Thesis Ref. No. ----------

SPECIES DIVERSITY AND SEASONAL DYNAMICS OF HELMINTHS OF CATTLE IN ADA'A AND BOSET DISTRICTS, CENTRAL OROMIA, ETHIOPIA

MVSc Thesis

BY

SOLOMON MOSU ENYEW

ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

JUNE, 2017

BISHOFTU, ETHIOPIA

SPECIES DIVERSITY AND SEASONAL DYNAMICS OF HELMINTHS OF CATTLE IN ADA'A AND BOSET DISTRICTS, CENTRAL OROMIA, ETHIOPIA

A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirement for Degree of Masters of Veterinary Science in Veterinary Parasitology

By

Solomon Mosu Enyew

June, 2017

Bishoftu, Ethiopia

Addis Ababa University
College of Veterinary Medicine and Agriculture
Department of Veterinary Pathology and Parasitology

SPECIES DIVERSITY AND SEASONAL DYNAMICS OF HELMINTHS OF CATTLE IN ADA'A AND BOSET DISTRICTS, CENTRAL OROMIA, ETHIOPIA

Submitted by: Solomon Mosu _____ _____
Name of Student Signature Date

Approved for submittal to thesis assessment committee

1. Dr. Bersissa Kumsa (DVM, MSc, PhD, Assoc. Prof.) _____ _____
   Major Advisor Signature Date
2. Dr. Teshale Sori (DVM, MSc, Assoc. Prof.) _____ _____
   Co-advisor Signature Date
3. Dr. Dinka Ayana (DVM, MSc, Assoc. Prof.) _____ _____
   Co-advisor Signature Date
4. Prof. Frans Jongjan (DVM, MSc, PhD, Professor) _____ _____
   Co-advisor Signature Date
5. Professor Yacob Hailu (DVM, MSc, PhD, Professor) _____ _____
   Department Chairman Signature Date

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Parasitology and Pathology

As member of the Board of Examiners of the MVSc Open Defense Examination, we certify that we have read, evaluated the Thesis prepared by **Solomon Mosu** and examined the candidate. We recommend that it be accepted as fulfilling the Thesis requirement for the degree of Masters in **Veterinary Parasitology**.

Dr. Fikru Regassa	_____	_____
Chairman	Signature	Date
Dr. Takele Sori	_____	_____
External Examiner	Signature	Date
Dr. Hagos Ashenafi	_____	_____
Internal Examiner	Signature	Date
Prof. Yacob Hailu	_____	_____
Department Chairperson	Signature	Date

DEDICATION

This thesis manuscript is dedicated to my family and academic advisors who are on behalf of my success.

STATEMENT OF THE AUTHOR

First, I declare that this Thesis is my bonafide work and that all sources of materials used for this Thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for MVSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University 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.

Brief quotations from this dissertation are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major School or the Dean of the School of Graduate Studies when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Solomon Mosu Enyew

Signature: _____

Place: Addis Ababa University, College of Veterinary Medicine and Agriculture

Date of Submission: _____

ACKNOWLEDGMENTS

I am highly indebted to my advisor Dr. Bersissa Kumsa for his incalculable guidance and time devotion to correct this paper. I have a great respect and heartfelt gratitude for his intellectual technical, and moral support starting from the commencement of the study to the end. My deep gratefulness goes to Dr. Teshale Sori and Dr. Dinka Ayana for their genuine comments and cooperativeness throughout the study. It is also my pleasure to acknowledge Professor Frans Jongejan for his unreserved technical advice, continuous supervision and professional guidance. This research was supported by Bill and Melinda Gates Foundation (BMGF) Parasite Mapping Project entitled "Survey of Ecto and Endoparasite Taxa Impacting on Small Scale Rural Livestock Farmers in Ethiopia," a collaborative investigation between College of Veterinary Medicine and Agriculture, Addis Ababa University and Clinglobal Limited, the Netherlands. I am thankful for the logistic and financial support.

I am grateful to thank W/ro Tigist Gizachew and Mr. Gebeyehu Alkadir for their assistance during laboratory work. Moreover, Boset and Ada'a district Administrative staff, Animal health technicians and livestock owners are highly acknowledged for their unreserved cooperation and willingness to collect samples for this MSc research during the field work.

Finally, I would like to express my sincere honor to my beloved family; their moral support was more than the spoken words.

TABLE OF CONTENTS

Contents **Pages**

TABLE OF CONTENTS (*Continued*)

LIST OF ABBREVIATIONS

AAU	Addis Ababa University
CSA	Central Statistical Authority
ELISA	Enzyme Linked Immuno-sorbent Assay
EPG	Eggs per gram of faeces
GDP	Gross Domestic Product
GIT	Gastrointestinal Tract
gm	Gram
GPS	Geographical Positioning System
ID	Identification Card
L_3	Third stage larval
MAFF	Ministry of Agriculture, Fishery and Food
masl	Meters Above Sea Level
ml	Milliliter
NaCl	Sodium Chloride
^oC	Degree Celsius
PAs	Peasant Associations
Spp	Species
STE	Sheath Tail Extension
WMO	Workshop on climate Monitoring

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Parasitic helminths are one of the major health constraints of cattle production and are usually associated with huge economic losses in Ethiopia. A cross-sectional study was conducted on 720 cattle selected using purposive sampling technique in Ada'a and Boset districts, Central Oromia Ethiopia from August 2016 to May 2017. The objective of the study was to identify the prevailing helminths species of cattle and assess the seasonal dynamics in the study districts. Simple floatation, sedimentation, McMaster egg counting techniques, coproculture recovery of infective larvae (L_3) of nematodes and larvae measurement using micrometer were all employed in this study. The overall prevalence of helminths in cattle was 57.4% (413/720) dominated by nematodes. The study identified *Haemonchus placei* (*H. placei)* (36.7%), *Trichostrongylus axei (T. axei)* (18.1%), *Oesophagostomum radiatum* (*Oe. radiatum)* (21.9%), *Bunostomum phlebotomum* (*B. phlebotomum)* (10%), *Strongyloides papillosus* (*S. papillosus*) (6.3%), *Nematodirus* spp. (0.7%), *Moneizia benedeni* (*M. benedeni)* (4.2%), *M. expansa* (1.1%), *Fasciola* spp. (5.7%), and *Paramphistomum* spp. (3.5%). Of these helminths *H. placei* was recorded as the predominant species affecting cattle of both study districts. Higher number of helminths species were recorded in highland PAs of Ada'a district than lowland Boset district. Significantly ($p < 0.05$) higher number of helminths was observed during the rainy season than the short rainy and dry seasons in both districts. Multivariable logistic regression analysis showed that cattle in Boset district were 1.77 times (OR = 1.77, 95%; CI = 1.28-2.46) more likely to become positive for helminths than those cattle found in highland PAs of Ada'a district. Likewise, during the short rainy season and during rainy season cattle were 1.8 times (OR = 1.80; 95% CI = 1.19-2.74) and twice (OR = 2.35, 95% CI=1.57-2.50), respectively, more likely to become positive for helminths than during the dry season of the year. The likelihood occurrence of helminths in diarrheic cattle was 8.7 times (OR = 8.7; 95% CI = 5.06-15.07) higher than those cattle with normal faecal consistency. Negative Binomial regression analysis showed that strongyle mean EPG count was significantly ($p < 0.05$) higher in cattle of Boset district than the mean EPG count of cattle in highland PAs of Ada'a district. Mean EPG count was higher during the rainy season (1001.0±51.1) than both short rainy

(705.5±43.2) and dry (631.7±35.4) seasons. Of the total 366 coprocultures, 264 (72.1%) were positive for *H. placei* followed by 158 (43.2 %) *Oe. radiatum*, 130 (35.5%) *T. axei* and 72 (19.7%) *B. phlebotomum*. In conclusion, results of the present study demonstrate that several genera and species of helminths are still widespread and very common with high prevalence that compromise the productivity and health of cattle, especially during the long and short rainy season in both Ada'a and Boset districts. Thus, it is very important to consider urgent control interventions to minimize the negative impact of helminths on health and economic benefits of cattle of the study districts.

Keywords: *Cattle, EPG, Ethiopia, Helminths, Highland, Lowland, L_3, Season*

1. INTRODUCTION

Ethiopian economy is predominantly based on agriculture which is considered as a primary factor in securing food self-sufficiency, generating employment and income for the poor (Philipp *et al*., 2015). The agricultural sector is a corner stone of the economic and social life of the people. Livestock is an integral part of the agriculture and the contribution of live animals and their products to the agricultural economy accounts for 40%, excluding the values of draught power, manure and transport of people and products (Asresie and Zemedu, 2015). It also plays a vital role by contributing 19% to the export earnings. In addition, crop production is almost exclusively dependent on livestock especially draft power of cattle. Cattle production, among the sector of livestock production systems, is a critical issue in Ethiopia (Ayele *et al*., 2003; Asresie and Zemedu, 2015).

Ethiopia has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zones that are suitable to host a very huge animal population (WMO, 2013). The country covers the largest livestock population in Africa with the estimated domestic animal number of 57.83 million cattle, 28 million sheep, 28.6 million goats, 1.23 million camels, 60.5 million poultry, 2.1 million horses, 0.4 million mules and 7.88 million donkeys (CSA, 2016). The livestock subsector has an enormous contribution to country's national economy and livelihoods of many people. Livestock in Ethiopia represent the pillar of the economy and plays vital roles in generating income to farmers, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values, and sustain livelihoods (Metaferia *et al.*, 2011).

In spite of a huge livestock resources and existing favorable conditions, full exploitation of cattle potential is mainly constrained and impeded at a great extent by widespread and high prevalence of endemic diseases mainly parasitic diseases (Johannes *et al*., 2016). Helminths, especially subclinical gastrointestinal nematode are known to be a major constraint to ruminant's well-being and productive performance (Holzhauer *et al*., 2011*;*

Charlier *et al*., 2014; Johannes *et al*., 2016) in many countries around the world (Holzhauer *et al*., 2011*;* Morgan *et al*., 2013; Lu and Judith, 2016).

Mortality of animals due to parasitic diseases may not be alarming at times but their indirect effects on livestock productivity and their zoonotic impact on human health are considerably greater. Indirect losses associated with helminths infection include the reduction in productivity potential such as decreased growth rate, weight loss, diarrhea, anorexia, and sometimes anemia (Ekong *et al*., 2012; Johannes *et al*., 2016). In young stock, gastrointestinal parasitism can reduce growth rate by 30%, even with a low level of worm challenge, and in adult cows, which are likely to be more immune to worms than calves, infections can cause a 1kg per day drop in daily milk yield (Taylor, 2014).

Helminths infection is the most important diseases of cattle in Ethiopia (Hailu *et al*., 2011; Jelalu and Yitagele, 2013; Balcha and Haftu, 2014; Yimer *et al*., 2015; Bedasa *et al*., 2016; Jelalu *et al*., 2017) that not only affect health of cattle but also affect the productivity and reproductive performance resulting in loss in body weight, digestive disturbance and emaciation (Taylor, 2014). Clinical disease (loss of appetite, scouring and poor condition) is generally only seen in young calves during their first grazing season, when control has been inadequate. Much more commonly, infected animals experience loss in production, which may be economically important, but can be difficult to detect without accurate observation and recording (Taylor, 2014; Johannes *et al*., 2016).

Cattle can acquire infections with any of several species of helminths while grazing in pasture, but amongst these the most common and important are gastrointestinal helminths. These worms are ubiquitous and can be found in all cattle where animals have access to grassland, even when they graze only for short periods (Morgan *et al*., 2013; Taylor, 2014; Lu and Judith, 2016). Cattle of all ages, but particularly young cattle, are affected by a diversity of gastro-intestinal parasites. These are roundworms (nematodes), which are primarily parasites of the gastrointestinal tract, the flukes (trematodes) in liver and rumen, and tapeworms (cestodes) in the small intestine (Taylor, 2014).

The majority of gastrointestinal helminth parasites of cattle which were investigated by different scholars in Ethiopia include *Haemonchus* spp., *Trichostrongylus* spp. *Strongyloides* spp., *Oesophagostomum* spp., *Bunostomum* spp. *Toxocara vitulorum, Trichuris* spp., *Capillaria* spp., *Paramphistomum* spp., *Fasciola* spp. and *Moniezia* spp. (Regassa *et al*., 2006; Hailu *el al.,* 2011; Jelalu and Yitagele, 2013; Bedasa *et al*., 2016).

Previous investigators noted the effect of seasonal variation on the species composition of helminth parasites (Jelalu and Yitagele, 2013; Sultana *et al*., 2013) and as a result the associated severity of infection vary considerably depending on local environmental conditions such as humidity, temperature, rainfall and vegetation cover (Jelalu and Yitagele, 2013; Taylor, 2014). In addition to diverse agro-climatic conditions, animal husbandry practice, nutrition and pasture management largely determine the incidence and severity of various parasitic diseases in cattle. The pattern of appearance of infective L_3 on permanent pasture is quite stereotypic, although the magnitude of risk can vary year to year, depending primarily on prevailing weather conditions (Taylor, 2014). The occurrence of GIT nematodes in cattle is higher in wet season than dry season of the year (Hailu *et al*., 2011; Jelalu and Yitagele, 2013; Sultana *et al*., 2013).

The occurrence of helminth parasites in cattle in many African countries including Ethiopia is usually higher than in other parts of the world. In Ethiopia, the problem is investigated by different scholars at different times, for instance, 77.6% prevalence was reported in Jimma town by Hailu *et al*. (2011). Likewise, Jelalu and Yitagele (2013) recorded 39.6% in Gedebano Gutazer Wolene district, Southern Ethiopia, whereas Tesfaye (2009) and Balcha and Haftu (2014) reported that gastrointestinal parasites are among the major constraints that adversely affect the health status of animals and cause enormous economic losses to the livestock industry of the country.

However, there is still scarcity of detailed information pertaining to occurrence, species composition and seasonal pattern of helminths of cattle in Ada'a and Boset districts, Central Oromia, Ethiopia. For this reason, this study was designed to provide information

on occurrence, species diversity and seasonal dynamics of helminths in cattle in highland PAs of Ada'a district and the lowland Boset district.

Therefore, the objectives of this study were:

- To determine the occurrence and species composition of helminths of cattle in highland PAs of Ada'a and lowland Boset districts of Central Oromia, Ethiopia.
- To assess the seasonal dynamics of helminths in cattle in the two districts

2. LITRATURE REVIEW

2.1. Helminths

Helminths are large, multicellular organisms that are visible to the eye once in the adult stage of their life cycle. They can either be free-living or parasitic. In their adult form, helminths are unable to multiply in their host, live in and feed on hosts which allow them to obtain nourishment while disrupting the hosts' nutrient absorption (Bowman, 2014).

The classification and identification of helminths are dependent on numerous factors including body shape, body cavity, body covering, digestive tubing, sex and type of attachment organs. The major groups of parasitic worms of vertebrates belong to the phyla Platyhelminthes (flukes, and tapeworms), Nematoda (roundworms) and Acanthocephala (thorny-headed worms). The definitive classification is based on the external and internal morphology of egg, larval, and adult stages (Bowman, 2014).

Platyhelminths (flatworms) include both trematodes (flukes) and cestodes (tapeworms). The platyhelminths are bilaterally symmetrical animals that are typically hermaphroditic. The digestive tract if present typically has only one opening, the mouth, with digested food being regurgitated from the oral opening (Colin *et al*., 2001; Bowman, 2014). The Cestoidea do not have a digestive tract in any of the life stages. The excretory system is a tubular network with clumps of flagellum-bearing cells, the so-called flame cells, which propel waste along the collection system to the excretory pore. Reproduction results in the formation of eggs that leave the body of the adult form via the genital pore (Bowman, 2014). Tapeworms are organized in a segmented plane, lack a body cavity and have a tegument body covering. They utilize suckers or bothridia, and rostellum with hooks for an attachment organ (Colin *et al*., 2001; Bowman, 2014). Trematodes (flukes) are characterized by an unsegmented plane for body shape, lack a body cavity and have a tegument for body covering. However, the digestive tube for trematodes ends in the

cecum. Trematodes are hermaphroditic and utilize oral suckers, ventral suckers or acetabulum for attachment organs (Bowman, 2014).

Nematoda are characterized by a cylindrical body shape and do indeed have a body cavity. Its body covering is a cuticle and the digestive tube ends in the anus. The sex of nematodes is dioecious (distinct male and female organisms). Lastly, their attachment organs range from lips, teeth, filariform extremities and dentary plates (Colin *et al*., 2001; Bowman, 2014).

2.2. Helminths of Cattle

2.2.1. Nematodes

Nematodes are commonly called roundworms because, as they are round when viewed in cross section. However, they are in fact cylindrical in structure and taper towards their anterior and posterior ends. They are bilaterally symmetrical, and while the sexes are separate in most species, a few are hermaphrodite (Bowman, 2014). Nematodes that parasitize domestic animals are found in all parts of the body but are most commonly found in the digestive and respiratory tracts and the circulatory system. Nematode parasites of domestic animals vary greatly in size ranging from small hair-like worms (up to 2 cm long) in the Superfamily Trichostrongyloidea to large, robust worms (up to 40cm long) in the Superfamily Ascaridoidea (Colin *et al*., 2001; Bowman, 2014).

Nematodes (round worms) are a group of worms (Sutherland and Scott, 2010) that are considered as an own systematic group called Nemathelminthes from which more than 28,000 species have been described. More than 16,000 roundworm species are parasites of many domestic and wild animals, humans and also plants. Roundworms of veterinary importance are all obligate parasites (Bowman, 2014, Junquera, 2015). They have a more or less long tubular form, and a digestive system with two openings, mouth and anus. The mouths of many species have species-specific structures such as teeth, cutting plates and hooks for attaching to the host and/or feeding on its tissues (Junquera, 2015).

The major parasites of ruminants are placed in the order Strongylida which includes the vast majority of nematode species causing gastrointestinal diseases in their host. The order contains the bulk of ruminant nematode parasites being found in the superfamily of Trichostrongyloidea (Table 1). A small number of important genera found in other superfamilies such as Ancylostomatoidea and Strongyloidea which are stouter than Trichostrongyloids (Sutherland and Scott, 2010).

Table 1. Taxonomic classification of GIT nematodes of cattle.

Order	Superfamily	Family	Genus	Species
Strongylida	Trichostrongyloidea	Trichostrongylidae	*Trichostrongylus*	*T. axei,* *T. colubriformis*
			Haemonchus	*H. contortus* *H. placei*
			Ostertagia	*O. ostertagi* *O. bisonis* *O. circumcincta* *O. trifurcate*
			Nematodirus	*N. battus* *N. spathiger* *N. helvetianus*
			Cooperia	*C. oncophora* *C. punctate* *C. pectinata,* *C. memasteri*
	Strongyloidea	Chabertiidae	*Chabertia*	*C. ovina*
	Ancylostomatoidea	Ancylostomatidae	*Oesophagostomum*	*Oe. radiatum*
			Bunostomum	*B. phlebotomum*
Ascaridida	Ascaridoidea	Ascarididae	*Toxocara*	*T. vitulorum*
Rhabditida	Rhabditoidea	Strongyloididae	*Strongyloides*	*S. papillosus*
Enoplida	Trichuroidea	Trichuridae	*Trichuris*	*T. globulosa*
			Capillaria	*C. hepatica* *C. bovis*

(Source: Colin *et al*., 2001; Murray, 2004)

Cattle host over 14 different species of gastro-intestinal roundworms. Different species live in different locations in the gut. As a few of these roundworms are usually present, the harm they cause is not always apparent and can be difficult to assess. Four species live in the abomasum: *Haemonchus placei, Ostertagia ostertagi, Ostertagia bisonis, Trichostrongylus axei,* Six species live in the small intestine: thread-necked worm

(*Nematodirus helvetianus*), four species of cattle bankrupt worms (*Cooperia* spp.), and cattle hookworm (*Bunostomum phlebotomum*), Four species live in the large intestine: nodular worm (*Oesophagostomum radiatum*), whipworm (*Trichuris discolor*), large-mouthed bowl worm (*Chabertia ovina*) and hairworm (*Capillaria bovis*). Some of roundworms listed above are rare or occur only in specific geographic areas. Other roundworms are common throughout the area wherever cattle are raised (Murray, 2004; Anne and Gary, 2012).

In the nematode, the sexes are separate and the males are generally smaller than the females which lay, eggs or larvae. During development, a nematode moults at intervals shedding its cuticle (Urquhart *et al*., 2001). The basic life cycle also consists of seven stages, an egg, four larval stages (L_1, L_2, L_3 and L_4) and two adult stages comprising separate males and females. Sometimes the sexually immature adult stages are called L_5's (Colin *et al*., 2001; Urquhart *et al*., 2001). Although some details vary, the life cycle of all the gastro-intestinal roundworms of cattle follows a similar pattern (Figure 1). Female roundworms lay microscopic eggs that are voided to the external environment with the faeces of cattle. Within a few days, a free-living larva develops and hatches from the egg. The hatched larva develops through a second, and then a third stage where it becomes capable of infecting cattle. Larval development on pasture takes only a few days in warmer months, but takes several weeks during cooler weather (Murray, 2004; Anne and Gary, 2012). Cattle become infected with roundworms as they graze on pasture or by ingesting feed or water contaminated with manure containing infective larvae. The larvae mature in the intestine and then mate. The females begin shedding eggs within two to four weeks after being ingested (Murray, 2004; Florian *et al*., 2013).

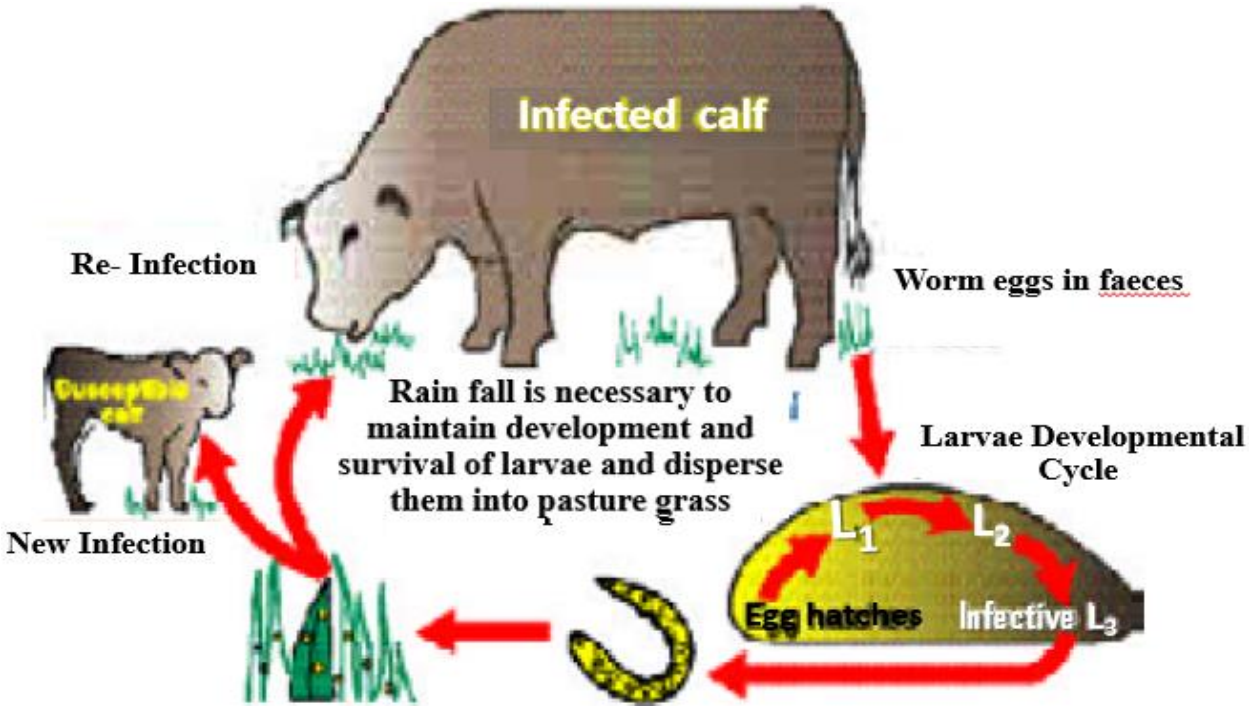


Figure 1. Life cycle of gastro-intestinal nematode (Williams and Loyacano, 2001)

The phenomenon of mixed infection has been suggested to be an important cause of morbidity and reduced production in livestock (Kumsa *et al*., 2011). Furthermore, immunosuppression of the host immune system by mixed infections increases host susceptibility to other diseases or parasites (Wang *et al*., 2006). Disease resulting from mixed infections with stomach and intestinal worms is called parasitic gastroenteritis. In severe infections, these changes in the gut can lead to the most obvious clinical sign, diarrhoea. Loss of nutrients and fluids is partially responsible for the loss of weight and body condition in affected animals, but the greatest cause of ill-thrift is reduced feed intake (Taylor, 2014).

Most GIT helminths' occurrence vary in their geographical distribution which depends particularly on climate (especially rainfall), vegetation and livestock density live in distinct sites of the intestinal tract (Tesfaye, 2009; Elsa *et al.*, 2012). As shown in (Table 2), there is also variation in the site and action of parasites in the cattle.

Table 2. Helminths located in the GIT, liver and bile duct of cattle and their effect.

Site	**Parasites**	**Action**
Rumen	*Paramphistomum*	Mucosal damage
Abomasum	*Haemonchus*	Blood sucking
	Ostertagia	Mucosal damage
	Trichostrongylus axei	Mucosal damage
Small intestine	*Trichostrongylus*	Mucosal damage
	Bunostomum	Blood sucking
	Cooperia	Mucosal damage
	Nematodirus	Mucosal damage
	Strongyloides	Mucosal damage
	Moniezia	Minimal
Large intestine	*Trichuris*	Blood sucking
	Oesophagostomum	Mucosal damage, nodules
	Chabertia	Mucosal damage
Liver	Immature flukes *Fasciola hepatica, Fasciola gigantica*	Destruction of tissue, fibrosis
Bile duct	Mature flukes *Fasciola hepatica, Fasciola gigantica*	Blood sucking, destruction of bile ducts, fibrosis
	Dicrocoelium	Minimal (fibrosis)
	Stilesia	Minimal

2.2.2. Trematodes and Cestodes

Trematodes and cestodes belong to the flatworms (also called *Platyhelminthes*). All the trematode species which are parasitic in livestock belong to the subclass Digenea (Bowman, 2014). Trematode species are estimated to be more than 20,000 worldwide. They are obligate parasites of either mollusks or vertebrates, including livestock and other domestic animals, as well as humans (Junquera, 2015).

Trematodes (known commonly as flukes) are dorso-ventrally flattened (Junquera, 2015), some being leaf-shaped and some long and narrow. The gastro-intestinal flukes have thick fleshy bodies and schistosomes are elongated and almost roundworm-like in appearance. Flukes are hermaphrodites (accept schistosomes) but they reproduce asexually and multiply in intermediate hosts (aquatic or amphibious snails) to complete their life cycles. Most of them are very discriminating in their choice of snail as intermediate host and the geographic distribution of trematode species is dependent on the distribution of suitable species of snails (Jorgen and Brian, 1994; Bowman, 2014). Flukes can be seriously harmful to grazing livestock particularly in humid regions that offer an adequate environment for the intermediate hosts. Most flukes have two suckers for attaching to the host, one close to the mouth and on the ventral side. They have a blind digestive system, without anus. But it is often not linear, as in most animals, but branched, ending in several blind ducts (called coeca) (Junquera, 2015).

Liver flukes are common in tropical and sub-tropical areas of the world (Jorgen and Brian, 1994). *Fasciola hepatica* is leaf-shaped and may reach a size of 30 x 30 mm. It occurs in the bile ducts and is cosmopolitan in its distribution. *F. gigantica* resembles *F. hepatica* but is easily distinguished by its characteristic shape and larger size (Muhammad *et al*., 2009). *Dicrocoelium dendriticum* is a small fluke, 6-10 mm long and 1-3 mm wide. It has an elongated lances-like body and inhabits the bile ducts of livestock (Jorgen and Brian, 1994).

Rumen flukes, paramphistomes are usually thick, short (4-12 mm), fleshy, maggot-like worms. They may infect all ruminants but young calves and lambs are the most susceptible. Not all the species are pathogenic, but clinical outbreaks of paramphistomiasis have been caused by *Paramphistomum microbothrium* (Africa), *Cotylophoron cotylophorum* (Asia), *P. ichikawar, C. calicophorum* (Australasia) and *P. cervi* (Europe) (Jorgen and Brian, 1994).

Paramphistomes require an aquatic snail as an intermediate host and the pre-parasitic stages of the life cycle are very similar to those of *F. hepatica* and *F. gigantica* (Nzalawahe *et al*., 2015). Infection of the cattle with liver flukes occurs by ingestion of encysted metacercariae on herbage, or less commonly by ingestion of suspended metacercariae in drinking water. Once ingested, the young flukes encyst in the small intestine, penetrate the gut wall and traverse the abdominal cavity to reach the liver capsule and the liver tissue. The immature flukes migrate in the liver parenchyma for 6-8 weeks before entering a bile duct where they mature and commence egg production. The severity of pathological manifestations usually depends on the number of metacercariae ingested over a period of time and the relative susceptibility of the animal (Joan *et al*., 2007; Anne and Gary, 2012).

The pre-patent period is reported to vary from approximately 56 days in cattle. The parasites appear as reddish/pink clusters between the papillae of the rumen and reticulum. Mixed infections with different trematode species are common since they share the same intermediate snail host and/or similar transmission sites (Keyyu *et al*., 2006; Phiri *et al*., 2006a; McGavin and Zachary, 2007).

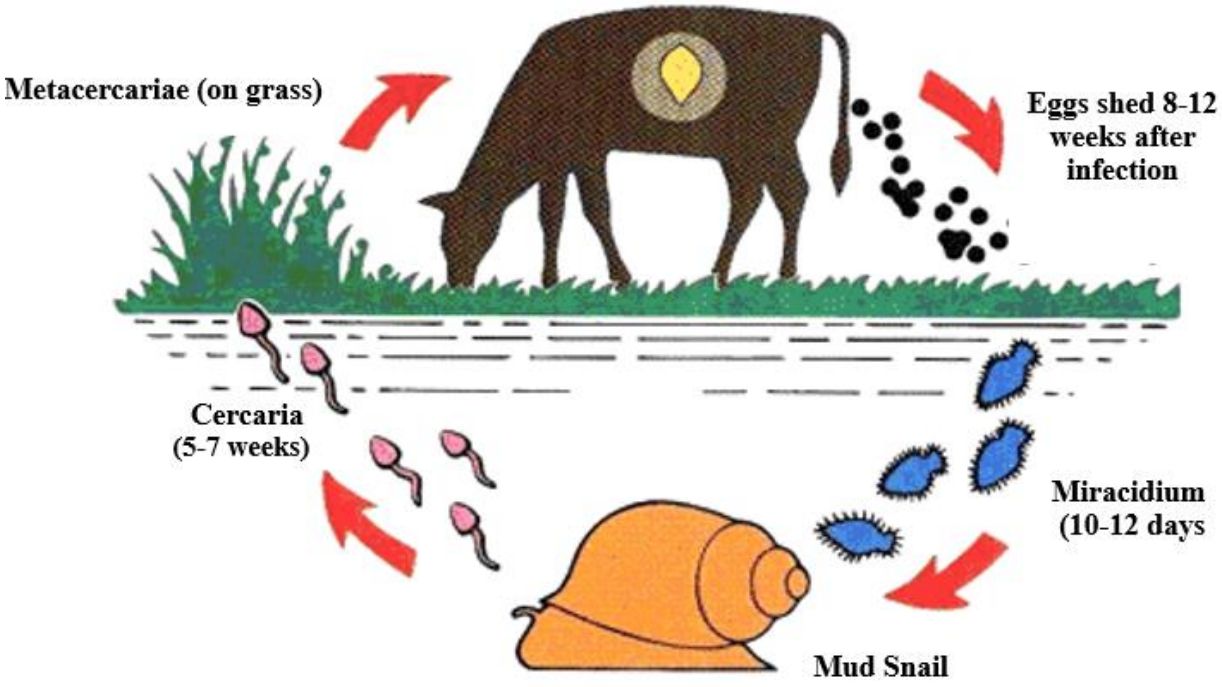


Figure 2. Life cycle of trematodes in cattle *(http://www.thebeefsite.com).*

Intestinal tapeworm comprises species of the genera *Moniezia* with cosmopolitan occurrence. *Stilesia hepatica* occurs in the bile ducts of ruminants and is very common in certain parts of Africa. They have indirect life cycles in which herbage mites of family *Oribatidae* act as intermediate hosts. Soil-inhabiting mites accidentally ingest eggs of the intestinal tapeworms present in the manure, and the larval stage called a cysticercoid develops in the mites. Ruminants become infected by ingesting herbage containing mites carrying the infective stage of the parasite. Intestinal and hepatic tapeworms are considered to be non-pathogenic, and no clinical signs are associated even with heavy infections (Jorgen and Brian, 1994; Zintl *et al*., 2014).

2.3. Epidemiology of Helminths of Cattle

2.3.1. Source of Infection

Pasture or water contaminated with manure containing infective larvae of helminth parasites is a source of infection for gastrointestinal helminthosis (Anne and Gary, 2012; Florian *et al*., 2013; Murray, 2004). Of the three larval stages of nematodes in the environment (L_1, L_2, and L_3) it is the L_3 which has a protective sheath that is the most resistant to variations in moisture, temperature and sunlight (Van Wyk *et al*., 2004, Van Wyk and Mayhew, 2013).

The occurrence of GI nematodes as observed through faecal egg counts is higher in calves than adult cattle (Jelalu and Yitagele, 2013; Chaparro *et al.,* 2016). Hence, adult cattle act as reservoirs of infection and constant sources of infection for the more susceptible young animals. Lactating and pregnant cows when compared to dry cows, bulls and oxen; demonstrate a higher prevalence and egg count of GI nematodes which confirms the periparturient egg-rise phenomenon and thus serves as a source of pasture contamination (Pfukenyi and Mukaratirwa, 2013; Chaparro *et al*., 2016).

Paramphistomes may survive for years, so there is a virtually constant source of infection for successive generations of snails. Intermediate hosts (genus *Planorbidae* and *Lymnaeidae)* are extremely adaptable and prolific breeders, which ensure a widespread availability of the snails within infested areas (Nzalawahe *et al*., 2015). Massive asexual multiplication of the parasites in infected snails and the survival of snails for several months may result in the shedding of large numbers of cercariae. Infected snails may also survive in mud for months. Clinical outbreaks of paramphistomiasis are usually confined to the drier months as the snail population becomes concentrated around natural sources of water and as these areas may provide the only dry season grazing. Older cattle seem to acquire immunity to the infection (Jorgen and Brian, 1994; Zintl *et al*., 2014; Nzalawahe *et al*., 2015).

2.3.2. Survival of helminths in the environment

Helminths species survival during unfavorable environmental conditions appeared to be achieved via a combination of arrested larval development (hypobiosis), an ageing residual population of adult worms in the host, and a small surviving population of infective larvae on the pastures (Horak *et al*., 2004). The development of infective larvae ingested by an animal during adverse environmental conditions may be temporarily arrested which helps the parasite to survive the dry seasons. The dynamics of infection and the stage composition of worm burden in different season can provide strong evidence that the parasite undergo arrested development (Horak *et al*., 2004; Meradi *et al*., 2016).

The development and survival of nematode eggs and free-living larvae are primarily affected by temperature and moisture and different parasites vary in their ability to survive extremes of temperature and moisture (Naomi *et al*., 2012; Leathwick, 2013). The difference in responses to environmental conditions, notably to extremes of temperature and moisture exist within and between nematode species (Horak *et al*., 2004). Desiccation from lack of rainfall kills eggs and larvae rapidly and is the most lethal of all climatic factors. Larvae may be protected from desiccation for a time by the crust of the faecal pat in which they lie or by migrating into the soil. Infective larvae may survive for up to 6 weeks or even longer in the manure pats, which act as a reservoir of infections during dry periods (Jorgen and Brian, 1994; Das *et al*., 2016).

In trematodes, freshly hatched cercariae from the snail can survive about one hour to encyst on a suitable object. If the water in which the snails live dries up, the snails can still be infected for months and in the presence of water the snails emerge and shed many cercariae. Metacercariae are extremely resistant to drying, infection may follow feeding of hay grown on infested meadows far removed from the scene of an outbreak (Anne and Gary, 2012; Bowman, 2012). They can survive prolonged periods (up to one year) on aquatic plants or even on hay grown in infested meadows, as they are resistant to drying. They can also be found free-floating in shallow, still water (Aksoy *et al*., 2005).

2.3.3. Seasonal influence and other risk factors

Helminths species, severity of infection and transmission dynamics are affected by changes in climate, geographical location and farm managemental factors (Tesfaye, 2009; Sutherland and Scott, 2010; Elsa *et al.*, 2012). The classes of stock present on a farm and the presence or absence of wildlife that may act as a reservoir of parasites can also have a major impact on the exact balance of parasite species present (Sutherland and Scott, 2010). Recent efforts have increasingly employed to assess the impact of predicted climate scenarios on future infection pressure for gastrointestinal nematodes in cattle, and to evaluate possible adaptive control measures (Verschave *et al*., 2016).

Climate has a direct impact on parasite abundance and larval availability through the influence of temperature and moisture on the development, migration, and mortality of the free-living stages. Higher temperature increase the development rate of eggs and early larval stages found in the fecal pat, but it may also increase the mortality of larval stages found on pasture, especially affecting larval survival during winter. The potential of the predicted temperature increase to affect development or mortality, however, varies between different nematode species and, therefore, the sensitivity of each nematode species to climate change also varies (Van Dijk *et al*., 2010). In addition to the difference in development of the free-living stages between nematode species, there exist species-specific needs for other life-history traits, such as egg hatching in *Nematodirus battus* (Van Dijk and Morgan, 2010).

Increased temperature variability can also drive increased or decreased infective-stage abundance depending on its relationship with important biological thresholds. The moisture level in temperate regions is not currently considered as a limiting factor for egg or larval development because this process occurs inside the dung (Van Dijk and Morgan, 2012). Future predictions report long periods of drought followed by short periods of heavy rainfall, which could lead to increased egg and larval mortality in desiccated feces and sudden increases in larval emergence and pasture infectivity (Van Dijk *et al*., 2010). If parasite abundance will in fact increase, a complex network of parasite population

dynamics and interactions will determine whether this will also lead to an increase in parasitic disease risk (Skuce *et al*., 2013).

Seasonal variation is an important factor for the development of helminths that can result in severe parasitic problems (Sultana *et al*., 2013; Jelalu and Yitagele, 2013; Knapp-Lawitzke *et al*., 2016). There is a marked seasonal fluctuation in number and availability of infective stages on pasture. This affects contamination of the environment and those controlling the survival, development, dissemination and availability of free-living stages and/or intermediate hosts (Jelalu and Yitagele, 2013). The interaction between moisture and temperature is an important factor for occurrence of liver flukes as it determines the survival and reproduction rate of the snails and the parasites (Muhammad *et al*., 2009). However, liver flukes have a versatile survival strategy to persist adverse environmental conditions such as hot and cold weather. Thus several mechanisms may be involved for persistence of infection from one season to the next: as adult flukes in mammalian hosts, as eggs on pasture, as larvae developing in snails and as metacercariae encysted on herbage (Bowman, 2014).

The seasonal variation in number of nematode infective larvae on pasture is similar to the pattern of egg shedding. After the winter decrease comes a small spring/early summer peak comprised of larvae that survived over winter as well as larvae derived from the increase in fecal egg shedding in cows (Murray, 2004). Calves are more susceptible than older cattle that frequently have been exposed to the parasites and have developed a degree of immunity. They are susceptible to be infected with parasites when they begin to graze (Yeshwas, 2013). The timing and size of these peaks and falls in helminths infection vary depending on environmental conditions. In some years, the spring peak may not occur at all (Murray, 2004).

During the rainy season, the rainfall and temperature are favorable for the development, survival and translation of infective larvae on herbage. These conditions result in increased availability of infective larvae on pastures, so the chances of cattle picking up infective stages of the parasites whilst grazing are high, leading to a buildup of high

worm burdens in the host (Magaya *et al.*, 2000; Akkari *et al*., 2012; Jelalu and Yitagele, 2013). This leads to an increase in FECs and be highest risk of occurrence of parasitic gastroenteritis (PGE) in cattle. However, it is also important to note that the most pathogenic GI nematode parasites, *Haemonchus* species, survive the dry season as inhibited early fourth-stage larvae (hypobiosis), whereas the other species from the genera *Cooperia* and *Trichostrongylus* survive the dry season mainly as adults (Magaya *et al.*, 2000; Akkari *et al*., 2012).

Generally, the most important predisposing factors of helminth infections in cattle are grazing habits, climate, nutritional deficiency, pasture management, age and immunological status of host, presence of intermediate host, number of infective larvae and eggs in the environment (Yeshwas, 2013; Abdurezak *et al*., 2015; Johannes *et al*., 2016). A combination of factors such as susceptibility of the host species, the pathogenicity of the parasite species, host-parasite interaction, and infective dose are also determining factors for the effect of helminth infections (Jelalu and Yitagele, 2013; Johannes *et al*., 2016).

2.3.4. Status in Ethiopia

Helminths are ubiquitous parasitic agents of livestock especially in ruminants. Different studies shown in (Table 3) that helminth infections are known to limit cattle production in many agro ecological zones of Ethiopia (Dinka *et al*., 2011; Hailu *et al*., 2011; Yeshwas, 2013; Jelalu and Yitagele, 2013; Balcha and Haftu, 2014; Abdurezak *et al*., 2015; Yimer *et al*., 2015; Bedasa *et al*., 2016).

Table 3. The status of helminths in cattle at different areas of Ethiopia.

Study Area	Helminth Parasite	Prevalence (%)	Author
Holleta Agricultural	*Paramphistomum* spp.	18	Bedasa *et al*., 2016
Research Center	*Toxocara* spp.	9.5	
	Fasciola spp.	8.5	
	Strongyle type	7.1	
	Trichuris spp.	1.8	
Dire Dawa	*Strongyloides*	24.05	Yimer *et al*., 2015
	Trichostrongylus	15.19	
	Trichuris	7.59	
	Cooperia spp.	4.43	
	Haemonchus spp.	1.9	
	Ostertagia spp.	1.9.	
Bahir Dar Zuria	Strongyle type	21.4	Yeshwas, 2013
and Gozamen Districts	*Fasciola* spp.	20.8	
	Paramphistomum spp.	17.9	
	Moniezia spp.	5.8	
Gedebano Gutazer	Strongyle type	37.9	Jelalu and Yitagele, 2013
Wolene district,	*Toxocara* spp.	22.4	
Southern Ethiopia	*Fasciola* spp.	16.1	
	Trichuris spp.	13.7	
	Paramphistomum spp.	9.9	
In and Around Gondar	*Toxocara* spp	57	Tigist *et al.*, 2012
	Strongyle type	56.07	
	Trichuris spp.	16,82	
In and Around Ambo	*F. hepatica*	23	Dinka *et al*., 2011
	Toxocara vetulorum	9.66	
	Paraphistomum cervi	9.38	
	Strongyle type	4.83	
	Moniezia benedeni	0.85	

2.4. Diagnosis

Accurate diagnosis of parasitic infections is of pivotal importance for both individual patient management and population-based studies, such as drug efficacy trials and surveillance of parasitic disease control programs (Giuseppe *et al*., 2010). The achievement of high quality diagnosis of parasitic diseases requires development of multivalent techniques which is important not only for individual diagnosis but also for population-based epidemiological investigations, such as anthelmintic drug efficacy trials, monitoring drug resistance, and surveillance of parasitic disease control and elimination programs (Papadopoulos *et al*., 2012). The clinical signs associated with GIT parasitisms are shared by many diseases and conditions; however, a presumptive diagnosis based on signs, grazing history, and season is often justified. In some countries, serologic diagnosis (ELISA) of important species, such as *Ostertagia* spp. in cattle, is also used and based on antibody titers in bulk tank milk samples in dairy herds. (Susan *et al*., 2014).

2.4.1. Coproparasitological examination

The fecal examination for diagnosis of helminth infections is the most common, relatively inexpensive and noninvasive laboratory procedure performed in veterinary practice. It can reveal the presence of parasites in several body systems. Parasites inhabiting the digestive system produce eggs that leave the body of the host by way of the feces. Occasionally, even adult helminth parasites may be seen in feces, especially when the host has enteritis (Anne and Gary, 2012).

Helminth infections are solely dependent on faecal microscopy for diagnosis. Confirmation of diagnosis usually can be made by demonstrating nematode or trematode eggs or tapeworm segments on faecal examination (Anne and Gary, 2012; Tankeshwar, 2013). In some cases, the helminths (eggs/ova) are present in sufficient quantities to be found by direct examination of a small amount of faeces, i.e. the direct smear (Tankeshwar, 2013).

Helminth eggs are often easier to find and identify because of their size and their distinctive morphological features (Tankeshwar, 2013). Many parasitic forms seen in feces have characteristic morphologic features (Figure 3) that, when combined with knowledge of the host, are diagnostic for a particular species of parasite. On the other hand, certain parasites produce similar eggs, oocysts, and so on, and cannot be identified to the species level (e.g., many of the strongylid-type eggs from livestock) (Anne and Gary, 2012). Such information is used as an indicator of pasture challenge at a herd level, as an indirect indicator of productivity, and is linked with the effectiveness of parasite control strategies (Susan *et al*., 2014).

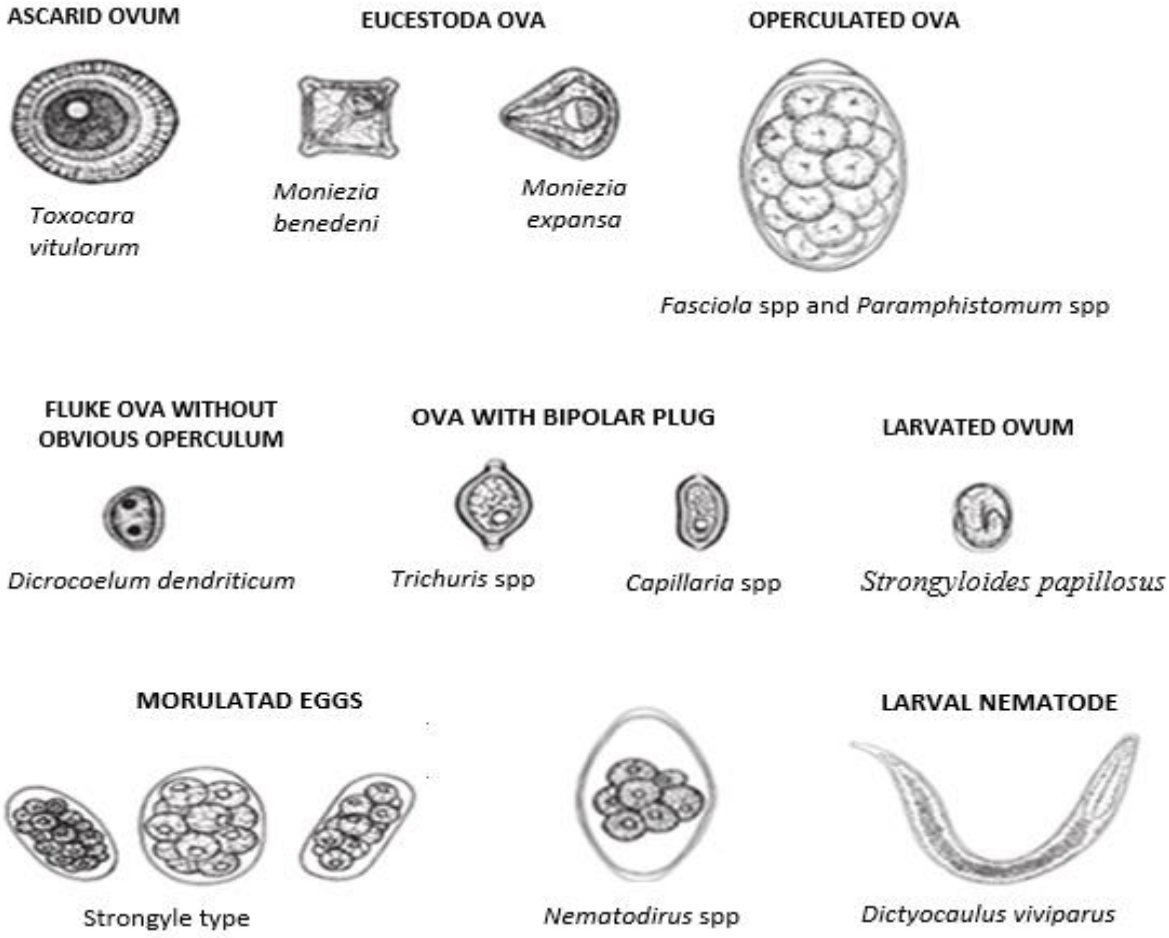


Figure 3. Helminth ova and larva in fresh faeces of cattle (Anne and Gary, 2012)

Grazing animals are infected with a variety of species of strongylid nematodes, which produce eggs that are not easily differentiated (Anne and Gary, 2012). Except for those worms with morphologically distinct ova such as *Strongyloides papillosus*, *Nematodirus* spp. and *Trichuris* spp, indication cannot be obtained about the identities of most of the common worm genera. Ova of *Ostertagia*, *Trichostrongylus*, *Oesophagostomum*, *Chabertia* spp. and, to some extent, *Cooperia* and *Bunostomum* spp. are either difficult or impossible to differentiate without measurements and computations (Van Wyk, and Mayhew, 2013). The most convenient method for identification of the strongylid nematode present in an animal or group of animals is culture of eggs to the third larval stage (Anne and Gary, 2012).

2.4.2. Identification of nematode infective larvae

Diagnosis of parasitic nematode infections of ruminants is largely still dependent on relatively inaccurate methods such as faecal worm egg counts (Tankeshwar, 2013). Though it is time consuming, measuring the L_3 of strongyles, or the length of the sheath tail extension (from the caudal tip of the larva to the tip of the STE – 'c' in Figure 4) can aid identification of nematode (Van Wyk, 2004; Van Wyk, and Mayhew, 2013).

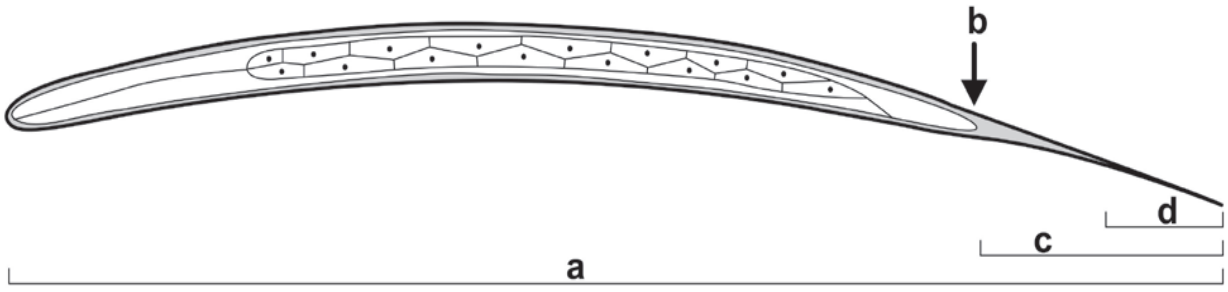


Figure 4: Diagram of a nematode infective larva, (a) total length, (b) tip of larva tail, (c) sheath tail extension (STE) and (d) filament (Van Wyk, and Mayhew, 2013).

As described by Van Wyk *et al*. (2004) and Van Wyk, and Mayhew, (2013), the length of the STE is a very important criterion for identification and, to facilitate its application in larval differentiation, the STE of every larva being evaluated is related to that of *T. axei*. To this end, the length of the STE of *T. axei* (±30µm) is represented by 'X', to which that of each L_3 encountered in a culture is related as follows:

Length of STE of L_3 being identified ('C' in Figure 4) = STE / X.

If the STE ends in a narrow, thin filament, calculating for the proportion of the total length of the STE that this comprises is important. With practice, it can be estimated without measurement. However, there is no exactly definable point of transition from the sheath filament to the cranial portion of the STE per species; it is invariably a more or less gradual process, with no precise point of inflexion (Van Wyk, and Mayhew, 2013).

2.5. Treatment and Control Strategies

Broad-spectrum anthelmintics currently available belong to five different chemical groups: benzimidazoles, imidazothiazoles, macrocyclic lactones, amino-acetonitrile derivatives and spiroindoles (Matthews, 2014; Susan *et al.*, 2014). The benzimidazoles set a new standard in efficacy and are still widely used. Fenbendazole, oxfendazole, albendazole, thiophanate, febantel, and netobimin are effective against most of the major GIT parasites of ruminants. The imidazothiazoles (levamisole, morantel, and pyrantel) also are highly effective, safe, broad-spectrum anthelmintics but have little activity against inhibited larvae in cattle. The macrocyclic lactones (avermectins and milbemycins) often administered by injection, are highly effective against adult and larval stages, including inhibited larvae of all the common GIT nematodes of ruminants and some of the important ectoparasites. Niclosamide, morantel, praziquantel, and the newer benzimidazoles (albendazole, fenbendazole, and oxfendazole) are effective against tapeworms (*Moniezia* spp.) in cattle (Susan *et al.*, 2014).

Sustainable and effective control of helminth infections of ruminants cannot always be achieved by drugs alone. It requires detailed knowledge on changes in environment and

livestock farming which have an impact on helminth infections (Morgan *et al*., 2013; Susan *et al.*, 2014). However, treatment with anthelminthic drugs may be used to reduce pasture contamination, particularly at times when seeding of the pasture with parasite eggs is a prerequisite for development of infective larvae (Susan *et al.*, 2014).

Optimizing intervention strategies must be based on accurate and efficiently collected information on levels of challenge, infection and production loss. This can be supported by (1) diagnosis of helminthoses with emphasis to multi-species infections and anthelmintic resistance; (2) prediction of the impact of global changes on the epidemiology of parasitic infections (3) explanation of current, and future predictions of seasonal trends in helminth infections of grazing livestock; (4) strategies for the sustainable management of helminth infections in a changing landscape. The development and implementation of innovative, refined approaches to control worm is a prerequisite for reducing the enormous helminth burden (Morgan *et al*., 2013). However, mixed grazing, alternating stock, rotational grazing, extensive grazing and stocking density, cutting and reseeding, and pasture composition are the major strategies that have shown successful control of helminths (Borovkov *et al*., 2013).

2.6. Anthelminthic Resistance

Anthelminthic resistance is an emerging issue globally with implications for effective parasite control (Cotter *et al*., 2015). It occurs when the worms are able to tolerate a drug at its normal dose, and this ability is passed on to their offspring. Resistance to anthelmintic drugs has been widely assessed in livestock worldwide, using diagnostic approaches with different levels of sensitivity (Papadopoulos *et al*., 2012; Cotter *et al*., 2015).

The Faecal Egg Count Reduction Test (FECRT) is the most widely used field-based method for estimating anthelmintic efficacy and as an indicator of the presence of anthelmintic resistant nematodes in cattle (Levecke *et al*., 2012; Lyndal-Murphy *et al*., 2014; Geurden *et al*., 2015), despite never having been validated against the gold

standard of controlled slaughter studies (Love *et al*., 2017). Reports of anthelmintic resistance given reliance on drugs for worm control on farms and threatens the viability of the livestock industry. Resistant worms are an increasing concern and there is an urgent need to alter control strategies. The most efficient way to limit the increase of anthelmintic resistance is the reduction of the selection pressure by drugs, and optimal timing to maximize their efficacy (Verschave *et al*., 2016).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in two districts of Central Oromia, Ethiopia; namely Ada'a and Boset districts (Figure 5). Ada'a is part of the East Shoa zone of Oromia regional state which is located 47 km South East of Addis Ababa and lies between longitudes 38º51' to 39º04' East and latitudes 8º46' to 8º59' North covering a land area of 1750 km^2. The district has a maximum altitude of 2300 masl above sea level and minimum altitude of 1500 masl. This study site has annual rainfall of maximum 1,200 mm and minimum 800 mm. It experiences bimodal patterns of rainfall with the main rainy season extending from June to September. A short rainy season occurs between March and May with an average rainfall of about 800 mm. The mean annual maximum and minimum temperatures are 30ºC and 8.5ºC respectively and the mean relative humidity is 61.3% (NMSA, 2015). It has a livestock population of 186,234 cattle, 45,678 sheep, 7,432 goats, 5,009 horses, 1,200 mules, and 25,654 donkeys (CSA, 2013).

Boset district, the second study site, is part of the rift valley of the East Shoa zone of Oromia regional state. Boset is bordered on the South by Arsi zone, on the West by the Awash River which separates it from Adama, on the North by the Amhara region, and on the East by Fentale. The area is located at 8°55′ N latitude and 38°37′ E longitude. The district has an altitude of maximum 1,400 masl and minimum 1,000 masl. This study site has annual rainfall of maximum 1,200 mm and minimum 550 mm. It has the minimum and maximum temperatures of 16.6°C and 31°C. The district has a mixed crop–livestock farming system (CSA, 2013; NMSA, 2015).

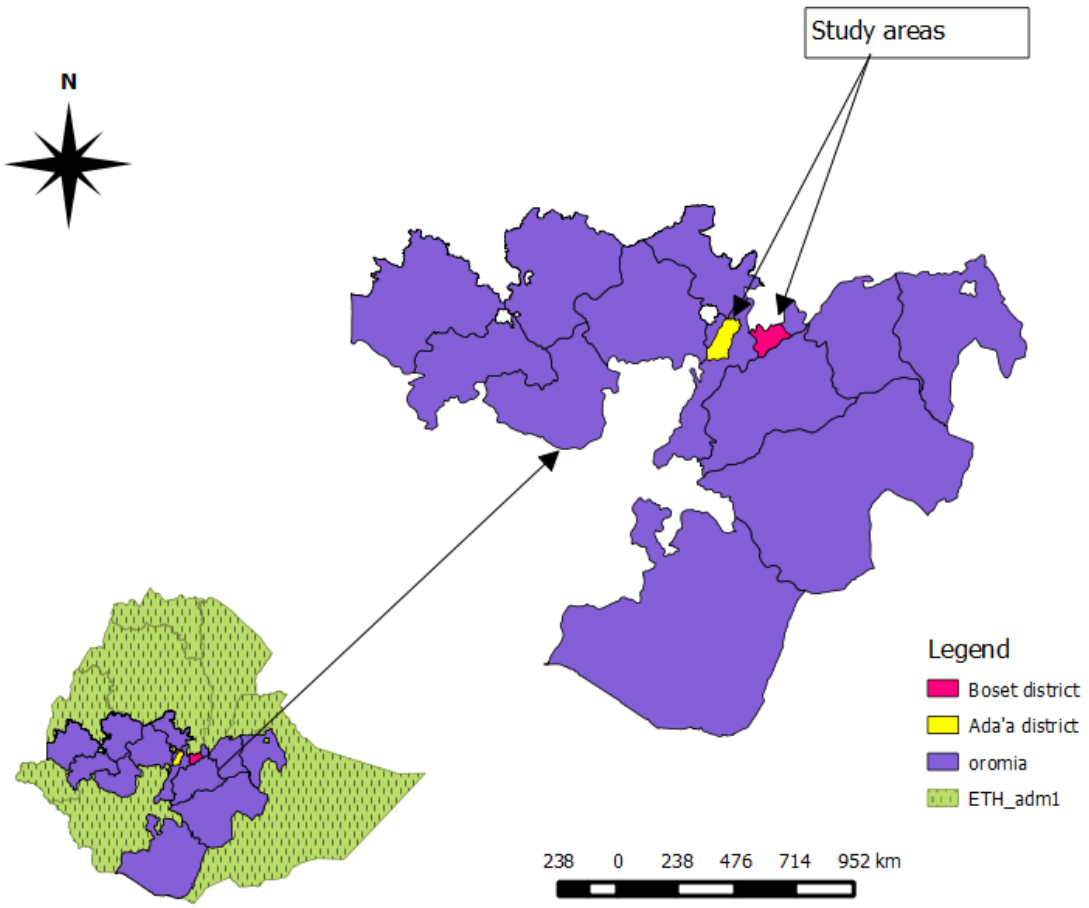


Figure 5: Map of Ethiopia showing the study districts in Central Oromia Regional State

3.2. Study Population and Design

The study was conducted on cattle reared under extensive management system on a communal grazing pasture land in the two study districts. All cattle between one and three years of age and not treated with any anthelmintic drug four weeks prior to sample collection were considered irrespective of their sex, and body condition. Relevant information such as name of the PAs and district with its altitude were recorded during faecal sample collection (Appendix 10). A cross sectional study design was used to study helminths of cattle during rainy, dry and short rainy seasons of the year from study animals in each district from August 2016 to May 2017.

3.4. Sampling Technique

The study peasant associations (PAs) in each district were selected purposively based on their agro-ecological attributes by recording the altitude of the sites using GPS Essentials (GPS Navigation on Android version 4.2.0). Consequently, PAs with altitude ranging from 2200-2300 masl in Ada'a district and PAs with 1200 masl to 1300 masl in Boset district were selected representing the highland and lowland agro-ecology in Central Oromia, respectively. Then a purposive sampling method was used to select study animals from each district on communal grazing site. Farms/owners who have cattle between one year and three years of age were selected. All cattle in this age group with no history of anthelmintic treatment four weeks prior to sample collection were included and sampling was conducted during the rainy season (August), dry season (December) and short rainy season (April) of the year in each site during the study period. Accordingly, 120 cattle were sampled in one visit from each districts. Thus, a total of 720 cattle were sampled during the study period.

3.5. Study Methods

3.5.1. Examination of sampled animals

The body condition of each of the study animal was scored using the guidelines established by Nicholson and Butterworth (1986) and Maurya *et al*. (2009) as shown in (Appendix 9). Accordingly, on the basis of observation of anatomical parts such as vertebral column, ribs, and spines, the study animals were categorized as poor (score, 1 to 3), medium (4 to 6), or good (greater than 6). The sex of animal and the consistency of faeces were recorded.

3.5.2. Faecal sample collection and examination

The faecal samples were collected directly from the rectum of purposively selected animals, by using a plastic glove and placed in screw cupped universal bottle. The bottles were labeled with a code containing the district name, altitude, season and animal

identity. The fecal samples were then transported with an icebox to Parasitology laboratory of College of Veterinary Medicine and Agriculture (CVMA) of Addis Ababa University (AAU). Faecal samples were processed by qualitative (floatation and sedimentation) and quantitative (Mc Master egg counting) coprological examination techniques on the day of collection or stored in refrigerator at 4°C to be processed on the next day.

Floatation technique: it was employed for qualitative examination of faecal samples. The procedure (Appendix 2) was conducted based on separating eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity. Floatation fluid, saturated NaCl solution, (Appendix 1) was used to concentrate nematode and cestode eggs and identification of helminth eggs was done by examining under a compound microscope as described in Soulsby, (1982).

Sedimentation technique: is qualitative coprological examination technique which was conducted to detect trematode infection in faecal samples. As most trematode eggs are relatively large and heavy compared to nematode eggs, this technique concentrated them in a sediment. The procedure (Appendix 3) was used to detect liver fluke *(Fasciola* spp.*),* rumen fluke (*Paramphistomum* spp.), *Dicrocoelium* spp. and *Schistosoma* spp. eggs in faecal samples. Identification of eggs was done by their distinct morphological and color differences as described in Soulsby (1982).

McMaster egg counting technique: it is a quantitative technique to determine the number of eggs present in one gram of faeces. Strongyle positive fecal samples after qualitative examination by floatation technique were subjected to modified McMaster egg counting technique (Appendix 4) as previously described by Over *et al*. (1992), Soulsby (1982) and MAFF (1997). The number of eggs per gram of faeces (EPG) were calculated by using the egg counting two chambers and then multiplying the total number counted in both chambers of McMaster by 50. Then EPG counts of strongyle type eggs were recorded in all positive animals for comparison of any change in the two agro-ecological districts during all seasons of the study period.

3.5.3. Coproculture and harvesting of L_3

Strongyle type egg positive fecal samples were further subjected to coproculture. The coproculture procedure (Appendix 5) was employed as previously used by Van Wyk *et al*. (2004, 2013).

The diarrheic faeces were finely mixed with sterilized bovine faeces. Then samples were transferred to petridish and were kept in an incubator at 27 ^{o}C for 7-10 days. The cultures were moistened sufficiently every 1-2 days to ensure that they do not dry out whilst being incubated, but without it becoming water-logged. After the 7 days period of incubation of the culture, the inside of the petridish were sprayed lightly with water before being placed in bright light that stimulates the L_3 to migrate up the inner surfaces of the vessel's walls. The cultures were transferred to a jar and larvae were harvested based on Borgsteede and Hendriks (1974) and Eckert (1960) by filling the jar with water, allowing it to stand for a few minutes to allow the air to escape from the culture, adding water to the jar until the water meniscus protrudes above the lip of the jar, placing an overturned petridish over the mouth of the jar and keeping the petridish in position whilst the jar is inverted (Appendix 5). Water was then added to the petridish and the preparation is left for a few hours for L_3 to migrate into the water and to settle. The water in the petridish is removed with a pipette for larvae harvest. Most cultures were capable of developing and maintaining significant populations of infective larvae (L_3). Then larvae in each harvest were identified immediately or stored in refrigerator at 4°C to be identified on the next day.

3.5.4. L_3 identification

The third stage larvae (L_3) of nematode species were prepared for examination by adding a drop of diluted Lugol's iodine solution to a drop of larval suspension from the harvested sample on a glass microscope slide (Van Wyk *et al*., 2004). Iodine solution added to the larvae suspension was used for immobilizing as well as staining the larvae. It was diluted to a level where L_3 do not stain darkly before a few minutes have elapsed. This is important, especially as it enables the free-living nematodes and L_3 of

Bunostomum spp. (which stain uniformly brown) to be differentiated more easily from the majority of the others, in which the cranial part of the larva stains less intensively than the rest. When the L_3 are too intensively stained it is difficult to observe their internal structures such as the shape of the oesophagus (Van Wyk *et al*., 2004; Van Wyk and Mayhew (2013).

Morphological identification of L_3 of most parasitic nematodes was conducted principally based on examination of the caudal and cranial extremities. However, other features such as the length and shape of oesophagus or cranial refractile spots were important identification keys (Annex 6) in some genera based on the description by Van Wyk and Mayhew (2013).

Furthermore, micrometry was employed to identify the species of harvested nematode L_3. Calibration of a compound microscope was made based on the procedure (Appendix 7) prior to measurement. A stage micrometer was used to determine, for each microscope objective lens, the number of divisions of the graticule in the ocular lens that span 30 µm; that is, the mean length of the sheath tail extension (STEs) of *T. axei*, defined as 'X' for the purpose of the present system of identification as described by Van Wyk *et al*. (2004), and Van Wyk and Mayhew (2013). The total larval length, sheath tail extension, and proportion of filament were recorded for each L_3 in the prepared larval suspension under glass slide. Finally, nematode species identification was done by comparing the recorded measurements for each L_3 with the standard measurement values investigated by Van Wyk and Mayhew (2013) as described in Appendix-8.

The length of the STE was a very important criterion applied for larval identification and differentiation. It was calculated for each larvae by using the formula previously used by Van Wyk, and Mayhew (2013). The length of the STE of *T. axei* (±30 µm) is represented by 'X', to which that of each L_3 encountered in a culture was calculated as follows:

Length of STE of L_3 being identified = STE / X.

3.6. Data Management and Statistical Analysis

Database was created in Microsoft Excel spreadsheet program by entering the raw data about individual animals. Season of sampling, district name, sex, body condition of animals and consistency of faeces were entered in to the spreadsheet. The types of helminth eggs identified from cattle by qualitative faecal examination techniques (faecal floatation and sedimentation technique) and eggs per gram of faeces estimated by McMaster egg counting technique were recorded. The species of third stage nematode larvae (L_3) recovered from individual coproculture and their count in pooled coproculture including micrometric measurement values were also recorded. STATA version 13 software was used for statistical analysis of the data (StataCorp LP, College Station, Texas USA). Helminths infection among districts, season of sampling, sex, body condition of the animal, and consistency of faeces were analyzed by Multivariate logistic regression model. However, Negative binomial regression models were applied for EPG counts analysis, because EPG counts were over dispersed with most animals having few eggs and a few having a high number of eggs. Association of identified parasite species with district and season was evaluated by Chi square test and Fisher's exact test statistics. Nematode species (identified third stage larvae) and their measurement values were summarizes by descriptive statistical tools such as percentage, mean, range, proportion and frequency tables. In all cases, the level of significance was set at $p < 5\%$.

4. RESULTS

4.1. Faecal Examination

An overall percentage of 57.4% (413/720) helminths in cattle was recorded using qualitative faecal examination techniques. Cattle positive for nematodes were 323 (44.9%), followed by trematodes 30 (4.2%) and cestodes 9 (1.3%). The other 51 (7.1%) cattle were positive for one or more of nematodes, trematodes or cestodes. The present study revealed that higher number of positive cattle were recorded during the rainy season (65.0%), followed by short rainy season (59.2%) and dry season (47.9%). Nematodes were the predominant helminths of the examined cattle during the rainy 125 (52.1%), dry 86 (35.8%) and short rainy 112 (46.7%) seasons of the study period. Nematodes were encountered as the predominant helminth in both agro-ecological districts. Nematodes and cestodes were higher in Boset district than Ada'a district. However, trematodes were not encountered in Boset district during all seasons of the study period (Table 4).

Table 4. Nematodes, trematodes and cestodes in faecal samples of cattle during different seasons and districts

Seasons and districts	No. examined	No. positive for class of Helminths (%)				
		Nematode	Cestode	Trematode	Mixed	Total
Season						
Rainy	240	125 (52.1)	4 (1.7)	4 (1.7)	23(9.6)	156 (65.0)
Dry	240	86 (35.8)	3 (1.2)	16 (6.7)	10 (4.2)	115 (47.9)
Short rainy	240	112 (46.7)	2 (0.8)	10 (4.2)	18(7.5)	142 (59.2)
Total	**720**	**323 (44.9)**	**9 (1.3)**	**30 (4.2)**	**51(7.1)**	**413 (57.4)**
District						
Ada'a	360	120 (33.3)	5 (1.4)	30 (8.3)	29 (8.1)	184 (51.1)
Boset	360	203 (56.4)	4 (1.1)	-	22 (6.1)	229 (63.6)
Total	**720**	**323 (44.9)**	**9 (1.3)**	**30 (4.2)**	**51(7.1)**	**413 (57.4)**

Results of coprological examination also classified helminths in the study area as single class, double class and triple class of helminths in positive cattle. The proportion of single class was higher than double as well as triple class positive cattle during all seasons of the study period. This study demonstrated (Table 5) that most of helminth infections were single class positive (87.7%) either by nematode, cestode or trematode followed by double positive (11.8%) and triple positive (0.5%). The proportion of positive cattle by any of the three classes of helminths was higher during the dry season (91.1%) followed by short rainy season (88.5%) and rainy season (85.9%) of the study period. The proportion of cattle positive for two classes of helminths was higher during the rainy season (14.7%) than short rainy (11.5%) and dry season (7.1%). However, the proportion of cattle positive for three classes of helminths (nematode, cestode and trematode) was observed only during the dry season 2 (1.8%).

Table 5. Proportion of nematodes, cestodes, trematodes and mixed class in cattle positive for helminths by coproscopy.

Helminths	**Season of the study**			
	Rainy (N=156)	**Dry (N=115)**	**Short rainy (N=142)**	**Overall (N=413)**
Single class				
Nem.	125 (81.1%)	86 (74.8%)	112 (78.9%)	323 (78.2%)
Ces.	4 (2.8%)	3 (2.6%)	2 (1.4%)	9 (2.2%)
Tre.	4 (2.8%)	16 (13.9%)	10 (7%)	30 (7.3%)
Overall	**129 (85.5%)**	**105 (91.3%)**	**124 (87.3%)**	**362 (87.7%)**
Double class				
Nem + Ces.	14 (9%)	2 (1.7%)	10 (7%)	26 (6.3%)
Nem + Tre.	9 (5.8%)	4 (3.5%)	8 (5.6%)	21 (5.8%)
Ces. + Tre.	-	2 (1.7%)	-	2 (0.5%)
Overall	**23 (14.7%)**	**8 (6.9%)**	**18 (12.7%)**	**49 (11.8%)**
Triple class				
Nem + Ces. + Tre.	-	2 (1.7%)	-	2 (0.5%)
Overall	**156 (37.8%)**	**114 (27.9%)**	**142 (34.4%)**	**413 (100%)**

N-Number of positive animals, Nem-Nematode, Ces-Cestode, Tre-Trematode

Multivariate logistic regression analysis showed that cattle during the rainy season and short rainy season were 1.81 times (OR = 1.81, 95% CI: 1.19-2.74) and 2.35 times (OR = 2.35, 95% CI: 1.57-2.50) respectively, more likely to be positive for helminths than cattle during the dry season of the study period. The odds of cattle to become positive for helminths in Boset district was twice higher (OR = 1.77, 95% CI: 1.28-2.46) than those cattle in Ada'a district. Diarrheic cattle had nine times (OR = 8.7, 95 % CI: 5.06-15.07) higher chance of becoming positive for helminths than those cattle with normal faecal consistency. However, statistically significant variation ($p > 0.05$) was never observed among cattle of different body condition scores and sex of cattle in helminths positivity (Table 6).

Table 6. Multivariate logistic regression analysis of helminths positive cattle by seasons, agro-ecological and host related factors.

Variables	No. Examined	Positive (%)	OR (95% CI)	p-value
Season				
Dry	240	115 (47.9)	1 *	
Short rainy	240	142 (59.2)	1.81 (1.19-2.74)	0.006
Rainy	240	156 (65)	2.35 (1.57-2.50)	0.000
District				
Ada'a	360	184 (51.1)	1 *	0.001
Boset	360	229 (63.6)	1.77 (1.28-2.46)	
Consistency of faeces				
Normal	573	283 (49.4)	1 *	0.000
Diarrheic	147	130 (88.4)	8.7 (5.06-15.07)	
Body condition				
Poor	132	79 (59.8)	1.70 (0.98-2.93)	0.058
Medium	430	256 (59.5)	1.44 (0.96-2.16)	0.080
Good	158	78 (49.4)	1 *	
Sex				
Male	342	203 (59.4)	1*	
Female	378	210 (55.5)	0. 79 (0.57-1.09)	0.151

* = Reference, OR- Odds Ratio, CI = Confidence Interval

The study revealed that 366 (50.8%) of cattle were positive for strongyle type eggs, 45 (6.3%) *Strongyloides papillosus* and 5 (0.7%) *Nematodirus* spp. eggs using qualitative coproscopy out of 720 faecal samples (Table 7). *Nematodirus* spp. and *Strongyloides papillosus* were identified by their egg morphology (Figure 6). *Moniezia benedeni* eggs and *Moniezia expansa* eggs were detected in 30 (4.2%) and 8 (1.1%) cattle respectively (Figure 7). Eggs of trematodes; *Fasciola* spp. and *Paramphistomum* spp. (Figure 8) were also detected in 25 (3.5%) and 41 (5.7%) animals, respectively.

Figure 6. Photograph of strongyle type eggs (left), *Nematodirus* spp. egg (middle), *S. papillosus* egg (right) under 40x objective lens by simple floatation technique.

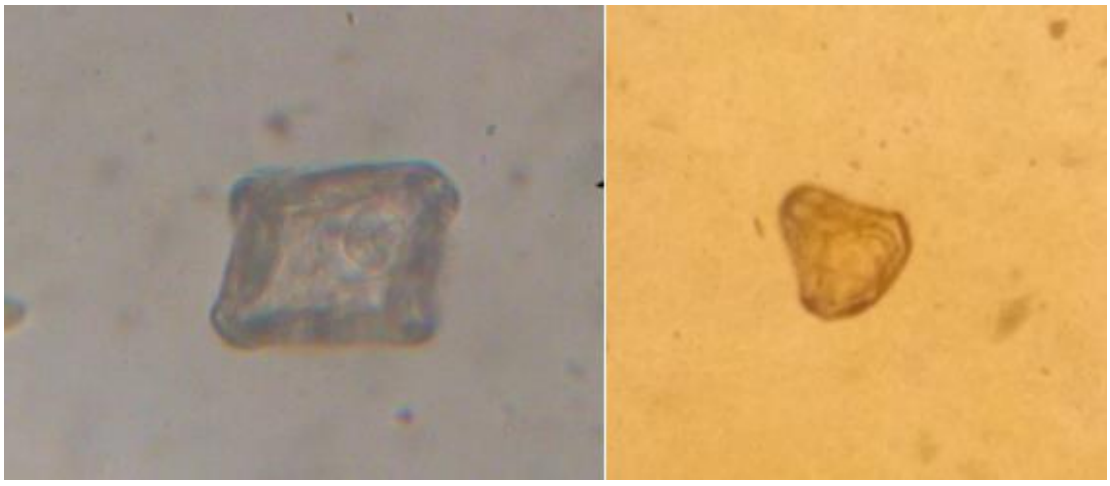


Figure 7. Photograph of cestode eggs; *M. benedeni* (left) and *M. expansa* (right) by simple floatation technique under 40x objective lens

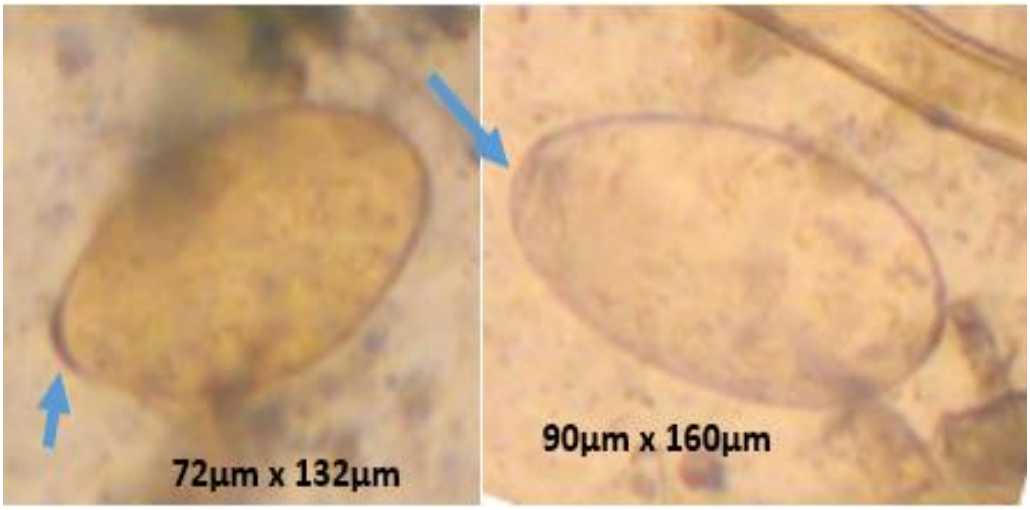


Figure 8. Photograph of trematodes (liver and rumen flukes) eggs; *Fasciola* spp. (left), *Paramphistomum* spp. (right) with one sided operculum (arrow) identified by sedimentation technique

In highland PAs of Ada'a district six types of helminths eggs including stronglye type eggs, *S. papillosus*, *Nematodirus* spp, *M. benedeni*, *Fasciola* spp. and *Paramphistomum* spp. were observed. However, in Boset district that represent lowland agro-ecology only four types of eggs of helminths including strongyle type eggs, *S. papillosus*, *M. benedeni* and *M. expansa* were identified (Figure 9). Stronglye type eggs were the predominant helminths eggs detected followed by *Fasciola* spp. in Ada'a district and *S. papillosus* in Boset district.

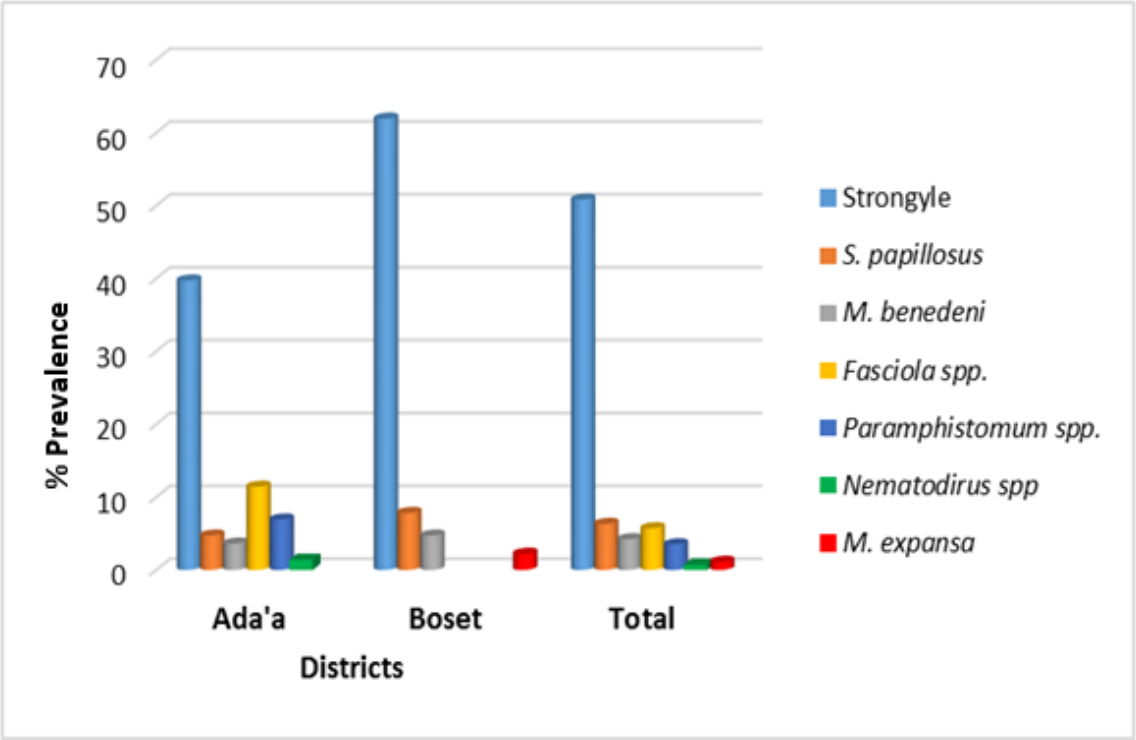


Figure 9. Eggs of helminth detected by coproscopy in Ada'a and Boset district

Statistically significantlly (χ^2 = 35.6, p = 0.00) higher overall frequency of strongyle in Boset district 223 (61.9 %) than in Ada'a district 143 (39.7 %) was observed. *Nematodirus* spp., *Fasciola* spp. and *Paramphistomum* spp. were found only in Ada'a district with 5 (1.4 %), 41 (11.4 %) and 25 (6.9 %) positive animals, respectively. *M. expansa* was recorded only in Boset district with 8 (2.2 %) positive animals (Table 7).

Significantly (χ^2 = 23.4, p = 0.00) higher frequencey of cattle positive for strongyle type eggs during the rainy season 145 (60.4 %) than during the short rainy 128 (53.3 %) and dry season 93 (38.8 %) was recorded. *Nematodirus* spp was found only during the short rainy season. Statistically significant association between *M. expansa*, *Fasciola* spp. and *Paramphistomum* spp. and season of the study period was never observed (Table 7).

Table 7. Ova of helminths detected by coproscopy in cattle of Ada'a and Boset ditricts during dffierent seasons of the study period

Helminths identified	No. of infected animals per district (%)			χ^2	p-value	No. of infected animals per season (%)			χ^2	p-value
	Total (N=720)	Ada'a (N=360)	Boset (N=360)			Rainy (N=240)	Dry (N=240)	S. rainy (N=240)		
Nematodes										
Strongyle	366 (50.8)	143 (39.7)	223 (61.9)	35.6	0.00	145 (60.4)	93 (38.8)	128 (53.3)	23.4	0.00
S. papillosus	45 (6.3)	17 (4.7)	28 (7.8)	2.9	0.09	25 (10.4)	12 (5.0)	8 (3.3)	11.2	0.00
Nematodirus spp	5 (0.7)	5 (1.4)	0 (0)	-	0.03	0 (0)	0 (0)	5 (2.1)	-	0.01
Cestodes										
M. benedeni	30 (4.2)	13 (3.6)	17 (4.7)	0.6	0.45	16 (6.7)	6 (2.5)	8 (3.3)	5.8	0.05
M. expansa	8 (1.1)	0 (0)	8 (2.2)	-	0.00	2 (0.8)	3 (1.3)	3 (1.3)	-	0.99
Trematodes										
Fasciola spp.	41 (5.7)	41 (11.4)	0 (0)	-	0.00	9 (3.8)	18 (7.5)	14 (5.8)	3.3	0.19
Paramphistomum spp.	25 (3.5)	25 (6.9)	0 (0)	-	0.00	6 (2.5)	10 (4.1)	9 (3.8)	1.1	0.58

N = number of animals examined, S. rainy = Short rainy

4.2. Worm Egg Count during Different Seasons in Ada'a and Boset Districts

The peak mean EPG of strongyle type eggs was recorded during the rainy season, drops during dry season and then it increased during the short rainy season (Figure 10). Statistically significantly ($p < 0.05$) higher overall mean EPG count was recorded during the rainy season (1001.0±51.1) than during the short rainy season (705.5±43.2) (Table 8).

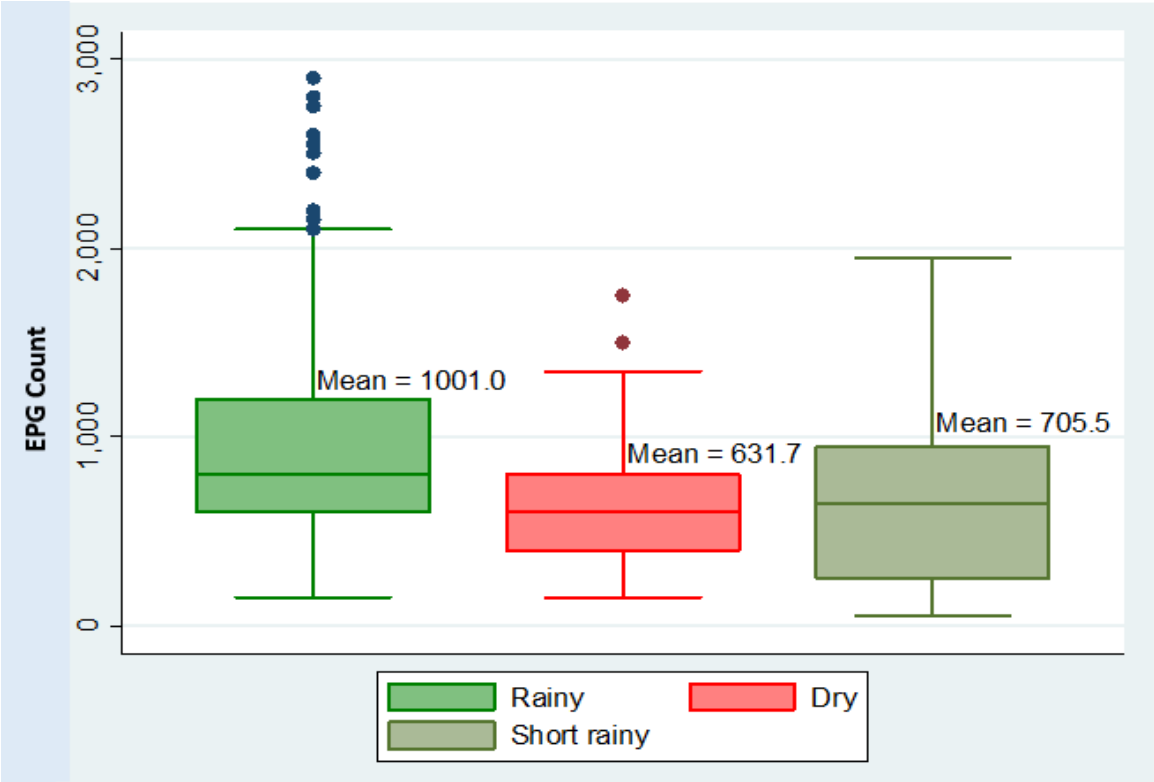


Figure 10. Box and Whiskers plot showing strongyle mean EPG count during different seasons of the study period

In the highland PAs of Ada'a district, higher mean EPG count was recorded during the rainy season than both during the dry and short rainy seasons (Table 8). But this difference was not statistically significant ($p > 0.05$). In Boset district significantly ($p = 0.000$) higher mean EPG count was recorded during the rainy season (1151.6±69.9) than short rainy season (811.5±60.7) and also it was significantly higher ($p < 0.05$) during the

short rainy than during the dry season. The overall mean EPG count was significantly ($p < 0.05$) higher during the rainy season than both during dry and short rainy seasons. The mean EPG count during the rainy season was on average 1.6 times higher than during the dry season and 1.4 times higher than the mean EPG count during the short rainy season (Table 8).

Table 8. Negative binomial regression analysis of mean ± SE of EPG count during different seasons in Ada'a and Boset districts

District	Season	Mean	SE	95% CI	Coef.	p- value
Ada'a	Rainy	731.7	50.9	629.4-834.1	*	
(N = 143)	Dry	641.9	60.2	518.9-764.9	-0.131	0.373
	Short rainy	625.9	59.2	507.3-744.3	-0.156	0.203
	Total	**667.8**	**33.7**	**601.3-737.4**		
Boset	Rainy	1151.6	69.9	1012.8-1290.5	*	
(N = 223)	Dry	626.6	44.1	538.4-714.8	-0.608	0.000
	Short rainy	775.7	61.5	653.1-898.4	-0.395	0.000
	Total	**891.0**	**39.7**	**812.8-969.2**		
Total	Rainy	1001.0	51.1	900.0-1102.1	*	
(N = 366)	Dry	631.7	35.4	561.3-702.1	-0.460	0.000
	Short rainy	705.5	43.2	620.0-791.0	-0.349	0.000
	Total	**803.8**	**28.1**	**748.6-859.0**		

* = Reference category; N = Number of animals positive for strongyle type eggs

There were variations in mean EPG count between the two agro-ecological districts. Significantly ($p = 0.000$) higher mean EPG count in cattle in Boset than those cattle in Ada'a district was recorded (Table 9). On average 1.31 times higher mean EPG count was recorded in cattle from Boset district than those cattle in Ada'a district.

Table 9. Comparison of strongyle type egg EPG count by agro-ecology and other host related factors.

Variables	No. positive	Mean ± SE	95% CI	Coef.	p- value
District					
Ada'a	143	667.8 ± 33.7	601.3 - 737.4	*	
Boset	223	891.0 ± 39.7	812.8 - 969.2	0.27	0.000
Sex					
Male	178	839.9 ± 42.3	756.4 - 923.3	*	
Female	188	769.7 ± 37.1	696.4 - 842.9	-0.07	0.314
Body condition					
Poor	71	798.6 ± 60.2	678.5 - 918.7	*	
Medium	226	790.7 ± 33.3	725.0 - 856.4	-0.15	0.120
Good	69	852.2 ± 80.7	691.1 - 1013.2	-0.17	0.165
Consistency of faeces					
Diarrheic	123	825.2 ± 51.5	748.6 - 859.0	0.09	0.254
Normal	243	793.0 ± 33.3	727.3 - 858.7	*	

* = Reference; SE = Standard error; CI = Confidence interval

4.3. Helminths Species Composition in Cattle by Coproscopy and Ova Culture

The identification of eggs of helminth species by coproscopy (flotation and sedimentation) and coproculture of individual cattle positive for strongyle type nematodes revealed ten helminths parasites of cattle; six nematode species (*Haemonchus placei*, *Oesophagostomum radiatum*, *Trichostrongylus axei*, *Bunostomum phlebotomum*, *Nematodirus* spp. and *Strongyloides papillosus)*, two cestodes (*Moniezia benedeni* and *Moniezia expansa*), and two trematodes (*Fasciola* spp. and *Paramphistomum* spp.). Out of the total 413 cattle positive for helminths, a total of 151 (36.6%) cattle were positive only for single species by either *H. placei, T. axei, Oe. radiatum, B. phlebotomum,*

Nematodirus spp., *S. papillosus*, *M. benedeni*, *M. expansa*, *Fasciola* spp., or *Paramphistomum* spp. However, a total of 262 (63.4%) cattle were positive for more than one parasite species. Most of cattle positive for helminths harbour two species of helminths 183 (44.3%), three species of helminths 68 (16.5%), four species of helminths 6 (1.5%), five species of helminths 4 (0.9 %) and six species of helminths (*S. papillosus*, *H. placei*, *T. axei*, *B. phlebotomum*, *Fasciola* spp, and *Paramphistomum* spp.) one animal (0.2%) (Figure 12).

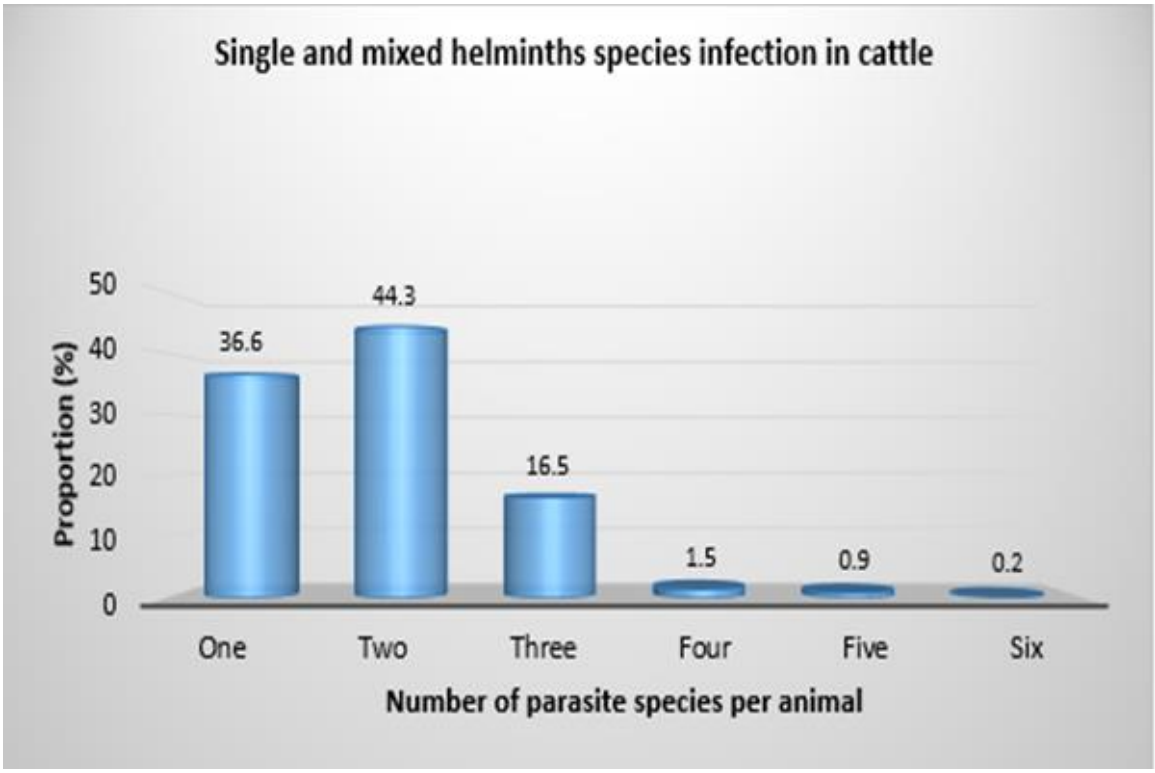


Figure 11. Overall proportion of single, two, or mixed helminths species in positive cattle by coproscopy and ovaculture.

Haemonchus placei 264 (36.7%) was identified as the predominant nematodes in cattle. *Oesophagostomum radiatum* 158 (21.9%) was identified as the 2nd common helminths in cattle followed by *Trichostrongylus axei* 130 (18.0%). Other helminths species were *Bunostomum phlebotomum* 72 (10%), *Strongyloides papillosus* 45 (6.3%), *Fasciola* spp.

41 (5.7%), *Moniezia benedeni* 30 (4.2%), *Paramphistomum* spp. 25 (3.5%), *Moniezia expansa* 8 (1.1%) and *Nematodirus* spp. 5 (0.7%) with their respective predominance (Table 10).

Pearson's chi square test statistics showed significantly ($p < 0.05$) higher prevalence of *T. axei* was recorded during the short rainy season 65 (27.1%) and the rainy season 59 (24.6%) than during the dry season 27 (11.2%). Significantly ($\chi^2 = 11.9$, $p = 0.003$) higher percentage of *Bunostomum phlebotomum* was detected during short rainy season than rainy and dry seasons. Significantly ($\chi^2 = 33.1$, $p = 0.000$) higher *Oesophagostomum radiatum* was recorded during the rainy season than during the dry and short rainy seasons. Significantly ($\chi^2 = 5.8$, $p = 0.05$) higher percentage of *M. benedeni* was recorded during the rainy season and the short rainy than during the dry season (Table 10).

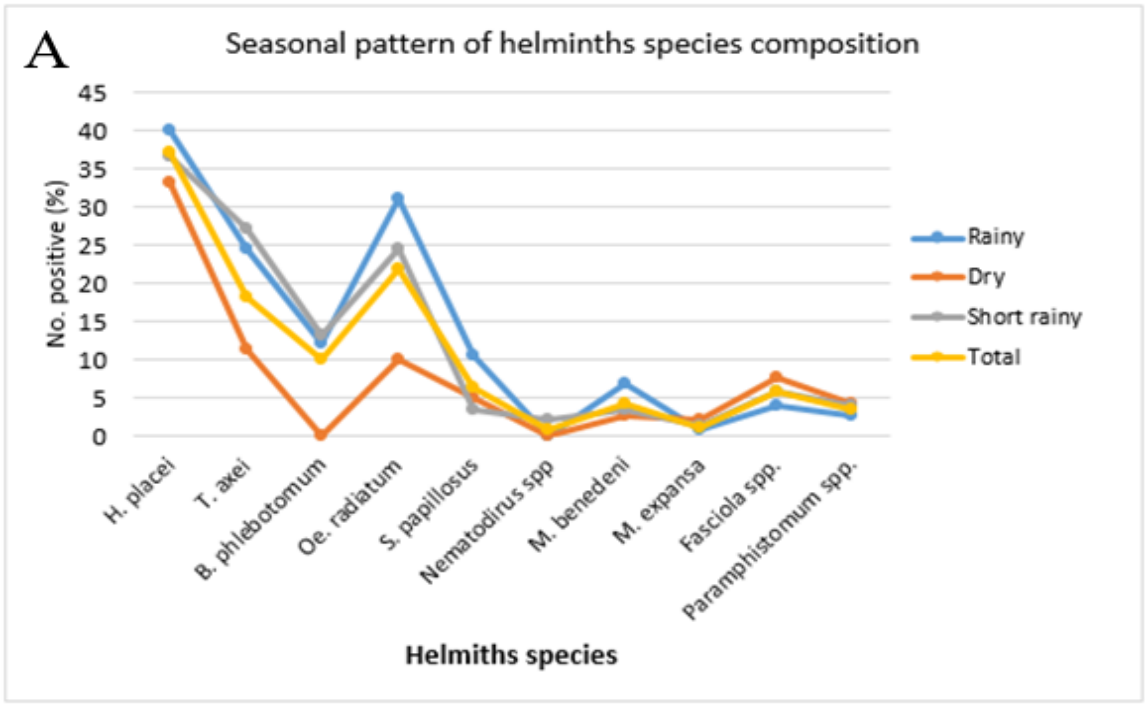
Table 10. Helminths species in cattle by coproscopy and ovaculture during different seasons of the study period.

Helminths species	No. infected animal per season (%)				χ^2	p-value
	Rainy (N=240)	Dry (N=240)	Short rainy (N=240)	Total (N=720)		
Nematodes						
H. placei	96 (40.0)	80 (33.3)	88 (36.7)	264 (36.7)	2.3	0.317
T. axei	59 (24.6)	27 (11.2)	65 (27.1)	130 (18.1)	14.4	0.001
B. phlebotomum	29 (12.1)	11 (4.6)	32 (13.3)	72 (10)	11.9	0.003
Oe. radiatum	75 (31.2)	24 (10)	59 (24.6)	158 (21.9)	33.1	0.000
S. papillosus	25 (10.4)	12 (5.0)	8 (3.3)	45 (6.3)	11.2	0.00
Nematodirus spp.	0 (0)	0 (0)	5 (2.1)	5 (0.7)	-	0.01
Cestodes						
M. benedeni	16 (6.7)	6 (2.5)	8 (3.3)	30 (4.2)	5.8	0.05
M. expansa	2 (0.8)	3 (1.3)	3 (1.3)	8 (1.1)	-	0.99
Trematodes						
Fasciola spp.	9 (3.8)	18 (7.5)	14 (5.8)	41 (5.7)	3.3	0.19
Paramphistomum spp.	6 (2.5)	10 (4.1)	9 (3.8)	25 (3.5)	1.1	0.58

N = Number of animals examined

Majority of helminths species recorded at higher frequency during the rainy and short rainy seasons than during the dry season (Figure 12A). However, *M. expansa*, *Fasciola* spp., and *Paramphistomum* spp were recorded with higher frequency during the dry and short rainy season than during the rainy season. *H. placei*, *T. axei*, *Oe. radiatum, B. phlebotomum, S. papillosus* and *M. benedeni* were detected in both Ada'a and Boset districts. However, *M. expansa* wasn't recorded in Ada'a district. *Nematodirus* spp.,

Fasciola spp., and *Paramphistomum* spp. were not recorded in Boset district (Figure 12B).



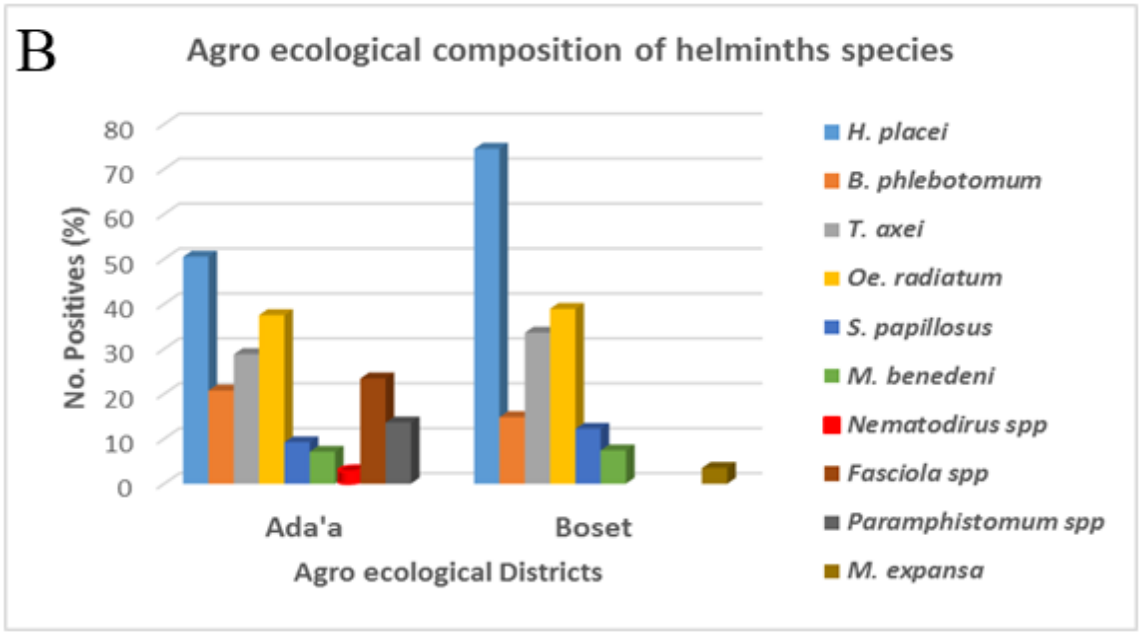


Figure 12. Seasonal pattern (A) and species composition (B) of helminths in cattle in Ada'a and Boset districts

4.4. Coproculture

4.4.1. Nematode third stage larvae (L_3)

H. placei, T. axei, Oe. radiatum and *B. phlebotomum* were recovered and identified from coproculture of 366 faecal samples positive for strongyle type eggs. Third stage larvae (L_3) of *H. placei* (Figure 13) were identified in 264 (73.3 %) of the examined cultures. L_3 of *T. axei* (Figure 14 and 17) were identified in 130 (36.1 %) cultures. L_3 of *Oe. radiatum* (Figure 15 and 17) were recovered in 158 (43.9 %) cultures. L_3 of *B. phlebotomum* (Figure 16) were recovered in 72 (19.7%) (Table 12).

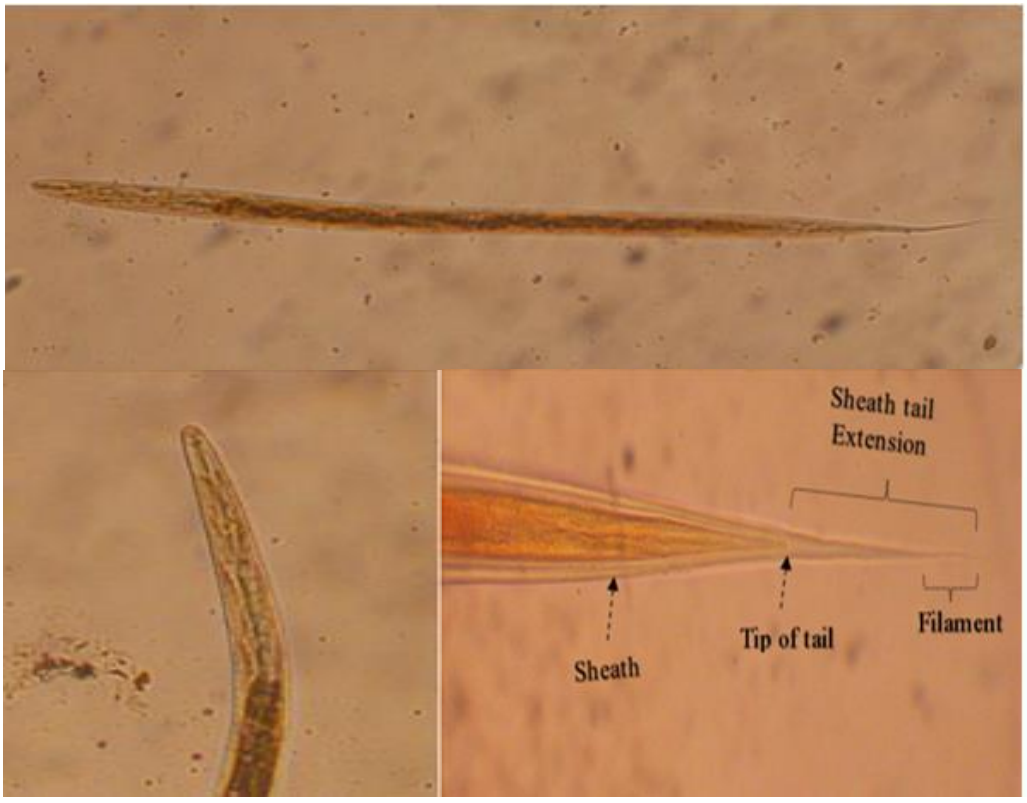


Figure 13. Photograph of *Haemonchus placei* third stage larva under 10x objective lens (top); Bullet shaped cranial end (bottom left), caudal end with moderate length of sheath tail extension ends in a relatively sharp point containing filament (bottom right)

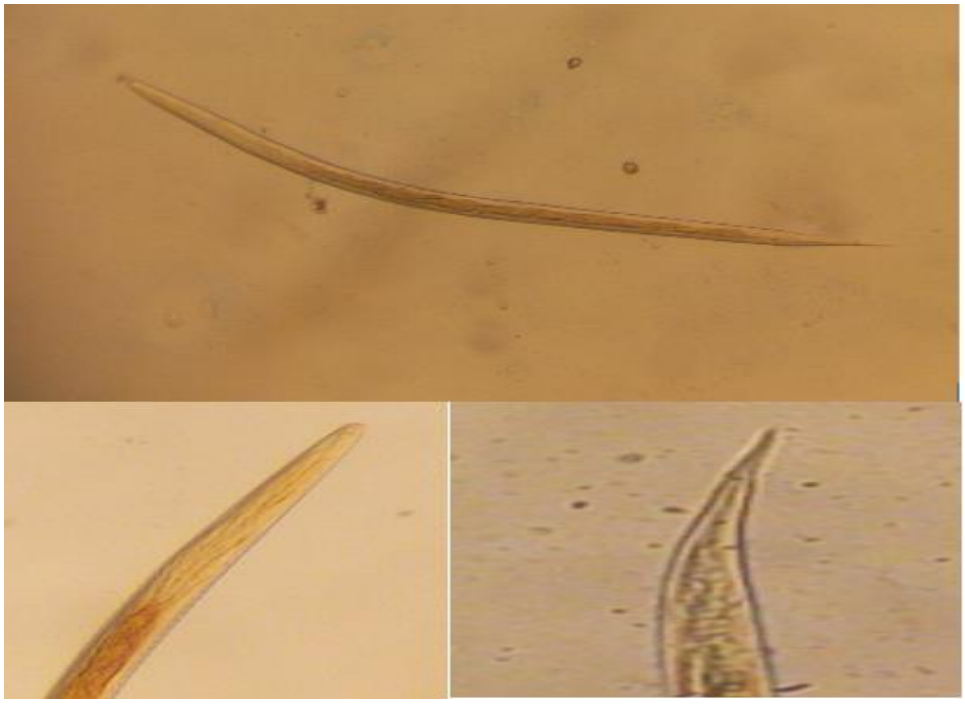


Figure 14. Photograph of *Trichostrongylus axei* third stage larva; Cranial end with no shoulder (bottom left), Caudal end without filament (bottom right)

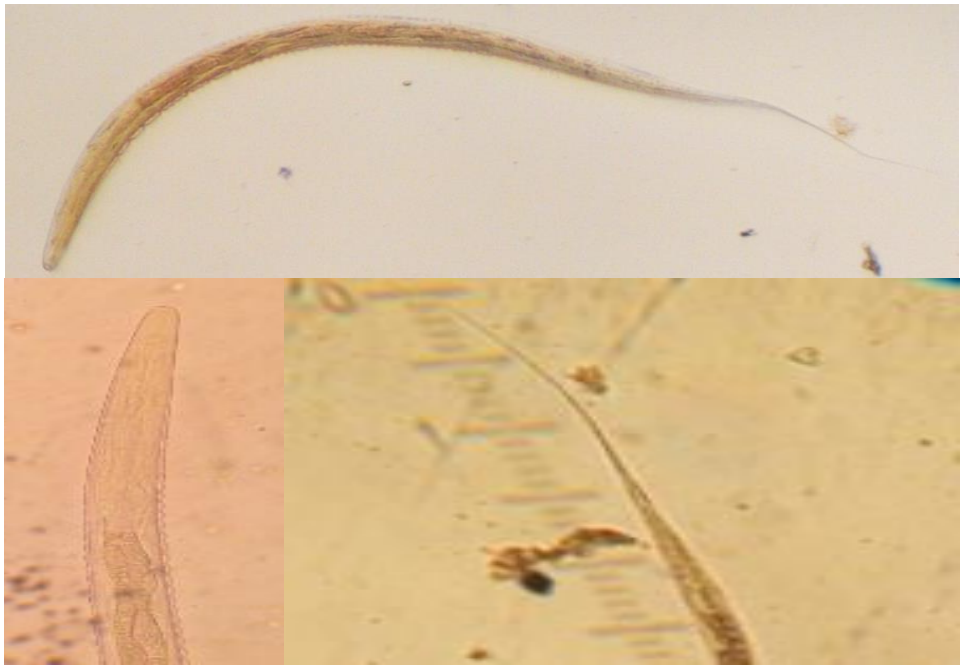


Figure 15. Photograph of *Oesophagostomum radiatum* third stage larva; broad cranial end (bottom left), caudal end with long filament (bottom right)

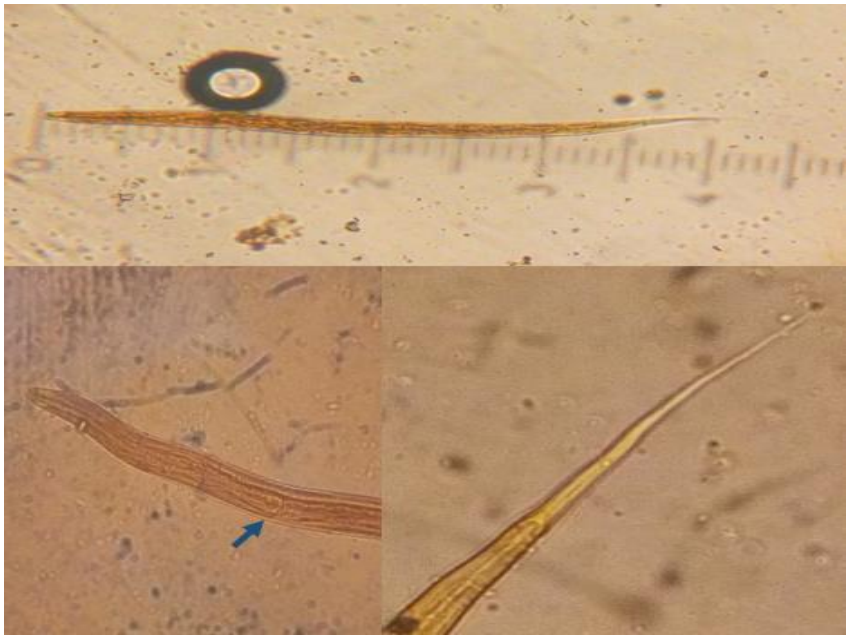


Figure 16. Photograph of *Bunostomum phlebotomum* third stage larva (516 µm) under calibrated microscope (top); cranial end (bottom left) with single caudal bulb (arrow), caudal end with filament (bottom right)

Figure 17. Micrometric measurement of nematode L_3 under calibrated objective lens (10x); STE (180 µm) of *Oe. radiatum* (top), length of *T. axei* (864 µm) (bottom).

Table 11. Summary of measurements for nematodes L_3 recovered from faecal cultures in cattle.

L_3 spp.	**No. L_3 (N=5135)**	**Overall Length (µm)**		**STE**		**X-value**		**Filament (%)**
		Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**	
H. placei	2563	728-865	826	80-109	87	2.7-3.6	2.9	20
T. axei	1342	624-792	721	25-38	32	0.8-1.3	1.1	0
Oe. radiatum	709	756-864	824	132-180	164	4.4-6.0	5.5	45-50
B. phlebotomum	521	501-600	555	60-84	71	2.0-2.8	2.4	50

L_3 = third stage larva of nematodes, N = number of larvae, STE = Sheath tail extension

Haemonchus placei and *Oesophagostomum radiatum* were identified as the predominant L_3 in highland PAs of Ada'a and lowland Boset districts. *Trichostrongylus axei* and *Bunostomum phlebotomum* L_3 were the least frequently recorded in both districts. The overall proportion of *H. placei* positive samples in Boset district were higher than the proportion of *H. placei* in Ada'a district. However, higher proportion of *Oesophagostomum radiatum, Trichostrongylus axei* and *Bunostomum phlebotomum* were recorded in Ada'a district than in Boset district. The overall proportion of *Haemonchus placei* was higher during the dry season (86.0%) than the proportion during the short rainy (68.7%) and rainy seasons (66.2%). *Bunostomum phlebotomum* with overall proportion of (19.7%) was the least recorded nematode L_3 than all the other L_3 of nematodes (Table 12).

Table 12. Frequency and percentages of nematode L_3 from coprocultures during different seasons in Ada'a and Boset districts

Season	District	Number of cultures	No. positive (%)			
			H. placei	***T. axei***	***B. phlebotomum***	***Oe. radiatum***
Rainy	Ada'a	52	38 (73.1)	19 (38.5)	16 (30.8)	22 (42.3)
	Boset	93	58 (62.4)	40 (43.0)	13 (14.0)	53 (57.0)
	Total	**145**	**96 (66.2)**	**59 (40.7)**	**29 (20.0)**	**75 (51.7)**
Dry	Ada'a	61	20 (32.8)	13 (21.3)	7 (11.5)	9 (14.7)
	Boset	62	60 (96.8)	14 (25.6)	4 (6.4)	15 (24.2)
	Total	**93**	**80 (86.0)**	**27 (29.0)**	**11 (11.8)**	**24 (25.8)**
Short rainy	Ada'a	60	35 (57.3)	38 (63.3)	15 (25.0)	21 (35.0)
	Boset	68	53 (77.9)	23 (33.8)	17 (25.0)	21 (30.9)
	Total	**128**	**88 (68.7)**	**61 (47.6)**	**32 (25.0)**	**42 (32.8)**
Over all	Ada'a	143	93 (65.0)	53 (37.1)	38 (26.6)	69 (48.3)
	Boset	223	171 (76.9)	77 (34.5)	34 (15.2)	89 (39.9)
	Total	**366**	**264 (72.1)**	**130 (35.5)**	**72 (19.7)**	**158 (43.2)**

5. DISCUSSION

5.1. Helminths Diversity, Agro-ecological and Seasonal Dynamics

Parasitic helminths are responsible for huge problems in health, reproductivity and productivity of cattle in Ethiopia (Hailu *et al*., 2011; Jelalu and Yitagele, 2013; Balcha and Haftu, 2014; Bedasa *et al*., 2016; Yimer *et al*., 2015). Helminths usually cause loss in body weight, digestive disturbance and emaciation (Ekong *et al*., 2012; Taylor, 2014; Johannes *et al*., 2016). The findings of this study, an overall of 57.4% positive for helminths in cattle, suggest that helminths are still a major problem of cattle in Ada'a and Boset districts. This observation is higher than 50.2% reported in Western Oromia (Regassa *et al*., 2006), 49% in West Arsi zone (Bacha and Haftu, 2014) and 39.6% in Southern Ethiopia (Jelalu and Yitagele, 2013). But the present finding is lower than 68.2% and 77.6% report by Bedasa *et al*., (2016) and Hailu *et al*. (2011) in Jimma and Holleta dairy farms of Agricultural research center, respectively.

The observation of very high overall percentage of nematodes (44.9%) than all the other classes trematodes (4.2%), cestodes (1.3%) and mixed class positive cattle 51 (7.1%) in the present study is not in line with the previous report of 42.3%, 5.2 %, 71.9% and 19.7% nematode, trematode and mixed infections respectively from Jimma by Hailu *et al*. (2011) and the previous report of trematodes as the most prevalent helminths in studies carried out in India, eastern Nigeria and Thailand respectively by Mir *et al*. (2013), Nwigwe *et al*. (2013) and Chonlawit *et al*. (2014). These differences could be associated with the variations in geographical, climatic and agro ecological conditions.

The observation of 50.8% strongyle type eggs in cattle in the present study is in agreement with the previous report of 56% in and around Gonder (Tigist *et al*., 2012) and 52.3% in Gursum Woreda of Eastern Hararghe Ethiopia (Abdurezak *et al*., 2015).

However, lower percentages by previous investigators including 32.4% from Jimma (Hailu *et al*., 2011); 4.8% in and around Ambo (Dinka *et al*., 2011); 22.9% in Gedebano Gutazer Wolene district, Southern Ethiopia (Jelalu and Yitagele, 2013); 41% in East Showa zone of Oromia regional state (Telila *et al*., 2014); 36.2% in West Hararghe Zone (Tulu and Lelisa, 2016) and 21.4% around Bahir Dar and Gozamen districts (Yeshwas, 2013) were reported. These variations could be attributed to differences in study seasons, age and management status of study cattle, climate and agro-ecology of study areas among different studies.

The findings of ten different species of helminthes (*H. placei*, *T. axei*, *Oe. radiatum*, *B. phlebotomum*, *S. papillosus*, *Nematodirus* spp, *M. benedeni*, *M. expansa*, *Fasciola* spp., and *Paramphistomum* spp.) of cattle in the present study is in agreement with previous reports by Hailu *et al*. (2011) in Jimma, Balcha and Haftu (2014) in West Arsi zone, Yimer *et al*. (2015) in Dire Dawa respectively. It is also in line with the previous report by Sultana *et al*. (2013), Muhammad *et al*. (2013), and Elele *et al*. (2013) in Bangladish, Pakistan, and Nigeria, respectively. This could be due to the similarity in climatic condition and grazing management of cattle. Waller (1997) stated that the species composition of helminths, their seasonal population dynamics and their importance for animal production is mainly dependent on the climate conditions of the area and the grazing management.

In present study the predominance of mixed helminths species (63.4%) than single helminth species (36.6%) in positive cattle was not in line with the previous report of the predominance of single helminth species than mixed infections by Marskole *et al*. (2016) and Olubukola *et al*. (2014). The possible explanation for this difference might be due to the variation in study methods and animals studied by researchers. The cited authors employed systematic random sampling method to identify helminths from slaughtered cattle but the present study was done by using purposively selected cattle grazing in a communal pasture land. Communal grazing pasture land has very great chance of

becoming contaminated by different species of infective larval stages of helminths which can contribute to a higher mixed infection (Morgan *et al*., 2013; Taylor, 2014; Lu and Judith, 2016).

The significantly ($p < 0.005$) higher overall percentage of positive cattle for helminths in lowland Boset district than the highland PAs of Ada'a district in the present study is in agreement with the previous report of significantly higher gastrointestinal parasite occurrence in lowland than high land agro-ecology by Regassa *et al*. (2006). The significantly higher distributions of helminths of cattle in highland PAs of Ada'a district than the lowland Boset district in this study is in line with the previous report of significant difference in helminthes between two agro-ecological zones in Western Hararghe by Tulu and Lelisa (2016). The lower percentages of trematodes (*Paramphistomum* and *Fasciola* spp.) in Ada'a district and their absence from Boset district in this study most probably attributed to the in availability of intermediate hosts to complete their life cycles due to unsuitable environmental conditions for the survival of intermediate hosts (*Lamnea* spp).

5.2. Seasonal Variation in Strongyle EPG Count

The findings of significantly higher EPG of strongyle type eggs during the rainy season than both during the dry season and short rainy season in the present study is consistent with the previous report of season as a determinant factor for strongyle EPG output by Keyyu *et al*. (2005), Jimenez (2007), Monica *et al*. (2008), Ibrahim *et al*. (2008), Getachew *et al*. (2008), Jelalu and Yitagele (2013), Takele *et al*. (2013) and Sultana *et al*. (2013). The most probable explanation for this finding could be due to decreased rate of egg production or due to death of some of the adult parasites during the dry season because of the unfavorable dry environmental conditions. It is also possible that the increased egg production of established parasites from arrested larval development during the dry season could contribute to this findings.

5.3. Nematode Infective Stage (L_3)

In the present study, the report of *Haemonchus placei* with 36.6% prevalence as the most abundant and common nematode species in cattle of highland PAs of Ada'a and lowland Boset district in Ethiopia is in agreement with previous report in Costa Rica by Jimenez *et al*. (2007). This is higher than the report of Jelalu *et al*. (2017), Adem and Anteneh (2011), Shirale *et al.* (2008), Balcha and Haftu (2015) who reported 10.9%, 11.6%, 6.57% and 11.7%, respectively. The second most prevalent species of nematode in this study was *Oesophagostomum radiatum* (21.9%) followed by *Trichostrongylus axei* (18.1%). This is in close agreement with previous reports (Jelalu, 2017; Adem and Anteneh, 2011) who found *Oesophagostomum radiatum* with 13.3% and 11% respectively. This variation could be due to climatic differences for transmission of the parasites, cattle management practices including feeding and watering systems. On the other hands, nematode infective larvae from coproculture of strongyle positive samples in this study dominated by *Haemonchus placei* (72.1%) and *Oesophagostomum radiatum* (43.2%) was in line with the report of (Belem *et al*., 2001) who found *Haemonchus* (66%) and *Oesophagostomum radiatum* (42.6%).

For the first time in Ethiopia, the present study identified the L_3 of *H. placei*, *T. axei*, *Oe. radiatum* and *Bunostomum phlebotomum* from cattle by measurement of total length of L_3, sheath tail extension and estimation of filament proportion by micrometer. The mean STE and length of *H. placei* L_3 (87µm, 826 µm), of *T. axei* (32µm, 721 µm), of *Oe. radiatum* (164µm, 824 µm) and *B. phlebotomum* (71µm, 555µm) is in agreement with previous studies conducted in other countries (Keith, 1953; Hansen and Shivnani, 1956; Borgsteede and Hendriks, 1974). Slight differences in minimum and maximum length of STE and other parameters of L_3 of nematode species reported could be attributed to the amount of moisture in the culture medium that could affect the morphology of larvae as has been reported by Rossanigo and Gruner (1996).

6. CONCLUSION AND RECOMMENDATIONS

The result of this study demonstrated high diversity of helminths species in cattle with very high overall prevalence of 57.4% suggesting that helminths are still great enough to compromise cattle productivity. The study identified ten different species of helminths including *Haemonchus placei*, *Trichostrongylus axei*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Fasciola* spp., and *Paramphistomum* spp. Helminths spp. recorded in the present study such as *Haemonchus placei*, *Oesophagostomum radiatum* and *Bunostomum phlebotomum* are already implicated as most pathogenic and cause huge economic losses in cattle around the world. *Haemonchus placei* 264 (36.7%), *Trichostrongylus axei* 130 (18.0%) and *Oesophagostomum radiatum* 158 (21.9%) were identified as the predominant nematodes affecting cattle of the study districts. Higher percentage and EPG of strongyle type nematode eggs were more common in cattle during the rainy season than during the dry season of the year. This predominance of helminths with great species diversity affecting cattle during all seasons in both highland and lowland agro-ecology warrant urgent attention at all levels.

Therefore, based on the findings of the present study and the above conclusion the following points are recommended:

- Young cattle should receive great attention to minimize pasture contamination with worm egg and to reduce the negative impacts of helminthosis on cattle production in the area.
- Parasite control practices need to be implemented by strategic treatment approach considering the seasonal dynamicity of helminths.
- Detail studies employing molecular methods and further investigation by including wide geographical areas to elucidate the epidemiological information on circulating helminths species and potential risk factors is mandatory.

7. REFERENCES

Abdurezak, M., Haimanot, D., Tadele, K., Tilahun, Z. and Girma, K. (2015). Prevalence of Gastrointestinal Nematodes of Sheep in Gursum Woreda of Eastern Hararghe Zone, Oromia Regional State, Ethiopia. *Researcher,* **7** (8).

Adedipe, O.D., Uwalaka, E.C., Akinseye, V.O., Adediran, O.A. and Cadmus, S.I. (2014). Gastrointestinal Helminths in Slaughtered Cattle in Ibadan, South-Western Nigeria. *Journal of Veterinary Medicine*, Article ID 923561, 6 pages, doi:10.1155/2014/923 561.

Akkari, H., Gharbi, M. and Darghouth, M. (2012). Dynamics of infestation of tracers lambs by gastrointestinal helminths under a traditional management system in the North of Tunisia. *Parasite,* **9**(4): 407-15.

Aksoy, D. Y., Kerimoglu, U., Oto, A., Erguven, S., Arslan, S., Unal, S., Batman, F. and Bayraktar, Y. (2005). Infection with Fasciola hepatica. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases,* **11**(11), 859-861.

Anne, M. Z. and Gary, A. C. (2012). Veterinary Clinical Parasitology. 8th Edition. Published by John Wiley & Sons, Inc. Wiley-Blackwell, Uk. Pp. 96-113.

Asresie, A. and Zemedu, L. (2015). Contribution of Livestock Sector in Ethiopian Economy: A Review. *Advances in Life Science and Technology, **29***:2225-3062.

Ayele, S., Assegid, W., Jabbar, M., Ahmed, M. and Belachew, H. (2003). Livestock marketing in Ethiopia: A review of structure, performance and development initiatives. *Socio-economics and Policy Research Working Paper 52*. ILRI, Nairobi, Kenya. pp 35.

Balcha, A. and Haftu, B. (2014). Study on Prevalence of Gastrointestinal Nematodes and Coccidian Parasites Affecting Cattle in West Arsi zone, Ormia Regional State, Ethiopia. *Journal of Veterinary Science and Technology,* **5**:5.

Bedasa, C., Getachow, M., Duguma, A. and Abera, S. (2016). Status of Helminthes Parasites of Cattle in Dairy Farms of Holleta Agricultural Research Center, Central Ethiopia. *Journal of Veterinary Science & Technology*, **7**(5): 365.

Belem, A.M., Ouedraogo, O.P. and Bessin, R. (2001). Gastro-intestinal Nematodes and Cestodes of Cattle in Burkina Faso. *Biotechnology, Agronomy, Society and Environment*, **5**(1): 17-21.

Borgsteede, F. and Hendriks, J. (1974). Identification of infective larvae of gastrointestinal nematodes in cattle. *Tijdschrift Diergeneeskunde, **99**:103-113.*

Borovkov, K., Day, R. and Rice, T. (2013). High host density favors greater virulence: a model of parasite–host dynamics based on multi-type branching processes. *Journal of Mathematical Biology*, **66**: 1123.

Bowman, D. (2012). Heartworms, macrocyclic lactones, and the specter of resistance to prevention in the United States, *Parasites Vectors* **5**:138.

Bowman, D. (2014). Georgis' Parasitology for Veterinarians. 10th Edition. Saunders, an imprint of Elsevier Inc. Elsevier's Health Sciences Rights Department in Philadelphia, PA, USA. pp. 122-230.

Central Statistical Authority (CSA, 2013). Agricultural sample survey. Report on livestock and livestock characteristics (private peasant holdings). Addis Ababa, Federal Democratic Republic of Ethiopia. Pp. 9-20.

Central Statistical Authority (CSA, 2016). Agricultural sample survey report on livestock and livestock characteristics, *Volume II*, Addis Ababa, Ethiopia.

Chaparro, J., Ramirez, N., Villar, D., Fernandez, J., Londono, J., Arbelaez, C., Lopez, L., Aristizabal, M., Badel, J., Palacio, L. Olivera, M. (2016). Survey of gastrointestinal parasites, liver flukes and lungworm in feces from dairy cattle in the high tropics of Antioquia, Colombia. *Parasite Epidemiology and Control;* **1**(1): 124-130.

Charlier, J., Vercruysse, J., Morgan, E., van Dijk, J., Williams, D. J. (2014). Recent advances in the diagnosis, impact on production and prediction of *Fasciola hepatica* in cattle. *Parasitology;* **141**(3): 326-35.

Chonlawit, Y., Suttipong, P., Tipabhon, S. (2014). Prevalence of gastro-intestinal parasites of cattle in Udon Thani, *Thailand. Khon kaen agricultural Journal*, **42**: 4.

Colin, J., Jorge, G., Sonja, C., Melissa, H., Russell, H. and Aimee, E. (2001). Parasites and parasitic diseases of domestic animals. The Merck Company Foundation. The University of Pennsylvania, Philadelphia, USA. Pp 30-78.

Cotter, J., Van Burgel, A.and Besier, R. (2015). Anthelmintic resistance in nematodes of beef cattle in south-west Western Australia. *Veterinary Parasitology,* **207**(3-4):276-284.

Das, M., Deka, D., Islam, S., Sarmah, P. and Bhattacharjee, K. (2016). Gastrointestinal n ematode larvae in the grazing land of cattle in Guwahati, Assam. *Veterinary World;* **9**(12): 1343-1347.

Dinka, A, Bedada, B. and Yacob, H. (2011). Study on Major Parasitic Problems of Rural Cattle in and Around Ambo, Western Oromia, Ethiopia. *Nigerian Veterinary Journal*, **31**(3): 208- 213.

Eckert, J. (1960). Diagnosis of gastro-intestinal worms in sheep by differentiation of the free-living third-stage larvae. *Journal of veterinary medicine,* **7:** 612-630.

Ekong, P. Juryit, R. Dika, N. Nguku, P. and Musenero, M. (2012). Prevalence and risk factors for zoonotic helminth infection among humans and animals, Jos Nigeria, 2005-2009. *The Pan African Medical Journal*, vol. 12, article 6.

Elele, K. Owhoeli, O. and Gboeloh, L. (2013). Prevalence of species of helminths parasites in cattle slaughtered in selected abattoirs in Port Harcourt, south-south, Nigeria," *International Research on Medical Sciences,* **1**(2):10-17.

Florian, R., Aaron, R. and Robin, B. (2013). Next-Generation Molecular Diagnostic Tools for Gastrointestinal Nematodes of Livestock with an Emphasis on Small Ruminants: A Turning Point? *Advances in Parasitology*, **83**:267-333.

Getachew, M., Feseha, G., Trawford, A. and Reid, S. (2008). A survey of seasonal patterns in strongyle faecal worm egg counts of working equids of the central midlands and lowlands, Ethiopia. *Tropical Animal Health and Production*, **40**(8): 637-642.

Geurden, T., Chartier, C., Fanke, J., Di-Regalbono, A., Traversa, D., von Samson-Himmelstjerna, G., Demeler, J., Vanimisetti, H., Bartram, D., Denwood, M. (2015). Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in Europe. *International Journal of Parasitology: Drugs Drug Resistance*, **5**:163-171.

Giuseppe, C., Laura, R., Maria, P. and Jurg, U. (2010). FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature protocols,* **5**(3): 503-515.

Hailu, D., Cherenet, A., Moti, Y. and Tadele, T. (2011). Gastrointestinal Helminth Infections in Small-scale Dairy Cattle Farms of Jimma Town, Ethiopia. *Ethiopian Journal of Applied Science and Technology*, **2**(1).

Hansen, M.F., Shivnani, G.A., 1956. Comparative morphology of infective nematode larvae of Kansas beef cattle and its use in estimating incidence of nematodiasis in cattle. *Transactions of the American Microscopical Society.* **75**: 91-102.

Holzhauer, M., van Schaik, G., Saatkamp, H. W., Ploeger, H. W. (2011*).* Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. *The veterinary record,* **169**(19): 494.

Horak, I., Evans, U. and Purnell, R. (2004). Parasites of domestic and wild animals in South Africa. XLV. Helminths of dairy calves on dry-land Kikuyu grass pastures in the Eastern Cape Province. *Onderstepoort Journal of Veterinary Research;* **71**(4): 291-306.

Ibrahim, M.M., Al Ghamdi, M.A., Al Gahmdi, M.S. (2008). Helminths Community of Veterinary Importance of Livestock in Relation to Some Ecological and Biological Factors. *Turkish Society for Parasitology,* **32** (1): 42-47.

Jelalu, K. and Yitagele, T. (2013). Prevalence of gastrointestinal parasitism of cattle in Gedebano Gutazer Wolene district, Ethiopia. *Journal of Veterinary Medicine and Animal Health,* **5**(12):365-370.

Jelalu, K., Yimer, M. and Adem, H. (2017). Major gastrointestinal nematodes of cattle in dairy farms in Dire Dawa administration, eastern Ethiopia. *Livestock Research for Rural Development*, **29** (1).

Jimenez, A.E., Montenegro, V.M., Hernandez, J., Dolz, G., Maranda, L., Galindo, J., Epe, C. and Schnieder, T. (2007). Dynamics of infections with gastrointestinal parasites and *Dictyocaulus viviparus* in dairy and beef cattle from Costa Rica. *Veterinary Parasitology*, **148**: 262-271.

Joan, L., Joe, B. and Stephen, L. (2007). Stomach fluke (paramphistomes) in ruminants. NSW department of primary industries, Prime facts 452. 5M publishing, Chicago, USA. At *http:// www.thedairysite.com*. Accessed on August, 2016.

Johannes, C., Valerie, D.W., Els, D., Mariska, V., Fiona, Vande, V. and Edwin, C. (2016). Decision making on helminths in cattle: diagnostics, economics and human behaviour. *Irish Veterinary Journal,* **69**:14.

Jorgen, H. and Brian, P. (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants. A Handbook. Published by the International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

Junquera, P. (2015). Parasites of Dogs, Cats, Horses & Livestock: Biology & Control. *http:// parasitipedia.net.* Accessed on August, 2016.

Keith, R.K. (1953). The differentiation of the infective larvae of some common nematode parasites of cattle. *Australian Journal of Zoology*, **1**: 223-235.

Keyyu, J., Kassuku, A., Msaliwa, L., Monrad, J. and Kyvsgaard, N. (2006). Cross-sectional prevalence of helminth infections in cattle of traditional, small scale and large scale dairy farms in Iringa district, Tanzania. *Veterinary Research Communications*, **30**: 45-55.

Keyyu, J.D., Kyvsgaardc, N.C., Monradd, J. and Kassuku, A.A. (2005). Epidemiology of gastrointestinal nematodes in cattle on traditional, small-scale dairy and large-scale dairy farms in Iringa district, Tanzania. *Veterinary Parasitology,* **127**: 285-294.

Knapp-Lawitzke, F., von Samson-Himmelstjerna, G. and Demeler, J. (2016). Elevated temperatures and long drought periods have a negative impact on survival and

fitness of strongylid third stage larvae. *International Journal of Parasitology*, **46**(4): 229-37.

Kumsa, B., Tadesse, T., Sari, T., Duguma, R. and Hussen, B. (2011). Helminths of sheep and goats in Central Oromia, Ethiopia during the dry season. *Journal of Animal and Veterinary Advances*, **10**(14): 1845-1849.

Leathwick, D. M. (2013). The influence of temperature on the development and survival of the pre-infective free-living stages of nematode parasites of sheep. *The New Zealand Veterinary Journal;* **61**(1): 32-40.

Levecke, B., Dobson, R., Speybroeck, N., Vercruysse, J., Charlier, J. (2012). Novel insights in the faecal egg count reduction test for monitoring drug efficacy against gastrointestinal nematodes of veterinary importance. *Veterinary Parasitology,* **188**: 391-396.

Love, J., Kelly, L., Lester, H., Nanjiani, I., Taylor, M. and Robertson, C. (2017). Investigating anthelmintic efficacy against gastrointestinal nematodes in cattle by considering appropriate probability distributions for faecal egg count data. *International Journal for Parasitology: Drugs and Drug Resistance,* **7**(1): 71-82.

Lu, H. and Judith, A. (2016). Eosinophils in Helminth Infection: Defenders and Dupes. *Trends in parasitology.* **32**(10): 798-807.

Lyndal-Murphy, M., Swain, A., Pepper, P. (2014). Methods to determine resistance to anthelmintics when continuing larval development occurs. *Veterinary Parasitology*, **199**: 191-200.

MAFF (1997). Manual of Veterinary Parasitology Laboratory Techniques. *Technical Veterinaria*., **12**:121-129. Bulletin, London No 18.

Magaya, A., Mukaratirwa, S., Mutisi, C., Kyvsgaard, N. & Thamsborg, S. (2000). Effects of fenbendazole slow-release (sr) bolus on productivity of indigenous cattle on communal pasture in Sanyati area, Zimbabwe', *Zimbabwe Veterinary Journal*. **31**: 29-40.

Marskole, P., Verma, Y., Dixit, A. and Swamy, M. (2016). Prevalence and burden of gastrointestinal parasites in cattle and buffaloes in Jabalpur, India. *Veteterinary World,* **9**(11): 1214-1217.

Matthews, J. B. (2014). Anthelmintic resistance in equine nematodes. *International Journal for Parasitology: Drugs and Drug Resistance*, **4**(3): 310-315.

Maurya V.P., Sejian V. and Naqvi S. (2009). Body condition scoring system-a strategic tool for optimizing productivity in animal farm. *Indian Farming,* **58**: 28-33.

McGavin, M. and Zachary, J (2007). Pathologic Basis of Veterinary Disease, 4th ed. *Canadian Veterinary Journal*; **48**(7): 724.

Meradi, S., Cabaret, J. and Bentounsi, B. (2016). Arrested development of abomasal trichostrongylid nematodes in lambs in a steppe environment (North-Eastern Algeria). *Parasite*, **23**: 39.

Metaferia, F., Cherenet, T., Gelan, A., Abnet, F., Tesfay, A., Ali, J. and Gulilat, W. (2011). Review to Improve Estimation of Livestock Contribution to the National GDP. Ministry of Finance and Economic Development and Ministry of Agriculture, Addis Ababa, Ethiopia. *IGAD Livestock Policy Initiative.* pp. 5-40.

Mir, M.R., Chishti, M.Z., Rashid *et al*. (2013). Point prevalence of gastrointestinal helminthiasis in large ruminants of Jammu, India. *International Journal of Scientific and Research Publications*, **3**(3): 5-8.

Monica, N.W., Koniba, T., Bassirou, B., Saidou, T., Sekouba, T. and Jakob, Z. (2008). Gastrointestinal parasite egg excretion in young calves in periurban livestock production in Mali. *Research in Veterinary Science,* 84:225-231.

Morgan, E., Charlier, J., Hendrickx, G., Biggeri, A., Catalan, D., von Samson-Himmelstjerna, G. and Demeler, J., *et al*. (2013). Global Change and Helminth Infections in Grazing Ruminants: Impacts, Trends and Sustainable Solutions. *Agriculture,* **3**: 484-502.

Muhammad, A.R., Muhammad, M.A., Saeed, M., Muhammad, S.A., Muhammad, N., Muhammad, Ali. and Hafiz, A.B. (2013). Prevalence of GIT helminths in cattle at the vicinities of tehsil jatoi, punjab, pakistan. *Science International,* **25**(2): 305-309.

Muhammad, K., Muhammad, S., Muhammad N., Zafar, I. and Muhammad, U. (2009). Bovine fasciolosis: Prevalence, effects of treatment on productivity and cost benefit analysis in five districts of Punjab, Pakistan. *Research in Veterinary Science*, **87**:70-75.

Murray, K. (2004). Intestinal Roundworms of Cattle. Practical information for Alberta's Agriculture Industry. Agri-Facts: Food Safety Division. Alberta Agriculture and Food. USA.

Naomi, J. F., Glenn, M., Ross, S. D. and Piran, C. L (2012). White and Michael R. Hutchings Livestock Helminths in a Changing Climate: Approaches and Restrictions to Meaningful Predictions. *Animals,* **2**: 93-107.

Nicholson, M.J. and Butterworth M.H. (1986). A guide to condition scoring of Zebu cattle. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia. pp. 1-29.

Nwigwe, J.O., Njoku, O.O., Odikamnoro, O.O. and Uhuo, C.A. (2013). Comparative study of intestinal helminths and protozoa of cattle and goats in Abakaliki metropolis of Ebonyi State, Nigeria. *Advances in Applied Science Research*, 4(2): 223-227.

Nzalawahe, J., Kassuku, A., Stothard, J., Coles, G. and Eisler, M. (2015). Associations between trematode infections in cattle and freshwater snails in highland and lowland areas of Iringa Rural District, Tanzania. *Parasitology*. **142**(11): 1430-1439.

Olubukola, D.A., Emmanuel, C.U., Victor, O.A., Oyeduntan, A.A. and Simeon, I.B. (2014). Gastrointestinal Helminths in Slaughtered Cattle in Ibadan, South-Western Nigeria. *Journal of Veterinary Medicine,* Article ID 923561, 6 pages,. doi:10.1155/2014/923561

Over, H., Jansen, J. and van Olm, P. (1992). Distribution and impact of helminth diseases of livestock in developing countries. *In: FAO Corporate Document Repository-Agriculture and Consumer Protection*.

Papadopoulos, E., Gallidis, E. and Ptochos, S. (2012). Anthelmintic resistance in sheep in Europe: A selected review. *Veterinary Parasitology,* **189**: 85–88.

Pfukenyi, D. and Mukaratirwa, S. (2013). A Review of the Epidemiology and Control of Gastrointestinal Nematode Infections in Cattle in Zimbabwe *Onderstepoort Journal of Veterinary Research*, **80**(1): 612.

Philipp, B., Joachim, V., Degnet, A. and Marc, M. (2015). Impacts of Large-scale Land Investments on Income, Prices, and Employment: Empirical Analyses in Ethiopia. *World Development,* **72**: 175-190.

Phiri, A., Phiri, I. and Monrad, J. (2006a). Prevalence of amphistomiasis and its association with Fasciola gigantica infections in Zambian cattle from communal grazing areas. *Journal of Helminthology*, **80**: 65-68.

Regassa, F., Sori, T., Dhuguma, R., Kiros, Y. (2006). Epidemiology of Gastrointestinal Parasites of Ruminants in Western Oromia, Ethiopia. *International Journal of Applied Research in Veterinary Medicine*, **4**: 51-57.

Rossanigo, C.E., Gruner, L. (1996). The length of strongylid nematode infective larvae as a reflection of developmental conditions in faeces and consequence on their viability. *Parasitology Research*, **82**, 304-311.

Soulsby, E. (1982). Helminths, arthropods and protozoa of domesticated animals. William Clowes Ltd. London. 7th ed., pp.136-346.

Sultana, M. R., Sarder, M. J., Mostofa, M., Islam, M. H. and Nahar, L. (2013). Incidence on gastro-intestinal nematodes of cattle in Rajshahi district and anthelmintic efficacy of some indigenous medicinal plants against gastro-intestinal nematodes of cattle in vitro and in vivo. *Reviews of Progress.* ***1(***10): 2321-3485.

Susan, E., Michael, A., Dana, G., Peter, D., Andrew, D., Peter, R., Katherine, E., Philip, T., Jagdev, M. (2014). Overview of Gastrointestinal Parasites of Ruminants. The Merck Veterinary Manual Reference Guide. Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, N.J., USA.

Sutherland, I. and Scott, I. (2010). Gastrointestinal Nematodes of Sheep and Cattle: Biology and Control. 1st ed. Blackwell Publishing, UK.

Takele, S.A., Yacob, H.T. and Getachew T. (2013). Epidemiology of gastrointestinal nematodes of Horro sheep in Western Oromia, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **5**(10): 296-304

Tankeshwar, A. (2013). Laboratory diagnosis of intestinal helminthic infections (infestati ons). In: *lab diagnosis of parasitic disease, parasitology note*. *http://microbeonline.com*, Accessed on September, 2016.

Taylor, M. (2014). Control of parasitic gastroenteritis in cattle. COWS Manual. A Technical Manual aiming to provide a sound basis for advice to industry. Eblex and Dairy Co., UK.

Telila, C., Abera, B., Lemma, D. and Eticha, E. (2014). Prevalence of gastrointestinal parasitism of cattle in East Showa Zone, Oromia Regional State, Central Ethiopia. *Journal of veterinary Medicine and Animal Health*, **6**(2): 54-62.

Tesfaye, T. (2009). Characterization of goat production systems and on- farm evaluation of the growth performance of Grazing goats supplemented with different protein Sources in metema woreda, Amhara region, Ethiopia. MSc Thesis presented to the Department of Animal Science, School of Graduate Studies, Haramaya University, Haramaya, Ethiopia.

Tigist, A., Basaznew, B. and Mersha, C. (2012). Occurrence of Gastro Intestinal Nematodes of Cattle in and Around Gondar Town, Amhara Regional State, Ethiopia. *Acta Parasitologica Globalis*, **3**(2): 28-33.

Tulu, D. and Lelisa, K. (2016). A Study on Major Gastro-Intestinal Helminths Parasites of cattle in Tulo District, West Hararghe Zone, South-Eastern Ethiopia. *Austin Journal of Veterinary Science & Animal Husbandry*, **3**(2): 1027.

Urquhart, G., Armour, J., Duncan, J., Dunn, A. and Jennings, F. (2001). Veterinary Parasitology, 2nd ed., Blackwell Science Ltd, UK. pp. 3-99.

Van Dijk, J. and Morgan, E. R. (2010). Variation in the hatching behavior of *Nematodirus battus*: polymorphic bet hedging? *International Journal of. Parasitology*, **40**: 675-681.

Van Dijk, J. and Morgan, E.R. (2012). The influence of water and humidity on the hatching of Nematodirus battus eggs. *Journal of Helminthology, 86, 287–292.*

Van Dijk, J., Sargison, N., Kenyon, F. and Skuce, P. (2010). Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. *Animal* 4(3): 377-392.

Van Wyk, J. and Mayhew, E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide, *Onderstepoort Journal of Veterinary Research;* **80**(1), 1-14.

Van Wyk, J., Cabaret, J. and Michael, L. (2004). Morphological identification of nematodes of small ruminants and cattle simplified, *Veterinary Parasitology,* **119**: 277-306.

Verschave, S. H., Charlier, J., Rose, H., Claerebout, E. and Morgan, E. R. (2016). Cattle and Nematodes under Global Change: Transmission Models as an Ally. *Trends in parasitology*, **32**(9): 724-738.

Waller, P.J. (1997). Anthelmintic resistance. *Veterinary Parasitology*, **72**: 391-412.

Wang, C., Qiu, J., Zhao, J., Xu, L., Yu, W., and Zhu, X. (2006). Prevalence of helminthes in adult dogs in Heilongjiang Province, the People's Republic of China. *Parasitology Research*, **99**(5): 627-630.

Williams, J. and Loyacano, A. (2001). Internal parasites of cattle in Louisiana and other Southern states. Louisiana State University Agricultural Center, USA.

WMO (2013). Workshop on Climate Monitoring including the Implementation of a Climate Watch System in RA I with focus on eastern and southern Africa. WCDMP-No. 81, Pretoria, South Africa.

Yeshwas, F. (2013). Epidemiology of gastrointestinal helminthiasis of crossbred calves in selected sites of Bahir Dar Zuria and Gozamen districts of Amhara region, North West Ethiopia. *International Journal of Pharmacy, Medicine and Biological Science*, **2**(2):2278-5221.

Yimer, M., Dinaol, B., Mesfin, A., Solomon, S. and Hassen, B. (2015). Prevalence of Gastrointestinal Nematode of Cattle in Selected Kebeles of Dire Dawa Districts Eastern Ethiopia. *Advances in Biological Research,* **9**(6): 418-423.

Zintl, A., Garcia-Campos, A., Trudgett, A., Chryssafidis, A., Talavera-Arce, S., Fu, Y., Egan, S., Lawlor, A., Negredo, C., Brennan, G., Hanna, R., De Waal, T. and Mulcahy, G. (2014). Bovine Paramphistomes in Ireland. *Veterinary Parasitology*, **204**(3-4): 199-208.

8. APPENDICES

Appendix 1. Formulation of floatation fluid and Iodine Stain

Floatation fluid

- Sodium chloride (salt) …….400 gm
- Water ……...1000 ml
- Specific gravity: 1.2

Iodine stain

- Iodine re-sublimed crystals……..10 gm
- Potassium iodide ……...50 gm
- Water ……1000 ml
- Dissolve the potassium iodine in the water. Then add and dissolve the iodine crystals.

Appendix 2. Simple floatation technique procedure

- Mix 4 grams of faeces with the flotation solution in a cup.
- Strain the mixture through a tea strainer or cheesecloth.
- Pour into a test tube until there is a reverse meniscus on the top of the container.
- Place a coverslip on the fluid drop at the top.
- Allow it to stand for at least 10 minutes
- Remove the coverslip, place it on a slide, and examine.
- If the test is allowed to stand for too long, the salt may crystallize on the edges of the coverslip so that it will not lie flat on the slide.

Appendix 3. Sedimentation technique procedure

- Mix approximately 3 gm of faeces with 40-50 ml of tap water.
- Filter the suspension through a tea strainer and pour it into a test tube.
- Allow to sediment for 5 minutes.

- Remove the supernatant very carefully.
- Re- suspend the sediment in 5 ml water and allow to sediment for 5minutes
- Discard the supernatant carefully.
- Stain the sediment by adding one drop of methylene blue or malachite green.
- Transfer a small drop of the stained sediment to a microscope slide using a pipette.
- Cover droplet with a coverslip.
- Examine under a microscope at 10 x 10 magnification

Appendix 4. McMaster egg counting technique procedure

- Mix 4 gm of faeces with 56 ml of flotation solution to yield a total volume of 60 ml
- Mix well and strain through a tea strainer or cheesecloth. The mixture does not have to be strained, but it will be much easier to read the slide if large pieces of debris are removed.
- Immediately fill each chamber of the McMaster slide with the mixture using a pipette or syringe. The entire chamber must be filled, not just the area under the grid. If large air bubbles are present, remove the fluid and refill.
- Allow the slide to sit for at least 5 minutes to allow the flotation process.
- Look at the slide with the 10x lens, focusing on the top layer, which contains the air bubbles. At this level, the lines of the grid will also be in focus. Count eggs in each line of both chambers. Each type of parasite should be counted separately. In some cases, eggs can be identified to genus or perhaps to species (e.g., *Strongyloides*, *Trichuris*, and *Nematodirus*), whereas others must be counted as a category of parasites (coccidia, strongylid eggs).
- To determine the number of parasite epg of feces, add the counts for both chambers for each parasite and multiplying the number of eggs counted by 50.
- Then interpret the degree of infection.

Appendix 5. Coproculture and L_3 harvesting procedure

- Mix the faecal samples finely and add sterilized bovine faeces if the faeces is diarrheic.
- Then transfer samples to petridish and keep in an incubator at 27 ^{o}C for 7-10 days.
- Moisten the cultures sufficiently every 2 days to ensure that they do not dry out whilst being incubated, but without it becoming water-logged.
- After the 7-day period of incubation of the culture, sprayed the inside of the petridish lightly with water before being placed in bright light to stimulates the L_3 to migrate up the inner surfaces of the vessel's walls.
- Transfer the cultures to a jar.
- Fill the jar with water until the water meniscus above the lip of the jar
- Allow it to stand for a few minutes to allow the air to escape from the culture
- Place an overturned petridish over the mouth of the jar and keeping the petridish in position whilst the jar is inverted.
- Add water to the petridish and the preparation is left for a few hours for L_3 to migrate into the water and to settle.
- Remove the water in the petridish with a pipette for larvae harvest.

L_3 harvesting from coproculture (A), Harvested faecal suspension kept in refrigerator (B)

Appendix 6. Keys for identification of L_3 nematode of cattle

No.	Description	Identification
1	Sheath tail absent or unobtrusive	2
	Sheath tail prominent	3
2	Sheath tail absent or unobtrusive	
	Oesophagus ≥40% of total length of larva, without a bulb; larva very thin; head relatively bullet-shaped; tail appears blunt like a sheath tail that has broken off, sometimes it appears as if the larva does have a sheath.	*Strongyloides*
	Helminth stains uniformly very dark brown with iodine. Oesophagus <40% of length of helminth and often rhabditiform (double bulb); thick, cigar-shaped helminth with a long tail that may appear as if with sheath; tip of head not smooth, but uneven and flattened	Free-living nematode
3	Sheath tail >0.5"*X*" and prominent; oesophagus <30% of total length of larva; fresh larva very active	
	Very small larva that stains ± uniformly with iodine; sheath tail 2.5"*X*", filament ±50%; oesophagus with a prominent single bulb caudally; head bullet-shaped	*Bunostomum*
	Oesophagus without a prominent bulb; larva stains light brown anteriorly and dark brown posteriorly with iodine	4
4	Larva stains light brown cranially and dark brown caudally with iodine; oesophagus without prominent bulb	
	Sheath tail without a filament and tapers fast (resembles a pencil point); head ± bullet-shaped, with no shoulder	*T. axei*
	Sheath tail with a filament terminally	5
5	Sheath tail with a filament terminally	
	Larva head with two refractile spots; larval tail with filament	6
	Larva head without refractile spots; larval tail with filament	7
6	Larva head with two refractile spots, bullet-shaped, somewhat flattened and tapers quickly to a point (±slanted sides)	*Cooperia* spp.

Appendix 6 ***(continued)***

No.	Description	Identification
7	Larva head without refractile spots; larval tail filamentous	
	>16 intestinal cells	8
	8 intestinal cells	9
8	Larva with 16 or more intestinal cells	
	Head somewhat flattened, with "shoulder"; sheath tail tapers fast to a relatively blunt point	*Ostertagia*
	Head ± bullet-shaped and tapers quickly to a point (±slanted sides); sheath tail ends in a relatively sharp point	*Haemonchus*
	Head broad and flattened (almost parallel sides); 28–32 intestinal cells	*Chabertia ovina*
	Head broad and flattened (almost parallel sides); 18–22 intestinal cells	*Oesophagostomum*
9	Large larva with 8 intestinal cells	*Nematodirus*
	Head bullet-shaped, but somewhat flattened at the tip; larva tail with finger-like projection medially	*N. helvetianus*
	Larval tail tapers to a fine point, with two notches dorsally	*N. battus*

(Source: Van Wyk *et al*., 2013).

Appendix 7. Microscope calibration procedure

- To calibrate the objectives, place the stage micrometer on the stage of the microscope and focus until the lines are sharp.
- Superimpose any convenient numbered line of the ocular micrometer (usually the 0 mark) on a convenient line of the stage micrometer
- Find the two lines that are exactly superimposed
- To complete the calibration, count the number of divisions of ocular and stage micrometer that superimposed.
- Divide number of divisions of ocular micrometer by stage divisions, resulting in a calibration factor per division for the ocular micrometer.
- Repeat this procedure for each objective lens to be calibrated on the microscope.

Appendix 8: Micrometric measurement values of nematode third-stage larvae (L_3) of cattle

Nematode	Total length		Length of STE		'X'-value		Filament (%)
	Mean	Range	Mean	Range	Mean	Range	
T. axei	685	619-762	32	25–41	1.1	0.8–1.4	0
H. placei	820	749-866	102	80–119	3.4	2.7–4.0	20
O. ostertagi	850	-	65	45–83	2.2	1.5–2.8	10
C. punctata	850	-	59	37–78	2.0	1.2–2.6	20
C. oncophora	890	-	94	65–116	3.1	2.2–3.9	20
B. phlebotomum	550	500-583	73	58–96	2.4	1.9–3.2	50
O. radiatum	800	726-857	163	136–185	5.4	4.1–6.9	40–45
N. helvetianus	-	-	250	203–283	8.3	6.8–9.4	50
N. battus	-	-	165	-	5.2	-	-

Total length- the length from cranial end to the tip of filament/sheath; Length of STE (sheath tail extension)- the length from tip of larva tail to the end of filament/sheath; 'X'-values- the proportion of STE of larva identified and STE of *T. axei* (±30µm); Filament (%)- proportion of the filament and sheath tail extension. (Van Wyk, and Mayhew, 2013).

Appendix 9. Description of body condition scores (BCS)

Poor

- Clearly defined bone structure of shoulder, ribs, back, hooks and pins easily visible.
- Little muscle tissue or fat present.
- Small amount of muscling in the hindquarters. Fat is present, but not abundant.
- Space between spinous process is easily seen.
- Fat begins to cover loin, back and fore ribs. Upper skeletal structures visible. Spinous process is easily identified.

Medium

- Fore ribs becoming less noticeable. The transverse spinous process can be identified by palpation. Fat and muscle tissue not abundant, but increasing in fullness.
- Ribs are visible only when the animal has been shrunk. Processes not visible. Each side of the tail head is filled, but not mounded.
- Ribs not noticeable to the eye. Muscling in hindquarters plump and full. Fat around tail head and covering the fore ribs.

Good

- Spinous process can only be felt with firm pressure. Fat cover in abundance on either side of tail head.
- Animal smooth and blocky appearance; bone structure difficult to identify. Fat cover is abundant.
- Structures difficult to identify. Fat cover is excessive and mobility may be impaired.

Appendix 10. Data collection sheet for individual samples.

Sample ID	Owner name	District	Season	Altitude	Sex	BCS	Faeces consistency	**Coproscopy and Coproculture result**			
								Floatation	McMaster(EPG)	Sedimentation	Coproculture (L_3)

Appendix 11. Data collection sheet for micrometric measurement of L_3

Date	Sample ID	Owner name	District	Season	Micrometric measurement of L_3 from harvested faecal suspension			
					Total length	Sheath Tail Extension	Filament (%)	L_3 Identified

Appendix 12. Strongyle type egg and *Nematodirus* spp. egg observed in mixed infection

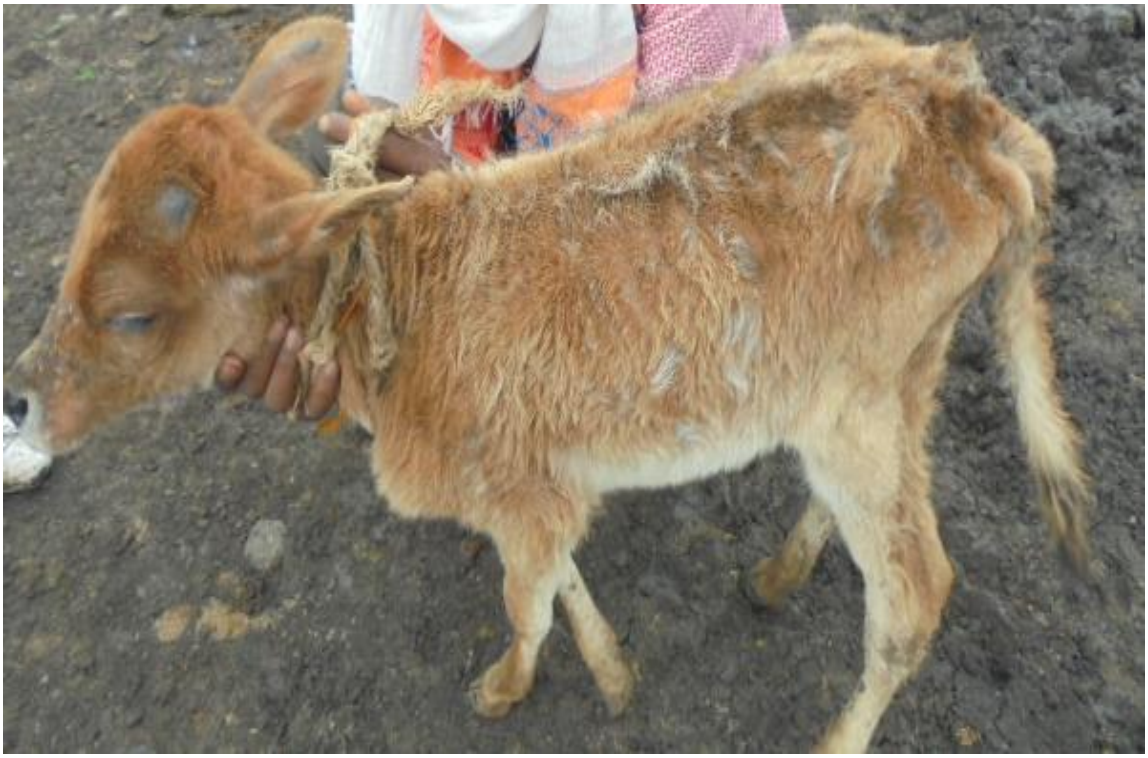
Note: Large size *Nematodirus* spp. egg containing 8 blastomers inside (upper right), Strongyle type egg containing 16 blastomers (lower left).

Appendix 13. Cattle graizing in communal pasture land in the study area.

Lowland Boset district during dry season (left) rainy season (right).

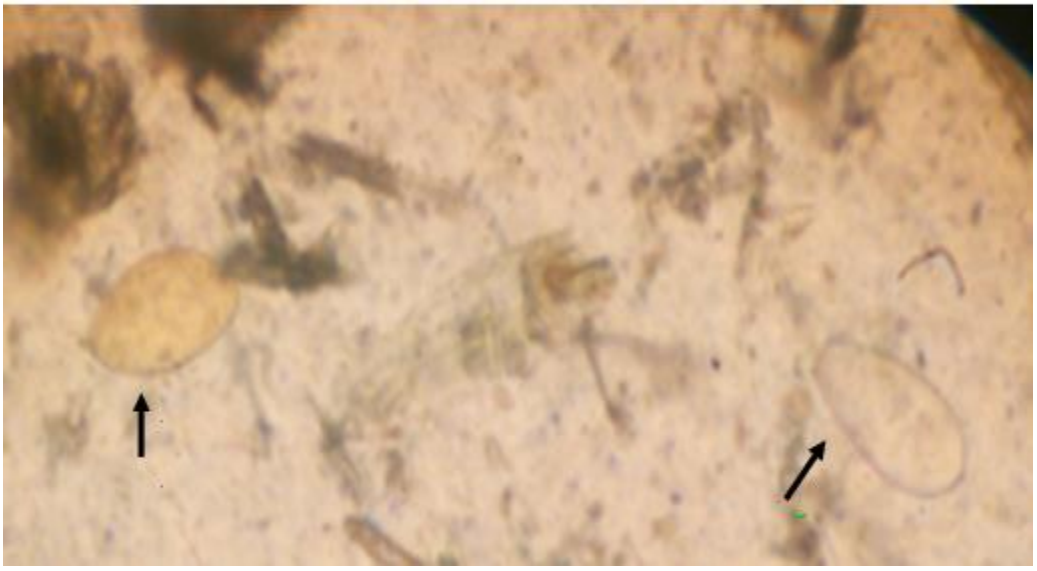
Highland PAs of Ada'a district during rainy season (left) and short rainy season (right).

Appendix 14. A 1½ year calf in highland PAs of Ada'a district infected with four gastrointestinal helminths.



Note: Identified helminths species were *H. placei, T. axei, B. phlebotomum* and *S. papillosus.*

Appendix 15. *Fasciola* spp. and *Paramphistomum* spp. eggs in mixed infection



Fasciola spp. (left) and *Paramphistomum* spp. (right)