0.0406
Sex ratio (F/M)	1.03±0.08 (0.839-1.23)[a]	1.00±0.08 (0.806-1.20)[a]	0.7964
Eggs per uterus	13.5±0.35 (12.8-14.3)[a]	14±0.35 (13.2-14.7)[a]	0.4272

Different superscript letters across rows are significantly different (P<0.05):
F/M=Female/Male, CI=confidence interval

4.3.*In vitro* Nematocidal Efficacy

The trapping devices of predatory nematophagous fungus **(***A. oligospora*) were seen around the body of *T. colubriformis* L3 starting from the third day of incubation period. Examination of either stained specimen or plates using compound and stereomicroscope clearly showed that several L3 were captured by massively grown fungal net on the seventh day following incubation with the fungus (Figure 7, A-D). *In vitro* efficacy evaluation revealed significant difference in the number of live larvae (P<0.05) between fungal treated (43.10±5.54, 11%) and non-treated control plates (279.30±14.11, 70%) (Figure 8).

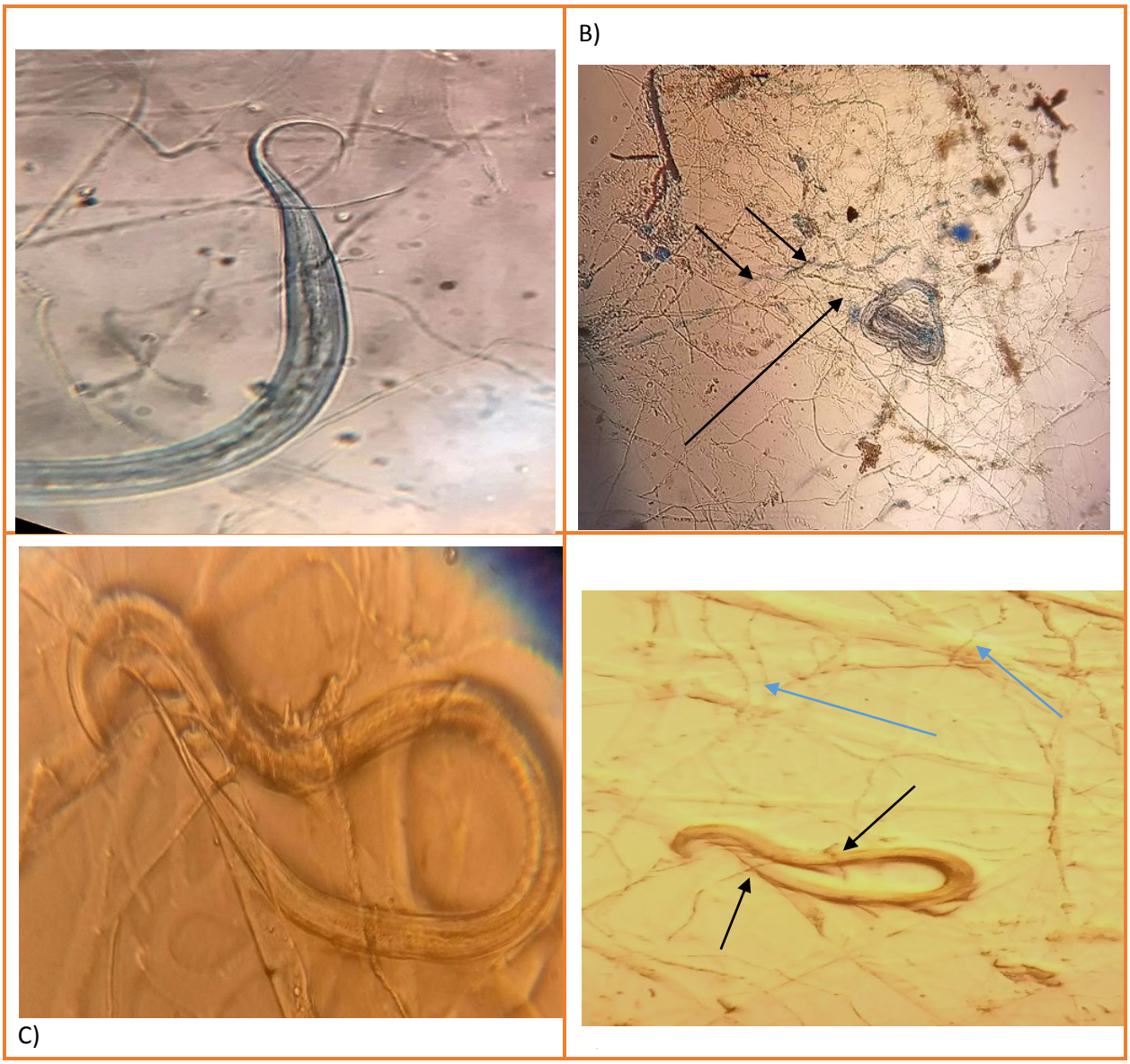

Figure 7: Nematophagous fungus (*A. oligospora*) trapping L3 of *T. colubriformis*

A) Few trapping device adhered at the tail area of the larva on the third day of incubation (arrow). B) Massive growth of trapping net that killed the larva on the 10th day of incubation ,C & D indicate trapped larva by trapping net and various fungal hyphae grown (blue arrow) on 10th day of incubation (Lacto-phenol cotton blue stained specimen at 10X magnification for A and B, direct microscopy for C&D). Photo by Tamirat Kaba.

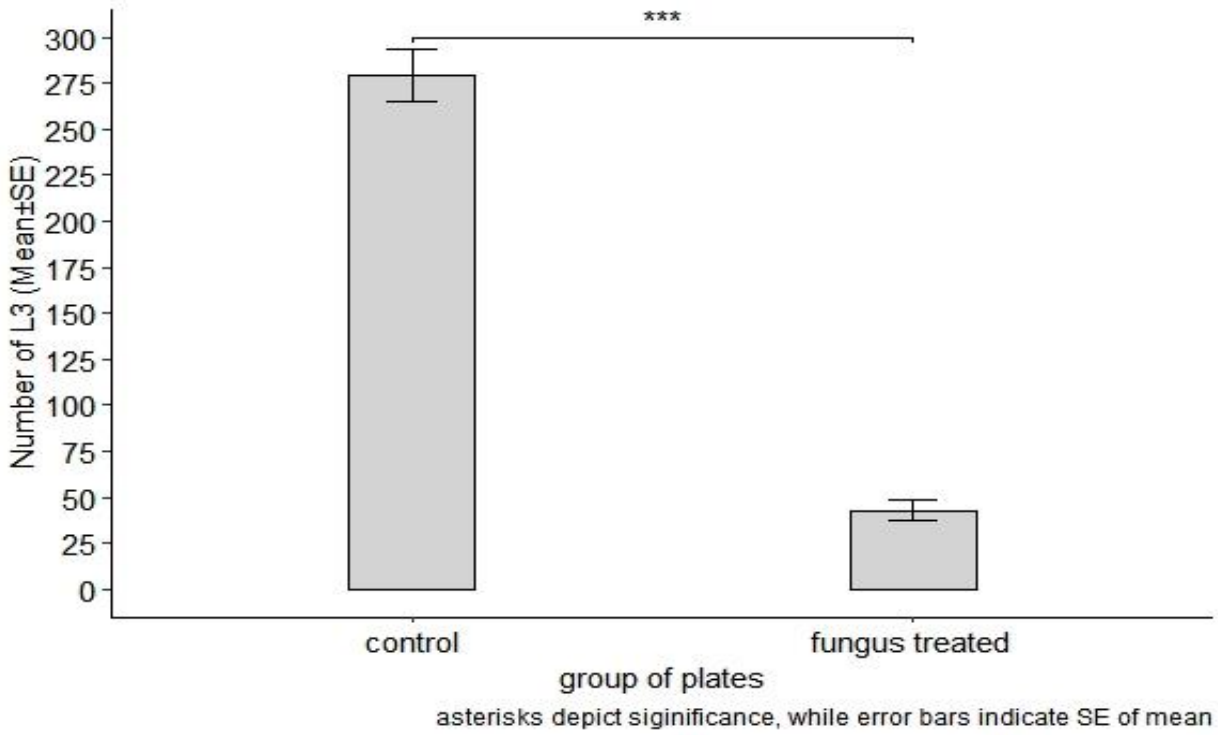

Figure 8: Average number of non-preyed L3 among fungus treated and control plates

5. DISCUSSION

This study aimed at demonstrating the difference in the relative performance of sheep and goats in terms of body weight and PCV and parasitological profiles following infection with *T. colubriformis*. It also intended to assess the *in vitro* efficacy of nematophagus fungal species as a biological control agent on the third stage larvae of the parasite.

5.1. Comparison of Body Weight and PCV between Sheep and Goats

The present study demonstrated the effect of experimental infection of *T. colubriformis* on body weight gain in both infected sheep and infected goats. These groups experienced significantly lower weight gain by end of study when compared to their uninfected counterparts and their initial measurement values. This is in agreement with the findings of Beriajaya and Copeman (2006). The reduction of body weight gain in both infected sheep and goats, which commenced at 21 dpi coincides with the timing by which significant number of adult worms emerge in to the lumen of the intestine and their associated pathogenic effect, which might have caused systemic changes such as reduction in appetite and disturbance in absorption of nutrients (Beriajaya and Copeman, 2006; Roeber *et al.,* 2013). However, the relatively stable weight gain noticed in infected sheep thereafter while continued reduction over 49 dpi without any gain by end of study in infected goat implies better performance of sheep under parasite challenge, possibly because of better immune response relative to goats or inherent species difference. The response of higher mucosal mast cell concentration, which resulted in reduction of significant number of adult *Teladorsagia circumcincta* in sheep than in goats (Macaldowie *et al*., 2003) holds the above explanation why sheep maintained weight gain in comparison with goats. This difference between sheep and goats could also be explained by the fact that goats which are selective feeders in nature were forced to live on grass hay instead of brows leaves which could be a confounding effect. Contrary to our findings, Beriajaya and Copeman (2006) indicated that reductions in weight gain were detected in both sheep and goats but the loss was higher in sheep than in goats. This difference could be attributed to differences in experimental protocols including animal breed, age and infective dose.

In this study, both infected sheep and infected goats presented significant reduction in PCV value when compared with their respective control group and with their initial values. This reduction started as early as 21 dpi in both infected groups, which coincides with emergence and development of worms in the intestinal lumen. Our findings support the results reported in previous studies (Cardia *et al*. 2011). Although pathogenesis of PCV reduction by non-hematophagous GINs, including *T. colubriformis* is unclear, hemorrhage associated with mucosal penetration by L3 in initial phase of infection and breaking of mucosa for the second time by immature worms are the speculated factors (Roeber *et al*., 2013). When the two species are compared, PCV values are much lower in infected goats than in infected sheep suggesting a kind of concordance with the body weight profile observed and tolerance of sheep compared to goats. Beriajaya and Copeman (2006) have also reported absence of reduction in PCV of sheep while significant reduction was seen in goats infected with *T. colubriformis*. Apart from host factors, such differences in body weight and mean PCV could be attributed to differences in the number of worms established.

5.2. Comparison of Parasitological Parameters between Sheep and Goats

The egg output within 3 weeks after infection in infected groups and absence of eggs in the feces of uninfected groups throughout study period, suggests the infective capacity of *T. colubriformis* and effectiveness of measures taken during study period. Comparisons of the results of this and previous similar studies concerning parasitological measurements between sheep and goats was not easy task, as variabilities often takes place in terms of feeding, infection modes (single versus multiple), previous exposure, age, sex, genetic factor of host and many more possibilities. The presence of eggs as early as 18 dpi in infected goat and absence of eggs on this day in infected sheep was interesting. The report of Moreno-Gonzalo *et al*. (2014) regarding shedding of *T. colubriformis* eggs earlier (17 dpi) than routine prepatent period (21 days) by infected goat supports the findings of this study, suggesting shorter pre patent period of *T. colubriformis* in goat when compared with sheep. The time by which first egg output appeared in infected sheep (21 dpi) concords with the reports of Chiejina and Sewell (1974) and Cardia *et al*. (2011). Besides, the work by Idika *et al.* (2012) that presents *T. colubriformis* prepatent period ranging from 21 to 22 days in West African Dwarf sheep agrees with this

study. The fact that signficant drop of FEC as early as 38 dpi in infected sheep, delayed response of this phenomenea; coupled with shorter pre patent period and significantly higher FEC by end of study in infected goat indicate higher susceptibility of goats in comparison with sheep, presumably because of variations in inherent gene or immune response. Although the present study did not investigate immunological parameters between the groups; available evidences indicates that immune responses to nematode infection by goats are inconsistence and sometimes could not be explained by parasitological parameters (Basripuzi *et al*., 2018). On the other hand, few workers have better explained immunological adaptation of sheep to the impacts of *T. colubriformis* (Pernthaner *et al.,* 2006; Cardia *et al*., 2011).

Better immunological responses or inherent genetic resistance by infected sheep than by infected goat in this study also evidenced by a significant lower worm burden and worm establishment rate in infected sheep than in infected goats. Contrary to this finding, Beriajaya and Copeman (2006) reported higher burden and establishment rate in sheep than in goats, This difference might have resulted from variations in animal feeding and management, infection modes (single versus multiple) or breed of experimental animals or probably strain of the parasites (Gonzalez-Garduno *et al.*, 2016). In this study, sheep derived isolate was used to infect all experimental groups that might have affected the results of parasitological parameters between sheep and goats. We were uncertain for the questions "what would happen if we had used goat strain for all experimental groups? Further research is required concerning these scenarios, as it is important in management of GINs in farming communities where sheep and goats are reared together. Worm establishment rate observed in the present study for infected sheep is in agreement with findings of Roy *et al*. (2004) and Almeida *et al*. (2010). However, it was much higher than that recorded by Cardia *et al*. (2011). For infected goat, establishment rate of present study was lower than that of Moreno-Gonzalo *et al*. (2014). This difference could be ascribed to factors already listed elsewhere in this discussion.

In this study, the mean utero eggs/female worm in infected sheep was higher than the finding of Kemper *et al*. (2010). In addition, in infected goat, it was lower than that reported by Moreno-Gonzalo *et al*. (2014). The reason for this variation could be the difference in the regulation of specific worms' biology by various host inherent resistant genotype or different mechanisms of

host immunity. Kemper and others (Kemper *et al*., 2010) who explained the ability of sheep to suppress female fecundity rather than to reject adult *Teladorsagia circumcincta* and vice versa in *T. colubriformis* support this explanation. Absence of significant variation (P>0.05) of utero eggs/female worm between infected sheep and infected goats in this study not only proves failure of both species to suppress fecundity of *T. colubriformis,* but also point the characteristics of sheep immunity to reject adult worms rather than suppression activity as explained by Kemper *et al*. (2010).

5.3. *In Vitro* Nematocidal Efficacy

In vitro predatory characteristics of various strains of nematophagous fungi against L3 of many GINs have been reported in several countries (Ghahfarokhi *et al*., 2004; Falbo *et al.*, 2013; Cai *et al.,* 2017a). In addition, *in vivo* trails (Graminha *et al.,* 2005; da Silveira *et al.,* 2017; Cai *et al.,* 2017b) show that several nematophagous fungi significantly reduce number of L3 in pasture. In this study, *A. oligospora* isolated from soil samples was used to assess its predatory capacity against L3 of *T. colubriformis*. In fact*, in vitro* trial of current study is the first report in its kind in the country, which testify efficacy above average against *T. colubriformis* L3. The efficacy of *A. oligospora* in this study is comparable with the efficacy of eleven isolates of *A. oligospora* (80-89%) reported in China (Cai *et al.,* 2017b). By contrast, 55-65.9% (Ojeda-Robertos *et al.*, 2019) and 90.3% (Alfaro Gutiérrez *et al.*, 2011) efficacy rates were reported in Mexico. These differences in efficacy rate of *A. oligospora* in present and previous studies could have been due to differences in source of samples for isolation of fungi. Several researchers have isolated *A. oligospora* from various natural habitats, including agricultural soils and animal fecal samples (Ghahfarokhi *et al*., 2004). This reasoning abide by the findings of Ojeda-Robertos *et al.* (2019) who highlighted variations in efficacy of *A. oligospora* isolated from soil and feces of water buffalos. This preliminary, small-scale *in vitro* trial only on *A. oligospora* considered in this study calls for further large-scale study on wide range of strains isolated from different natural habitats.

6. CONCLUSIONS AND RECOMENDATIONS

In conclusion, monospecific experimental infection of *T. colubriformis* in indigenous breed of small ruminants have resulted in worm establishment and, hence eggs output. However, shorter pre patent period, higher FEC in the last two weeks, much lower PCV, diminished weight gain and higher worm burden by end of the study were characteristic of infection in goats when compared to sheep. This suggests that the relatively low infection of goats under natural conditions may be due to their feeding behavior rather than their inherent resistance compared to sheep. Given the similarity in the in-utero egg count and the female/male ratio of the parasites, what has contributed to the high fecal egg count in goat is the worm establishment rate/ worm burden. The goats were less able to control worm burden compared to sheep thereby suffering from much reduced weight gain and PCV as compared to sheep infected with similar doses of the parasite. *In vitro* biological trial of present study shows that nematophagous fungus, *A. oligospora* isolated from soil samples has effectively reduced the survival of infective larva of *T. colubriformis*.

Therefore, based on the above conclusions the following recommendations are forwarded:

- Further research is needed by using parasite isolates derived from goats to strengthen the present finding
- Adequate attention has to be given to goats in the control of helminth parasitism as they can suffer much more than or at least equal to sheep given equal exposure
- Further study must be done to evaluate the nematophagus effect of *A. oligospora* after passage through the animals and on the contaminated pasture.

7. REFERNCES

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8. ANNEX

Annex 1: Fecal Floatation Technique

Procedures

1. Fecal pellets were directly collcetd from the rectum of animals
2. A 3g of sample broken and dissolved in 15ml of flotation fluid (saturated saodium chloride at the concentration of 400g NaCl in 1000ml distilled water).
3. After mixing throughly, the solution was strained using test seive (250µm pore size), and the filtrate was poured in to 15ml plastic test tubes until a reverse meniscus was formed and a coverslip was adapplied on top of it.
5. The tubes with coverslip on top were allowed to stand for 10 minutes.
6. Finaly the covreslip were removed and mounted on clean microscopic slide for egg identification under light microscope at 100X total mainification.

Annex 2: Fecal Sedimentation Test

Procedures

1. A 3g of fecal pellet were broken and mixed throughly in 50ml of water in plastic cup
2. The mixture was poured in to beaker after straining through 250µm seive. The filtrate were allowed to sit for one hour. After an hour, supernatants were decanted and more water was added and mixed to repeate sedimentation for the second time.
3. Once supernatant were decanted for the second time, the sediment was mixed up and 0.1% methylene blue was added.
4. A drop of mixture was transferred to clean slide to examine eggs under microscope at 100X maginification power.

Annex 3: Standard Baermann technique for lungworm identification

1. Individual animal's fecal sample were collected directly from the rectum in clean disposable plastic glooves. The feces were proceced right away after collection.
2. A 10g of fecal pellets was gathered in double layer gauze and tied up at the top using rubber band.

3. Two applicator sticks were passed through the rubber bands.
4. A funnel was used to suspend the sample by placing the applicator sticks on the edges of the funnel at two sides. The two sides of the sample and bottom part were not contacting the wall of funnel. A rubber tubes were inseretd in to the bottom part of the funnel, and the tubes were clamped firmly at position just below the insertion point.
5. Then, the funnel was filled with lukewarm water, and the samples was allowed to sit overnight at room temprature.
6. After 24h, the clamp was released and the first few drops of fluid was collected and allowed to sediment in test tubes. After 5 hours of sedimentation, the supernatant was discarded and sediment was transferred to clean microscopic slide and examined under microscope.

Annex 4: Coproculture and larva recovery with Modified Baermann method

About 40g fecal pellets were collected directly from the rectum of animals using sterile gloves. The feces were broken up finely using mortar and pestle to give a crumbly mixture. The fecal mixture of 20 g was then, packed in a glass jar container and moistened with distilled water. The top of jar was loosely covered with petri dishes and incubated at room temperature for 12 days. Distilled water was sprinkled on the fecal mixture in the jar when it appeared dry. On the final day of incubation, the jar was filled to the brim with lukewarm water. The jar was then inverted onto a petri dish. Afterwards, the petri dish was filled with lukewarm water and allowed to stand for 30 min for L3 migration from the fecal culture. Water containing L3 in the petri dish was pipetted into a universal plastic cup.

Annex 5: Modified McMaster Technique

The test was performed with 2 g of fresh feces and 28 ml of saturated sodim chloride solution. The fecal samples were mixed well with floatation fluid and strained through laboratory seive (250µm pore size). Aliquates were transferred and filled in to both chambers of McMaster slide uing pipete. After filling the cahmbers, the slide was allowed to sit for 5 minutes before counting. Eggs in both grids of chambers were counted, sumed up and multiplied by 50 to get the number of eggs in a gram of feces.

Annex 6: Female worm collection to harvest eggs

Intestinal samples from the abattoir were processed as soon as the samples arrived at parasitology laboratory. The contents of intestines were collected in to glass bucket after cutting the intestine in the longetudinal direction. The mucosa were also rinsed in the tap water to harvest any adult worm that attached to the mucosa. The contents and mucosa washs were mixed together in flask and then the mixture was passed through laboratory seive, with 250µm pore size. The materials retained on the seive were washed and collected in to flat bottom flask. Then, the solutions were agitated, shaked and poured in to 1000ml volume cylinder to stand for 20 minutes. After 20 minutes, the supernatants were discarded and the sediments were resuspended in tap water for agitation and shaking to clear the debris. This clearing process was repeated for three times before individual female *T. colubriformis* were collected from the mixture in to Petri dishes that contained normal saline (0.85% NaCl). The collection of worms inolved, picking up the worms using small needle that had hook on its tip, whilist observing under steromicroscope. For clarity, individual worms collecetd in the Peteri dishes were mounted on glass slide with small drop of saline with out cover clip and staining reagent in order to examine their generic feature under light miroscope with 10X objective (Annex 11). After examination, the worms were retained back in to the Peteri dishes conatining saline solution. All worms harvested in single collection period were pooled together and crushed in small pistil and mortar to liberate eggs from their uteri.

Annex 7: Worm burden investigation

As soon as evisceration completed, the two ends of the intestine (abomaso-duodenual and illeio-cecal junctions) were firmly ligated using ruber bands, and the intestine was separeted from the rest of compartments. In the lab, one end was oppened and the content milked out to the tray. Intestinal lumen was also flushed with tap water. The content and washing were mixed throughly, and strained through 150µm pore size seive in jet of water with high water flow speed. The solid material (debris along with worms) retained on the seive were transferred to plastic containaier that contained 70% ethanol for preservation. The volume of contents and ethanol was standardized and made up 1L final volume in all samples. After mixing throughly

the contents, 10% of the total volume of preseved samples were obtained in to flat bottom flasks. In order to count worms, 4ml of mixture was transferred at a time to glass Petri dishes that had grid marks to enhance counting. The worms in 10% intestinal wash were counted as male and female using stereomicroscope. To get the worm burden, 10 multiplied the total number counted in 10% aliquot. Furthermore, female to male ratio was obtained from this count.

Annex 8: In utero egg count

For these procedures, 30 matured female worms were randomly picked up from preserved in samples. The worms were, then immersed in lacto phenol solution for 5 minutes for clearing their cuticle. Then, individual worms was mounted on glass microscopic slide with small drop of saline. Eggs in their uteri were counted using 10X objective, and the number of eggs per female represented fecundity.

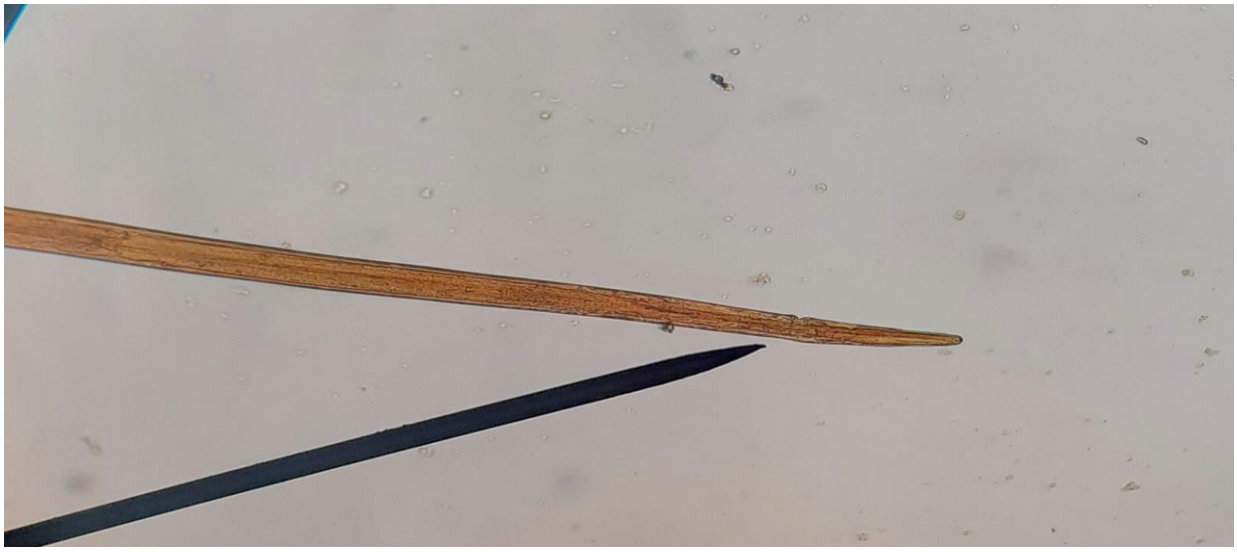
Annex 9: Worm establishment rate

This measurement was calculated from the results of the worm count and indicate total number of worms recovered divided by the total number of L3 given, multiplied by 100.

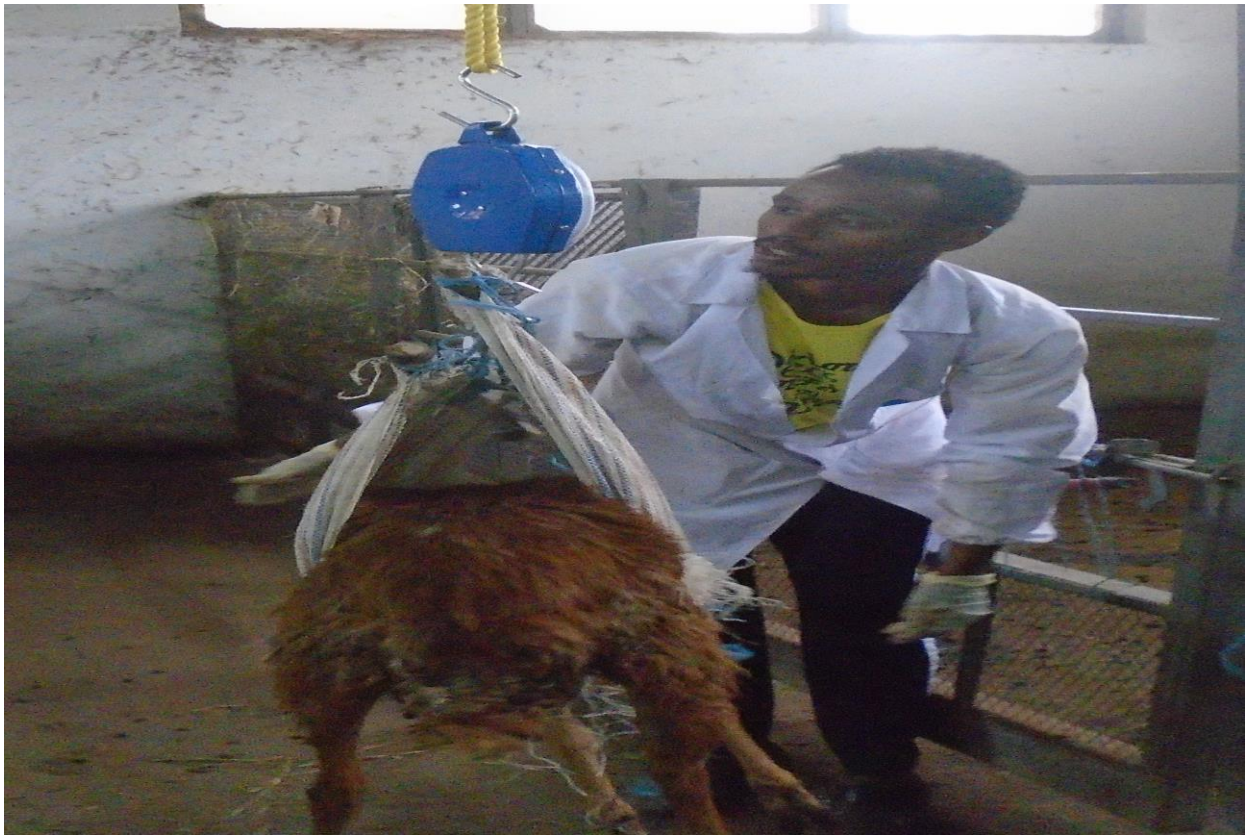
WER=Total worm count/Total L3 infected*100

Annex 10: A photo showing animals were exercising and getting sunlight at confined area

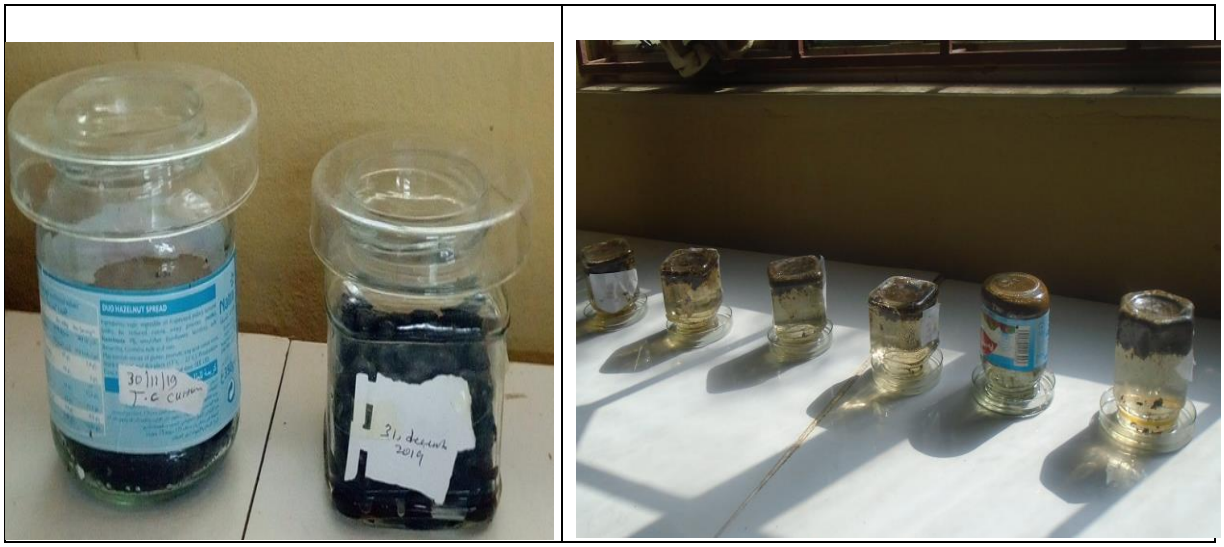
Annex 11: Image depicting excretory notch at the esophageal region (indentation visible just on the top of pointer)



Annex 12: Image showing weight measurement using hanging balance



Annex 13: Fecal culture at room temperature (left) and recovery of infective larva (right)



Annex 14: Data collection format sheet for fecal egg count

Animal ID	Treatment group	Day	FEC
O-18	Infected sheep	0	0

Annex 15: Data collection format sheet for PCV and body weight measurements

Animal ID	Treatment group	Day	PCV (%)/body weight (Kg)
C-12	Uninfected goat	14	30%/29Kg

Annex 16: Data collection format sheet for post mortem worm investigation

Animal ID	Treatment group	No. female worm	No. of male worm	No. of immature worm	burden	Female to male ratio	Establishment rate
O-18	Infected sheep	1900	2200	0	4100	1900/2200	4100/10000*100

Annex 17: Data collection format sheet for female worm fecundity

Animal ID	Treatment group	No. of worm	No. of eggs per uterus
O-9	Infected sheep	1	15
“	“	2	10
“	“	3	9
“	“	4	6
“	“	5	12
“	“	6	14
“	“	“	15
“	“	“	11
“	“	“	16
“	“	30	13