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**IMPACT ASSESSMENT OF STRATEGIC MASS DEWORMING OF DONKEYS IN
SELECTED CENTRAL LOW LAND AREAS OF OROMIA REGION, ETHIOPIA**

MSc Thesis



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

BY

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Bishoftu, Ethiopia

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
AM	Avermectins and milbemycins
AR	Resistance Anthelmintic
BCS	Body Condition Score
BZ	Benzimidazoles
CI	Confidence Interval
<i>D.arnfieldi</i>	<i>Dictyocaulus arnfieldi</i>
DHWP	Donkey Health and Welfare Project
DHWP	Donkey Health and Welfare Project
DVM	Doctor of Veterinary Medicine
EARO	Ethiopian Agricultural Research Organization
EPG	egg per gram of faeces
ERP	Egg Reappearance Period
EMSA	Ethiopian Metrological Service Agency
FAO	Food and Agriculture Organization
FECRT	Faecal Eggs Count Reduction Tests
GI	Gastrointestinal
IVM	Ivermectin
ML	Macrocyclic lactones
MOARD	Ministry of Agriculture Rural Development
MOX	Moxidectin
MSc	Masters of Science
<i>O.equi</i>	<i>Oxyrus equi</i>
O.R	Odia Ratio
<i>P.equorum</i>	<i>Parascaris equorum</i>
PAS	PeasantAssociations
PhD	Doctor of Philosophy
rpm	revolution per minute
<i>S.edentatus</i>	<i>Strongyle edentatus</i>
<i>S.equinus</i>	<i>Strongyle equinus</i>
<i>S.vulgaris</i>	<i>Strongyle vulgaris</i>

S.westeri

spp.

T.axei

WAAVP

Strongylid westeri

Species

Trichostrongylus axei

World Association for the Advancement of Parasitology

ABSTRACT

A cross sectional study was conducted in two purposively selected central low land Oromia regions Boset districts (intervention area) one of the project operational areas of Donkey Health and Welfare Project (DHWP) and AdametuluJidoKombolcha districts (control area) agroecological from December 2014 to mid July 2015. The study was carried out in different seasons (dry and beginning of the rainy seasons). The objectives of the study were to determine prevalence of nematode parasites, identifications of non-nematode parasitic health related problems and evaluation of animal owner perception on strategic mass deworming of donkey with ivermectin. Faecal samples were collected randomly from 580 (intervention area) and 771 (control area) working donkeys and subjected to parasitological examination including simple flotation, McMaster egg counting technique and faecal culture. The overall prevalence of major nematode parasites were *Cyathostomum* (81.7% and 100%) , *Strongylus vulgaris*(68.3% and 97%), *Trichostrongylus axei* (62.2% and 92.5%),*Dictyocaulus arnfieldi* (21.2% and 49.4%), *Parascaris equorum* (15.9 % and 23.6%) and *Oxyuris equi* (7.6% and 10.4%) in intervention and control areas , respectively ($p<0.05$). The mean eggs counts of *Strongylus* revealed (304.6 and 1293.00) during dry season and (307.32 and 1456.53) during the beginning of rainy season in intervention and control areas, respectively ($P<0.05$). The impact of strategic mass deworming in the intervention area also revealed lower number of nematode parasites, species and epg of *Strongylus* in intervention area as opposed to the control area. There was no statistical significance difference ($p>0.05$) in *O.equi*, body conditions and visible mucus membrane in both study areas. Among non nematode parasitic health related problems, knits of *Gastrophilus* (31.9% and 32.7%) and abnormal mucus membrane (31.9% and 32.7%) were major between control and intervention areas, respectively Based on these, it can be concluded that parasite burden and faecal egg counts were lower in intervention area as opposed to control area. Taking into account the existing health problems of working donkeys in both areas coupled with current trend in strategic mass deworming of donkeys, alternate use of the anthelmintics with supplementary feeds, improved management practices and efficacy study of ivermectin and study of the relationship of faecal egg counts with parasite burden were recommended.

Key words: Assessment, Control area, Ethiopia, Ivermectin, Impact, Intervention area, Nematode, Oromia, Prevalence, Strategic mass demorming,

1. INTRODUCTION

Working equids (horses, mules, and donkeys) have an essential role in the livelihoods of millions of people worldwide. These equids perform numerous activities on a daily basis, including the transportation of goods, people, and construction materials, as well as being used in agricultural and tourism activities (Pritchard, 2010).

It is estimated that the total world equid population is approximately 112 million (58.5 million horses, 43.0 million donkeys, and 10.5 million mules), although this is very likely to be a gross underestimate (FAO, 2013). Working equids currently have a limited place within many animal health systems, and are largely absent from agricultural policy, research, and education programs (Curran *et al.*, 2005; Pritchard, 2010). According to central statistical agency, (CSA, 2013) total equid populations in Ethiopia are 10.32 million approximately about 1.91 million horses, 6.75 million donkeys, 0.35 million mules.

Working equids suffer from low productivity as a result of prevalent infectious diseases, and diseases associated with poor management practices. The health and welfare of working equids is often compromised in many low-income countries as a result of the impoverished situations their owners live in, the challenging environmental and climatic conditions, the unavailability of appropriate medications and vaccines, and the widespread use of ineffective or harmful traditional therapies (Pritchard *et al.*, 2005). Helminth parasites are ubiquitous in grazing equines that have been associated with poor growth, weight-loss and clinical disease (Love *et al.*, 1999). Consequently, large efforts have been made to control these parasites by developing parasite control programmes based on regular use of anthelmintic drugs (Matthee *et al.*, 2002).

Parasite control in equines in the 1960 was to deworm every six to eight weeks (Drudge and Lyons, 1966). This was based on the time that strongyle egg counts per gram of feces (epg) began returning after treatment with compounds like thiabendazole which was effective when first marketed. Also this was the period when the most pathogenic parasites, large strongyles were still quite prevalent and caused severe problems to equines worldwide (Duncan, 1974). Frequent usage of effective anti-parasitic drugs since then has virtually eliminated *Strongylus* spp (Herd, 1990; Nielsen *et al.*, 2012).

The first finding of resistance against modern broad-spectrum anthelmintics in strongyle nematodes of horses was in 1965 only four years after thiabendazole had been introduced onto the market (Drudge and Lyons, 1966). In South Africa broad-spectrum anthelmintics has been used for strategic and targeted nematode parasites control for longer period of time (Van Wyk *et al.*, 1997). The currently available literature relating to the potential benefits of anthelmintic use in working equines is limited and unclear. Three studies reported a measurable benefit from anthelmintic treatment using “body condition scoring” as the sole measure of assessment (Bliss *et al.*, 1985; Fesheha, 1990; Khallaayoune, 1990). On the contrary, three studies conducted questioned sole measure of benefit of anthelmintic treatment using only body condition scoring (Wallace, 2004; Mengistu *et al.*, 2005).

The Donkey Health and welfare project (DHWP) in Ethiopia is working in different parts of the country to help improve the health and welfare of the animals. In the areas of interventions, anthelmintic treatments are commonly undertaken free of charge to combat helminth parasitism in equines starting from 1996. Initially, they used tactical means of deworming . Currently, they implement strategic mass deworming parasites control methods for working equines in Oromiya regions and other parts of Ethiopia (Getachew *et al.*, 2010; Abebew *et al.*, 2011). The hypotheses tested in this study were to verify the usual practice and trend exercised by the donkey health and welfare project that strategic mass deworming of donkeys twice a year in selected low land areas of central Oromiya region was effective nematode parasite control methods.

Hence , the objectives of this study were to determine species of nematode parasites affecting working donkeys in both control and intervention areas, to determine egg per gram of faeces of strongylus, to see the effect of the season on each species of nematode parasites and egg per gram of faeces in both control and intervention areas, to identify risk factors associated with the occurrence of each nematode parasite, to identify other non-nematode parasite health related problems of working donkeys, to assess the impact of strategic mass deworming in intervention area and to assess the donkeys owner perception on strategic mass deworming of donkeys.

2. LITERATURE REVIEW

2.1 Nematode Parasites of Working Donkey

2.1.1 Strongyles (Strongylidae)

Strongyles live as adults in the large intestine of equids (horses, donkeys, zebras, wild asses and their hybrids) and are commonly categorized as large and small strongyles. The strongyles as a group comprise almost one-half of the over 100 species of internal parasites found in horses (Krecek *et al.*, 1987).

Strongyles have a direct life cycle. They lay eggs that are passed in the faeces of the host to the environment. Under favorable environmental conditions, optimum temperature and humidity, the embryo develops within the egg to the first stage larvae which hatches. Further development occurs to the second stage and third stage on the pasture. The rate of development and survival of the free-living stages depends on the surrounding temperature and humidity or rainfall (English, 1979 ; Soulsby, 1982; Urquhart *et al.*, 1996). The third stage larva is the infective stage, and infects equids if ingested. In the host, development continuous with advancement to the fourth stage and the fifth stage or adult. The prepatent period varies between the large and the small strongyles and among the species. It varies from 6-12 months for large strongyles (Soulsby, 1982; Urquhart *et al.*, 1996). Although the prepatent period of individual small strongyles has not been studied, some studies have shown that it is in the range of 5-18 weeks (Soulsby, 1982; Love and Duncan, 1992; Urquhart *et al.*, 1996) (Table1).

Table 1: Summary of prepatent period (PPP) of nematode parasites in equines.

Species of nematode parasites	ppp
<i>Strongylus vulgaris</i>	6-7 months
<i>S. edentatus</i>	10-12month
<i>S. equinus</i>	8-9 months
<i>S. westeri</i>	8-14 days
<i>T. tencolli</i>	Similar to Cyathostome
Cyathostomes	2-3 months
<i>T. axei</i>	25 days
<i>D. arnfieldi</i>	2-4 months
<i>P. equorum</i>	10 weeks minimum
<i>O. equi</i>	5 months

Source, (Urquhart *et al.*, 1996)

2.1.2. Large strongyles.

Large strongyles encompass the genera *Strongylus*, *Craterostomum*, *Oesophagodontus*, *Triodontophorus* and *Bidentostomum* (Lichtenfels, 1998). The commonly known large strongyle species infecting equids are *Strongylus vulgaris*, *S. edentatus*, *S. equines*, *Triodontophorus brevicauda*, *T serratus*, *T minor*, *T tenuicollis*, *T nipponicus*, *Craterostomum acuticaudatum*, *C. tenicauda* and *Oesophagodontus robustus* (Soulsby, 1982; Urquhart *et al.*, 1996).

Work done to identify the species of large strongyles infecting working donkeys in various regions have revealed similar species to those described in horses (Table2). *S. vulgaris* and *Triodontophorus* species are considered the most pathogenic of the large strongyles in horses (Slocombe, 1985; Austin, 1994; Proudman and Matthews, 2002). However, it is the epidemiology and pathogenic effect of *S. vulgaris* that has been well studied in horses in the northern hemisphere under temperate climate (Duncan, 1974) because of the potential severe tissue damage, including occlusion of the blood vessels (cranial mesenteric arteries and its branches) during parentral larval migration which often results in colic and sometimes death

(Duncan, 1973; Drudge and Lyons, 1977).

Although variations were seen, studies have shown higher prevalence and intensity of *S. vulgaris* infection in donkeys than the other strongyle species (Table 2). Almost all studies reported a 100% prevalence of *S. vulgaris* in donkeys (Pandey and Eysker, 1989; Getachew, 1999; Matthee *et al.*, 2002). A study conducted in Zimbabwe (Pandey and Eysker, 1989) showed that the intensities of infection of adults in the large intestine and larvae in the cranial mesenteric arteries and its branches were much higher than those recorded for horses from Morocco (Pandey, 1981; Poynter, 1970; Ogbourne, 1975).

2.1.3. *Cyathostomum*(small strongyles)

Cyathostomum formerly called trichonemes or cyathostomes (Lichtenfels *et al.*, 1998) are the most common other genera of strongyles infecting equids. The adult worms live in the large intestine and have a non-migratory life cycle (Soulsby, 1982; Urquhart *et al.*, 1996). According to Reinemeyer, (1986) *cyathostomum* ova usually comprise over 95% of the strongylid ova in horse faecal samples. Infections with these nematodes typically include very large populations and numerous species. A total of 52 species of *cyathostomum* have been recorded in horses, donkeys and zebras (Lichtenfels *et al.*, 1998).

The species of cyathostomins described in donkeys are similar to those described in horses of the over 50 species of cyathostomins 10 species have been reported only from donkeys or zebras (Lichtenfels *et al.*, 1998). Previous prevalence studies on cyathostomins were from donkeys in different parts of the world (Table 2).

Table 2: Previously reported prevalence of Strongylus species from donkeys in different parts of Africa.

Species of strongylus	Areas	Prevalence (%)	References
<i>S. vulgaris</i>	Sululta and Gefersa	100	Zerihun <i>et al.</i> , 2011
	Hawassa	51.8	Nuraddis <i>et al.</i> , 2011,
	Control	100	Abebew <i>et al.</i> , 2011
	Boset,	100	Abebew <i>et al.</i> , 2011
	Dugda Bora	100	Ayele <i>et al.</i> , 2006
Strongylus	South Africa	100	Wells <i>et al.</i> , 1998; Matthee <i>et al.</i> , 2000
Strongylus	Gambia	83	Mattioli <i>et al.</i> , 1994
<i>Strongyle edentatus</i>	Boset	89.9	Abebew <i>et al.</i> , 2011
	Meki	89.9	Abebew <i>et al.</i> , 2011
	Hawassa	30.8	Nuraddis <i>et al.</i> , 2011
	Dugda Bora	66.6	Ayele <i>et al.</i> , 2006
	Sululta and Gefersa	73.8	Zerihun <i>et al.</i> , 2011
<i>Strongyle equinus</i>	Project areas	44.4	Abebew <i>et al.</i> , 2011
	Control area	33.3	Abebew <i>et al.</i> , 2011
	Hawassa	12.3	Nuraddis <i>et al.</i> , 2011
Tridontophorus spp	Dugda Bora	50	Ayele <i>et al.</i> , 2006
	Project area	44.45	Abebew <i>et al.</i> , 2011
	Control area	55.5%	Abebew <i>et al.</i> , 2011
	Hawassa	29.7%	Nuraddis <i>et al.</i> , 2011
	Sululta and Gefersa	52.8%	Zerihun <i>et al.</i> , 2011
Cyathostomum	Sululta and Gefersa	100%	Zerihun <i>et al.</i> , 2011
	Project area	100%	Abebew <i>et al.</i> , 2011
	Control area	100%	Abebew <i>et al.</i> , 2011
	Boset	100%	Getachew <i>et al.</i> , 2010
	Hawassa	94.9%	Nuraddis <i>et al.</i> , 2011
	Dugda bora	100%	Ayele <i>et al.</i> , 2006

As part of their life cycle, cyathostomins undergo a period of arrested development as early third stage larvae in the large intestinal mucosa of the horse (Eysker *et al.*, 1984; Eysker *et*

al., 1990; Love, 1995; Paul, 1998; Proudman and Matthews, 2002) which could be extended for more than 2 years (Reinemeyer, 1986). In temperate regions winter is an unfavorable period for larval development and survival (Ogbourne, 1975) but in many tropical and subtropical areas it is the dry season (English, 1979; Mfitilodze and Hutchinson, 1988). Fecundity is the rate of egg production per matured adult female worm per day (Urquhart *et al.*, 1996). It is usually estimated in terms of faecal worm egg count if worm burden and daily faecal mass are known (Kao *et al.*, 2000). According to the study by Love and Duncan (1992), the general pattern of faecal worm egg output were similar in foals, yearlings and adults with the counts showing a sharp increase at about 100 days after first grazing and then remaining fairly constant until treatment on day 186. But the mean worm egg count in adult ponies was lower than those detected in foals and yearlings, which they attributed to modified cyathostomum development as a consequence of host age and/or previous exposure to parasites. A similar result was obtained by Smith, (1978) in which he recorded significantly lower faecal worm egg counts when he infected ponies for a second time. Miller, (1953) indicated that the characteristic of eggs production of females cyathostomins markedly vary from about 10 or 12 eggs per female per day.

Older as well as recent studies support the hypothesis that not only seasonal conditions but also resistance acquired through exposure or size of the parasite population promotes cyathostome arrested development (Chapman *et al.*, 2002; Love and Duncan, 1992). It is difficult to consider each of these factors separately since each is dynamic and likely to be independent to some extent.

2.1.4. *Oxyuris equi*

The equine pinworm *O.equi* is a common horse and donkeys parasite with a worldwide distribution (Urquhart *et al.*, 1996). In the past, research on equine nematodes has mainly been focused on members of the superfamily Strongyloidea, especially cyathostomes, since resistance of these parasites against anthelmintics represents a global problem (Coles *et al.*, 2006).

O. equi follows a direct life cycle with adults inhabiting mainly the right dorsal colon and in case of heavy infections also the adjoining parts of the colon of equines (Urquhart *et al.*, 1996).

O. equi infections generally are recognized by horse owners owing to the pruritus caused by these semi-liquid substances when desiccating, which can result in scratching and damage to the tail (Reinemeyer and Nielsen, 2009).

Horses and donkeys become infected by ingestion of eggs carrying larvae 3 on fodder, grass and bedding. Hatched larvae 3 will invade the crypts of Lieberkuhn in the ventral colon and caecum, and within 3–11 days post infection development into the fourth larval stage takes place. The larvae 4 will move slowly towards the dorsal colon and from day 50 post infection moulting into fifth larval stage begins and it will take another hundred days to reach sexual maturity. Nevertheless, the whole prepatent period may also take place within only 4½–5 months. Most of the pathogenic effects of *O. equi* in the intestine are due to the feeding habits of the larval stages, mainly of larvae 4 within the mucosal crypts, which result in small erosions of the mucosa and, in heavy infections, these may be widespread and accompanied by an inflammatory response (Urquhart *et al.*, 1996; Reinemeyer and Nielsen, 2009). Previous *O. equi* infection was reported from donkeys in different parts of the world (Table 3).

Table 3: Summary of the previously reported prevalence of *O. equi* from donkeys in different parts of the world.

Countries (Town)	Prevalence (%)	References
Boset	4.6	Abebew <i>et al.</i> , 2011
Meki	6.5	Abebew <i>et al.</i> , 2011
Hawassa	31.8	Nuraddis <i>et al.</i> , 2011
Berehi	16.2	Getachew, 2006
South Wollo	4.5	Alemayehu and Etaferahu, 2013
Turkey	6.45	Sinasia and Mustafa, 2009
Boset	12.6	Getachew, 2006
South Africa	17.7	Well <i>et al.</i> , 1998
Dugda Bora districts	6	Ayele <i>et al.</i> , 2006
Sululta and Gefersa	9.8	Zerihun <i>et al.</i> , 2011

2.1.5. *Parascaris equorum*

Research on ascaridinae from African zebra and zebras living in the Vincennes zoological garden in France, and those from horses confirmed that they belong to the same species, *P. equorum* (Ansel *et al.*, 1974). Ascarid infections have been reported in donkeys from various regions of the world (Table 4). *P. equorum* is common parasites of horses and donkeys throughout the world and is a major cause of unthriftiness in young foals (Urquart *et al.*, 1996).

Table 4: Prevalence studies of *P. equorum* from donkeys in different parts of the world.

Countries	Prevalence (%)	References
Hawassa	52.8	Nuraddis <i>et al.</i> , 2011
Projeect	22.2	Abebew <i>et al.</i> , 2011
Meki	49.7	Abebew <i>et al.</i> , 2011
South wollo	10.4	Alemayehu and Etaferahu, 2013
Burkina Faso	43	Vercruysse <i>et al.</i> , 1986
Dugda Bora District	50	Ayele <i>et al.</i> , 2006
Chad	72	Graber, 1970
Boset	46	Getachew, 1999
Ada	61.3	Getachew, 2006
Akaki	62.2	Getachew, 2006
Bereh	37.1	Getachew, 2006
Germany	2.8	Gothe and Heil, 1984
India	8.4	Kotwal <i>et al.</i> , 2000
Kenya	17	Lewa <i>et al.</i> , 1998
Morocco	37	Khallaayoune, 1991
South Africa	25	Mathee <i>et al.</i> , 2000
Turkey	20	Burgu <i>et al.</i> , 1995
USA	13	Tolliver <i>et al.</i> , 1985
Zimbabwe	50	Pandey and Eysker, 1990
Norther Wollo	48.8	Mulate <i>et al.</i> , 2005
Turkey	22.58	Sinasiand Mustafa, 2009
Sululta and Gefersa	53.2	Zerihun <i>et al.</i> , 2011

2.1.6. *Dictyocaulus arnfieldi*

This ubiquitous parasite of donkeys is rarely associated with signs of clinical diseases in horses, its prevalence is difficult to establish since infectious are rarely became patent although it is frequently incriminated as a cause of chronic coughing (Urquhart *et al.*, 1996). Its previous prevalence studies conducted from donkeys in different countries are listed in (Table5).

Table 5: Compilation of the prevalence of *D. arnfieldi* from donkeys in different parts of the world.

Countries(Towns)	Prevalence (%)	References
Around Hawassa Town	3.6	Nuraddis <i>et al.</i> , 2011
Dugdabora Districts	32	Ayele <i>et al.</i> , 2006
Sululta and Gefersa	42.8	Zerihun <i>et al.</i> , 2011
North America	50-80	Klei , 1986
around Jimma town	35.3	Basaznew <i>et al.</i> , 2012
Turkey	9.67	Sinasi and Mustafa, 2009
Control areas	66.6	Abebew <i>et al.</i> , 2011
around Bahir Dar	20	Esheta, 2000
Denmark	87.5	Andersen and Fogh, 2010.
Morocco	48	Pandey, 1980
Ethiopia	83	Feseha <i>et al.</i> , 1991
Sudan	70.5	Hassan <i>et al.</i> , 2004
Kentucky, USA	54	Lyons <i>et al.</i> , 1985
Project	22.2	Abebew <i>et al.</i> , 2011

2.1.7. *Strongyloides.westeri*

Members of strongyloids are common parasites of the small intestine in very young animals and although generally of little pathogenic significance, under certain circumstances may give rise to severe enteritis (Urquhart *et al.*, 1996). Previous years prevalence studies from different areas are listed in Table 6.

Table 6: Compiled data as to the prevalence of *T.axei* and *S.westeri* from donkeys in different parts of the world.

Parasite species	Areas	Prevalence (%)	References
<i>T. axei</i>	Dugda Bora	40	Ayele <i>et al.</i> , 2006
	Sululta and Gefersa	100	Zerihun <i>et al.</i> , 2011
	Boset	91.3	Getachew <i>et al.</i> , 2010
	Project area	33.3	Abebew <i>et al.</i> , 2011
	Control area	33.3	Abebew <i>et al.</i> , 2011
	Gambia	83	Mattioli <i>et al.</i> , 1994
	Moroco	80.9	Pandey <i>et al.</i> , 1990
<i>S. westeri</i>	Boset	17.5	Getachew <i>et al.</i> , 2010
	Control	77.8	Abebew <i>et al.</i> , 2011
	Sululta and Gefersa	42.8	Zerihun <i>et al.</i> , 2011
	Hawassa	20	Nuraddis <i>et al.</i> , 2011

2.2 Nematode Parasites Control Methods

2.2.1 Strategic mass deworming

The goal for control of equine parasites infections is to minimise the number of eggs or larvae and resultant infective larvae on the grazing areas, and there by prevent clinical and subclinical disease. There are various approaches on how to achieve this goal, but traditionally control has relied on regular treatment with anthelmintic drugs (Duncan *et al.*, 1974). The traditional way to approach the equine parasites has been to identify one or a few species of particular importance and then design control strategies targeting these in all animals (Drudge and Lyons, 1966) introduced the interval-dose program principle where horse owners were advised to deworm every 8 weeks to prevent parasite transmission. The frequent treatment regimen was recommended because the early benzimidazole drugs available at the time were not efficient against migrating stages of the large strongyle parasites.

Kenya has an active livestock industry whose main method of control of nematodes is the use of anthelmintics and all broad spectrum anthelmintics have a share of the lucrative market. A study by Kinoti *et al.*, (1994) in the Central and Rift Valley regions showed that

anthelmintics are used on a considerable scale and that large farms treat their animals at least quarterly, with exotic breeds being treated more regularly than the indigenous breeds.

Problems of strategic mass deworming

Today the major parasite enemy of equines or any animals or humans are no longer a particular species but rather anthelmintic resistance in general, and various treatment strategies have been suggested to decrease the rate of development of resistance. The trends of equine parasites control appear to be constantly changing (Nielsen, 2012).

In South Africa horses were treated between five and seven times annually (Matthee *et al.*, 2002) but equines in Ireland were treated at 4–6 week intervals all year round (Meara and Mulcahy, 2002). A survey from the United States in 1998 revealed that 49.2% of all equine operations dewormed at least four times a year (Love, 2003). In Tanzania, a study showed the use of benzimidazole for parasites control in an institutional farm which, despite stoppage of usage for about 10 years, showed no reversion to susceptibility (Kassuku *et al.*, 1997). In Zimbabwe, Boersema and Pandey (1997), in a study on commercial sheep farms, benzimidazole, levamisole and rafoxanide were extensively used for strategic helminthes parasites control. The shift in paradigm involves moving away from treatment strategies that aid for nil transmission to new strategies based on the parasite survey, more targeted or selective application of anthelmintic treatments not to all animals (Vanwyk, 2006).

The concept of parasite refugia has been widely accepted as playing a very central and important role in preventing the development of anthelmintic resistance in livestock (Kaplan, 2004). Those parasite stages (encysted) not exposed to the drug at the time of treatment are in refugia since they escape the drug and are not under selection for resistance. Parasite stages on pasture (eggs, larvae1, larvae2 and larvae3) are always in refugia as are parasites in horses not receiving treatment. Parasite stages in refugia serve as a means of dilution by which small numbers of resistant worms surviving the treatment within equines can be quickly outnumbered by worms in refugia leading to a very slow accumulation in the population of genetic alleles encoding drug resistance. To increase levels of parasite refugia, treatment regimens based on the principles of selective therapy have been recommended in equine establishment for more than 15 years (Duncan and Love, 1991).

The basis for this strategy is the general knowledge about parasite distributions within a herd

of hosts (Galvani, 2003), meaning that some equine may harbour low parasite burdens, while others carry more moderate burdens and a few individuals may be heavily infected with parasites. Thus, it remains clear that some horses may not require as many treatments as others and a treat all approach (mass deworming) will involve many unnecessary treatments and any drugs resistance (Vanwyk *et al.*, 2006).

For equines, the only means of selectively identifying individuals for treatment has been to perform quantitative faecal egg counts on all equines and then treat those exceeding a predetermined cut-off value. There is a general perception that the correlation between equine egg counts and worm numbers is mediocre to poor (Duncan, 1974). Results indicated that faecal egg count and larval culture have value as qualitative diagnostic tools for detecting presence or absence of a given parasite group (Nielsen *et al.*, 2010)

Some investigations verified the absence of strongyle eggs for about 8 weeks after treatment for horses that were administered ivermectin. Early clinical studies on reduction of strongyle egg numbers by ivermectin treatment and subsequent egg reappearance period (ERP) established the 10 to 11 weeks required for rebound after the 0.2 mg/kg dosage. The 0.3 mg/kg dosage extended the strongyle ERP to 14 weeks (Asquith and Kulwich, 1981).

Data from a Brazilian study suggests that AM resistance has developed in cyathostomins and reports of shortened egg reappearance periods after ivermectin treatment have been published recently from USA. With increasing levels of resistance to benzimidazoles and pyrantel being reported worldwide, equine parasite control now relies heavily on the avermectin/milbemycin (Kaplan, 2004). Ivermectin (IVM) is the mostly used macrocyclic lactone (ML) for the deworming of livestock. In horses receiving an oral (paste or liquid) dose of 0.2 mg/kg body weight (BW) of IVM, a high efficacy (95%-100%) against adult and most fourth stage larvae of cyathostomes, large strongyles and ascarids has been demonstrated (Asquith and Kulwich, 1981).

2.2.2. Grazing management methods

One approach involves the use of two or more different host species on pasture alternately, with the changeover taking place when the pastures have become helminthologically safe for the alternate host. The most commonly used species are cattle and equines and alternate or

mixed grazing systems depend on the host specificity of the parasite species involved (Stromberg and Averbek, 1999). Cattle and equine parasites are relatively host specific so the pasture contaminated by cattle may be considered clean for equine and vice versa and where infection occurs it rarely develops to patency (Thomas, 1982). The same author advocated a three year alternation of cattle, sheep and crops. The interval of change over should not be so long that one host is infected by its own parasites. In practice, control is best achieved in temperate climates by exchanging spring pastures grazed by sheep with beef cattle over the previous year, preferably combined with anthelmintic treatment at the time of exchange (Urquhart *et al*, 1996). The disadvantage of this method is that not all ruminant gastrointestinal nematodes are host specific, an example being *T. axei* which can be found in cattle, sheep, equines and can also infect other hosts including goats and pigs. Another example which is of practical importance in the temperate areas is *Nematodirus battus* infection in lambs and calves (Bairden and Armour, 1987 on an alternate grazing system of husbandry).

Another grazing management method is rotational grazing which involves resting the pastures long enough for any residual contamination to decline to negligible levels before susceptible livestock is introduced. Barger *et al.*, (1994) showed the potential of rotational grazing to control nematode infections in goats in a wet tropical climate. He suggested that such systems are most likely to work in the tropics where the life expectancy of infective larvae in pasture is short (6-8 weeks), than in temperate areas where larvae can survive in reasonable numbers for as long as 3 to 9 months (Barger, 1999).

Cropping is another form of efficient utilisation of the pasture which, after it becomes highly contaminated, is planted with crops or has a harvest of hay or silage taken from it. Rotational grazing is a specialised form of pasture spelling which is impractical where farmers own small pieces of land. The use of strip grazing, which involves confining animals along narrow strips in the field, is now less popular in temperate regions though the use of tethering is becoming popular in tropical areas where small scale farmers keep sheep and goats tethered, moving to a new grazing area every one to two days.

2.2.3. Combination of anthelmintic treatment and grazing management

In temperate regions of the world, a large number of recommendations aimed at improving efficiency of parasite control in livestock are based on combining anthelmintic treatment with some form of grazing management (Barger, 1997). In comparison, there are relatively few examples of such schemes in the tropics/subtropics but in these regions the potential for grazing management is possibly greater (Waller, 1997).

Grazing management schemes usually incorporate a limited number of anthelmintic treatments and the most widely used is the "dose and move" system. This system is sometimes referred to as an evasive strategy and is used in various agro climatic zones but requires meteorological information to define periods of high risk when chemoprophylaxis becomes necessary (Michel, 1969). There are disadvantages to this system because the new generation of worms which appears after the move to clean pasture will consist entirely of the progeny of the parasites which survived treatment, thus this approach may select for anthelmintic resistance.

2.2.4. Use of nutrition supplementation

This has gained importance recently because of widespread anthelmintic resistance. Houtert *et al.*, (1995) studied interactions between dietary supplementation and degree of nematode control on production responses in young grazing Merino sheep. They found that supplementary feeding with sunflower cake appeared more effective than treatment with anthelmintics in reducing production losses attributable to nematode infections. This confirmed the study of Jorgensen *et al.*, (1992) whom reported that supplementary feeding of young cattle with lucerne markedly reduced faecal egg counts in the ensuing period. Though this method is gaining acceptance as an alternative control strategy, dietary supplementation is more expensive than treatment with anthelmintics (Houtert and Sykes, 1996).

2.2.5 Control by using genetically resistant animals

Genetic selection can also be used to alter the genotype of animals within the flock to make them more resistant to a disease. It is being increasingly recognised that the animal genetic resources of the tropics/subtropics are likely to provide the foundation of sustainable and

environmentally sound solutions to helminthoses in these regions (Waller, 1997). The trait sought in selection studies is resistance, which is the ability of the host to regulate gastrointestinal nematodes. Another trait that has been considered is resilience, which has been defined as an enhanced ability of sheep to withstand the impact of nematodes on productivity when remaining undrenched. There is ample evidence that breeding for parasite resistance is possible (Albers and Gray, 1987).

2.3 Non-Nematode Parasites Health Related Problems of Working Donkeys

2.3.1 Gasterophilids

Members of this genus are commonly referred to as bot flies. The adults of this species are about 18 millimeter long a dark, irregular, transverse band runs across either wing. The life cycle of the various species differ only slightly. In temperate areas, adult flies are most active in late in late summer. From the life cycle it is obvious that in temperate areas, almost the entire population will be present as larvae in the stomach during the winter since adult fly activity ceases with the advent of the first frosts in autumn (Soulsby, 1978; Urquhart *et al.*, 1996; Zurek, 2004). Kumar *et al.*, (2014) reported 13% prevalence of knits of *Gastrophilus* in Mekelle city.

Horse bot flies undergo complete metamorphosis, including three larval instars. Only one generation is produced per year. The stages of the life cycle are not restricted to certain seasons due to the varied climates found in different geographical locations. Ovipositing on the rear legs appears to be discriminated by most flies whereas age, breed, size, and sex do not appear to be a factor (Cogley, 2000).

2.2.2. Wound

Management and care for donkeys seems to many people to be unnecessary as donkeys are one of the few domesticated animals that appear to do rather well with minimal management (Pearson *et al.*, 1997). Wounds are one of the primary welfare concerns of working equids. Wound is most common health problems of working donkeys in developing countries (Atawalna *et al.*, 2015). Previously reported prevalence of wound in working donkeys across different countries are listed in Table 7.

2.2.3. Lameness

Lameness is one of the health related problems in the working donkeys. It causes of loss of performance in cart pulling donkeys (Putnam *et al.*, 2014; Amene *et al.*, 2015).The prevalence of lameness in working donkeys across different parts of the world were reported by different researchers (Table7).

2.2.4. Sarcoid

Equine sarcoids are the most common skin tumors in horses and other equids like donkeys, mules and zebras (Lazary *et al.*, 1988; Reid *et al.*, 1994; Chambers *et al.*, 2003; Knottenbelt, 2005; Nel *et al.*, 2006; Yu, 2006). Equine sarcoids are observed worldwide, independent of breed, coat color, sex or age (Tarwid *et al.*, 1985). The most common sites recorded for sarcoid infestation are the ventral body regions, head, neck and all body regions, which contain thin skin layer (Teifke and Lohr, 1996).Sarcoids in donkeys were reported by different researchers in Egypt, Ghana (Mohamed *et al.*, 2012, Atawalna *et al.*, 2015) (Table7). Sarcoids have also been detected in 33 (3%) of 1090 working donkeys at Debre Zeit (Yilma *et al.*,1991).

Table 7: Common health problems of the donkeys reported from different parts of the world.

Health problems	Countries	Prevalence (%)	References
Lame	Hawassa	40.2	Amene <i>et al.</i> , 2015
Lame	Debrezeit	3.1	Morgan, 2006
Lame	Pakistan and India	98	Broster <i>et al.</i> , 2009
Lame	Pakistan	98	Reix <i>et al.</i> , 2014
Lame	Mekelle city	9.9	Kumar <i>et al.</i> , 2014
Lame	Dugda bora	6	Ayeleet <i>et al.</i> , 2006
Lame	Ghana	3.5	Atawalna <i>et al.</i> , 2015
Wound	Ghana	18.6	Atawalna <i>et al.</i> , 2015
Wound	Yilmana densa	42.2	Girma <i>et al.</i> , 2014
Wound	Hawassa	77.5	Curran <i>et al.</i> , 2015
Wound	Hawassa	79.4	Biffa and Moges, 2006
Wound	Jordan	59	Burn <i>et al.</i> , 2007
Wound	Morocco	54	Sells <i>et al.</i> , 2010
Wound	Central Ethiopia	40	Pearsons <i>et al.</i> , 2002
Sarcoid	Ghana	6.2	Atawalna <i>et al.</i> , 2015
Wound	Dugda bora	16.4	Ayeleet <i>et al.</i> , 2006
Dental disease	Ghana	0.95	Atawalna <i>et al.</i> , 2015
Dental disease	Ada'a and Dugda	63.25	Mengistu <i>et al.</i> , 2015
Dental disease	Mexico	16	Fernando Martinez <i>et al.</i> , 2006
Abnormal mucus membrane	Mekelle city	20	Kumar <i>et al.</i> , 2014
Abnormal mucus membrane	Meskan district	51.7	Solomon and Rahmeto, 2010
Sarcoids	Mekelle city	1.3	Kumar <i>et al.</i> , 2014
Sarcoids	Debre zeit	3	Yilma <i>et al.</i> , 1991
Sarcoids	Dugda bora	3.1	Ayele <i>et al.</i> , 2006
Sarcoid	Ghana	6.2	Atawalna <i>et al.</i> , 2015

3. MATERIALS AND METHODS

3.1 Description of the Study Areas

The current study was conducted from December 2014 to the first half of July 2015 in Boset project operational areas (DHWP) under the auspices of the Donkey Sanctuary and control area (AdametuluJidocombolcha). According to Ethiopian agricultural research organization (EARO), (1998) ecologically, the study sites are classified into lowland (kolla) e. g. Boset and Adametulu and Jidocombolcha based on temperature and length of plant growing period. The rainfall pattern is bimodal i.e. long and short rainy seasons. The long rainy season starts on June and extends up to the month of September whereas, the short rainy season occurs from February to March.

AdamituluJidoKombolcha

The current study was conducted in Adami Tulu and Jido Kombolcha district(control area) which is located in central rift valley of Ethiopia at 160 km away from Addis Ababa (capital of Ethiopia). The district lies at latitude of 7.58° north and 38.43° east longitudes. This district was considered as control areas where no interventions have been carried out. Its agro-ecological zone is semi-arid and sub-humid in which 90% of the area is lowland while the remaining 10% is intermediate with altitude ranges from 1500–2000 meter above sea level. The mean annual rainfall ranges from 750-1000mm and the distribution is highly variable between and within years. The mean annual temperature ranges from 22-28 degree centigrade. Mixed crop-livestock farming system characterizes the agriculture of the district. There are many private plant irrigation farms. Lake desta abjata and Jidoriver are sources of water and grazing is also available during the long dry seasons to those animals near to it.

Boset

Boset(intervention area) is situated in the Great Rift Valley 135 kilometer southeast of Addis Ababa. It lies at latitude 08' 43'north and longitude 039' 41'east. This area was considered as intervention area. The region has an altitude range of 1000-1500 metres above sea level and a hot dry/moist agro-ecological zone. According to Ethiopian National Metrological Service agency (ENMSA), (2013) the mean annual rain fall and maximum (minimum) temperature

are recorded to be 550-1200 millimetre and 31degree centigrade (16.6), respectively.

Agriculture is a mixed crop-livestock production system but livestock herding is the dominant activity. Very few areas bordering the Awash River practice irrigation. Thin grasses and bushes with patches of woodlands cover most areas. There is no permanent pasture and animals graze on the road and hillside, and on weeds of arable lands. Animals spend most of their time within the surrounding bushes and acacia trees, which is not suitable for crop production, and the availability of grass is very scarce and almost non-existent during the long dry season (October to June). Crop residues from cereal such as teff, and maize or sorghum stover are the major feed source for the prolonged dry-period. The areas are not waterlogged but there are small ponds and water wells in the rural areas used as water sources both for humans and animals. The dominant soil types are cambisol/yermisols (Solonchaks) of sandy texture and vertisols very rich in clay (EARO, 2004).

3.2. Target Populations and Study Animals

Target population for control areas were working donkeys in Adami Tulu and Jido Kombolcha district where as target population for intervention area were working donkeys treated with ivermectin at least once in October 2014 by strategic mass deworming in Boset districts based on donkey owner interview information. Donkeys which used for pack purposes were the study animals in both interventions and control areas. Cart donkeys availability in both study areas were not significant so that they were not included as study animals.

3.3. Study Design

The type of the study design was repeated cross sectional. The sampling method for study animals was random sampling. The list of all peasant association (PA) within each district of both areas was obtained from the districts agricultural office and Sampling of PAS was based on the random sampling methods for intervention area. Similarly, sampling of PAS was based on random type of sampling for control area.

3.4. Age and Body Condition Estimation

During sample collection various potential risk factors including sex, age, and body condition score of donkey was recorded. The age of the selected donkeys was determined from birth records of owners and by dentition (Crane and Svendsen, 1997). Body condition score (BCS) was subjectively estimated based on the guides published by (Svendsen 1997). Accordingly, donkeys were grouped into three age categories: donkeys from 1-3 years of age were classified as young; 3-10 years were considered as adult; and those beyond 10 years were classified as old (annex 4). These age classes were based on age of first work, productive age and the life span of Ethiopian donkeys (Svendsen, 1997; Yosef Shiferaw *et al.*, 2001). Regarding BCS, the studied animals were grouped poor, moderate, ideal, fat and obese (NEWC, 2002) (Annex 5).

3.5. Sampling Strategy

3.5.1. Intervention

There are 8 peasant associations where DHWP has conducted deworming strategy for the last 10 years based on the information that I have got from DHWP from Bisoftu campas. Among 8 peasant associations, 4 were selected by random sampling method. Each of this kebele has their own water point so that sampling of the donkeys were done at their corresponding water points by simple random sampling method.

3.5.2. Control area

Those kebeles which near were to the veterinary clinics were excluded because of the probability of getting anthelmintics that was given by Brooke Ethiopia to the professional who are responsible at the veterinary clinics based on the initial survey I have conducted before starting the research. Those kebele's which are near to Alagae college and accessible to transportation were selected for undertaking laboratory examination as soon as possible for qualitative faecal examination before hatching in to the first larval stage. Hence, 10 peasant associations were selected randomly. The numbers of the donkeys sampled from each kebele were according to the proportion of their total donkey number. Sampling of the donkeys was done at water points which are belonged to each kebele's.

3.5.3. Sample size

Random sampling method was used to select the study animal from each representative water point. Sample size was determined according to Thrusfield, (2005).

$$n = 1.96^2 P_{exp} (1 - p_{exp}) / d^2$$

Where:

n=required sample size

P_{exp}=expected prevalence

d=absolute precision

The prevalence of nematode gastrointestinal parasites from earlier study conducted in Boset that is one of the interventions areas of DHWP which has been reported to be 22.8% in donkeys (Abebew *et al.*, 2011) so that this value is used as the expected prevalence in the equation for sample size determination with 95% confidence interval and 5% desired absolute precision. The computed sample size value using 22.8% expected prevalence is obtained to be 272 which is the minimum sample size to be taken for intervention area. By using the same formula, sample size in control area, taking expected prevalence of 70% in and around South Wollo reported by Alemayehu and Etaferahu, (2013) was calculated to be 323 which is the minimum sample size to be taken in the control area. Hence, the sample size was determined to be 280 and 370 in intervention and control areas, respectively during the long dry season. The sample size was increased to 300 and 401 for both intervention and control areas, respectively during the beginning of the rainy season.

Table 8: Total number of donkeys sampled from the study areas.

Names of districts	Names of kebeles	Total number of donkeys	Donkeys sampled during dry season	Donkeys sampled during beginning of rainy seasons
Boset (interventions area)	Chelekelto	670	65	77
	Tiyowanga	1182	115	122
	Bulketatuludimto	442	43	43
	Tedecha	586	57	57
Subtotal intervention areas		2880	280	370
Adame tulu JidoKombolcha (control area)	Naka	596	33	32
	Reje	271	15	16
	Haleke	343	19	19
	Baraobicho	470	26	28
	Elealeaabo	415	23	24
	Chetogeto	380	21	21
	Lelisodenbia	651	36	36
	Jelaluto	1175	65	67
	Destabjata	2385	132	158
Subtotal control areas		6686	370	400

3.6. Data Collection

3.6.1. Faecal sample collection and laboratory processing.

Faecal samples were collected directly from the rectum of the donkeys. In all faecal sampling, samples were subjected to both gross and microscopic examination for the presence of adult helminthes or larvae, mucus, blood and consistency of faeces in all study areas.

Samples were collected in clean universal bottles and labelled. The collected samples were then kept in a refrigerator at about 4°C until faecal worm egg recovery and counts which were conducted within 48 hours of faecal sample collection. All faecal samples were transported to the Donkey Health and Welfare Project sites (Bishoftu), Alagae College Veterinary Parasitology Laboratory.

Faecal samples were collected two times from both interventions and control areas from December to March during the dry season (first sampling), June to the mid of July during the beginning of the rainy season (second sampling) for the prevalence studies of nematode parasites and epg counts. In all faecal samples collection, animals data (age, body condition score, sex concurrent diseases, management factors (housing, feeding and work type) were also recorded from each animal at the same time.

Qualitative Microscopic Examination of Faeces For Nematode Eggs.

Simple floatation was conducted for initial surveys of faecal egg counts. The principles, procedures and were attached to Annex 1 (Jorgen and Brian, 1994).

Quantitative McMaster Egg Counting Technique for Epg

A quantitative faecal examination was conducted using a McMaster egg counting technique to count *Strongylus* parasite eggs (epg). A flotation fluid (sodium chloride) was prepared at the laboratory and used to separate nematode eggs from faecal material in a counting chamber with two compartments (Annex 1). The number of eggs per gram of faeces was calculated as follows: add the egg counts of the two chambers together and multiply by 50 (Jorgen and Brian, 1994).

Larvascopy by Modified Baermann Technique.

Modified Baermann technique was used to isolate lungworm larvae, *S.westeri* and infective stage larvae of strongyles from faecal cultures. The faecal samples were cultured in an incubator at 27°C for seven days and recovered by the Baermann technique (MAFF, 1984). The larvae were identified to their specific genera and/or species according to the key description given by Bevilaqua *et al.*, (1993). Procedures of Baermann technique, preparation of faecal cultures, principles and equipments were written in details in Annex 3. Since there was interruption of light, the recommended 7 days were extended up to 10 days so that eggs could reach to their infective stages (larvae 3) (Lichtenfels *et al.*, 2008).

3.6.2. Physical Examination:

Each randomly selected donkey from both areas has been physically examined for any external body problems including wound, lameness, external parasites, sarcoids, dental abnormalities and recorded on a semi structured examination sheet. Age of the donkeys was determined by using the dentition method described by Pearson, (2000). Body condition score estimations have been made according to the method described by Donkey sanctuary, (1997).

3.6.3. Questionnaire survey

The semistructured questionnaire was subjective assessment based on the donkey owner perception and memory to get data on impact assessment of strategic mass deworming in donkeys from intervention area given by DHWP and on the general information of the donkeys. All sampled donkeys owner were interviewed from both intervention and control areas only during the long dry season. The questionnaire was pretested before the actual survey for time, resource and relevance of the type the questions included. The different questions were used to generate data from donkeys' owners on the strategic dewormings of donkeys. The details of the questions to both groups are attached to Annex 2.

3.7. Data Management and Analysis

Data of direct physical examination, questionnaire and laboratory results from both control and intervention areas were properly coded and entered into Microsoft Excel-2007 spreadsheet. The data was filtered for any invalid entry and then transferred to SPSS 20 version for windows package (2007) for statistical analysis. Descriptive and analytic statistics were made and results of the analysis were presented through tables. The association between prevalence of each studied parasite and the study variables (season, age, sex, study areas and body condition score) was analyzed by Chi-square test of independence. One way analysis of variance (ANOVA) was used to observe the variations of total mean epg of *Strongylus* with study areas and season. In all the analyses, confidence level was held at 95% confidence interval and P values ($p < 0.05$) were considered as significant. Multivariate logistic regression with Bonferroni multiple comparisons test was also applied that was very useful to see interaction among variables. The variables to be tested were age, sex, body condition score, and season and study areas.

4. RESULTS

4.1. Laboratory

4.1.1. Simple floatation

Parasites were identified based on their egg characteristics to nematode parasites according to Soulsby (1982).

O. equi

The overall prevalence of *O. equi* infection by simple floatation parasitological technique was found to be 7.6% and 10.4% in intervention and control areas, respectively. There was a statistical significance difference ($p < 0.05$) in prevalence between intervention and control areas (Table 9 and 10). On the contrary, there was no a statistical significance difference ($p > 0.05$) in infection between seasons and sexes in both intervention and control areas (Table 9 and 10).

Analysis of logistic regression revealed age and body condition scores to be significant risk factors ($p < 0.05$) for the occurrence of *O. equi* between intervention and control area (Table 9 and 10). Hence, the likelihood of occurrence of infection of *O. equi* among different risk factors in intervention area were young age (odd ratio (OR) = 10.3, 95% CI = 1.25, 84.98) and animals with poor body conditions (OR = 8.84, 95% CI = 1.13, 68.97) and moderate (OR = 1.79, 95% CI = 0.23, 14.1) (Table 9 and 10).

Similarly, the likelihood of occurrence of infection of *O. equi* among different risk factors in control area were young age (OR = 14.35, 95% CI = 3.18, 64.69), adult age (OR = 2.4, 95% CI = 0.54, 10.69) and animals with poor body conditions (OR = 5.87, 95% CI = 2.2, 15.69) and moderate (OR = 2.12, 95% CI = 0.97, 1.65) (Table 9 and 10).

P. equorum

Over all prevalence of *P. equorum* in both intervention and control area by simple floatation parasitological technique was 15.9%, 23.6% in intervention and control area, respectively

(Table 9 and 10). Infection prevalence of *P. equorum* was statistically significant ($p < 0.05$) between control and intervention areas (Table 9 and 10). On the contrary, there was no statistical significance difference ($p > 0.05$) in infection prevalence in relation to seasons and sexes in both intervention and control areas (Table 9 and 10). The logistic regression analysis of the risk factors indicated the presence of strong association in the occurrence of infection (OR=1.64, 95% CI=1.24, 2.16) of *P. equorum* between intervention and control area (Table 10 and 11). Age and body condition scores were also found to be the risk factors ($p < 0.05$) by the analysis of logistic regression in both intervention and control areas (Table 9 and 10). Hence, the likelihood of occurrence of infection of *P. equorum* among different risk factors were young age (OR = 5.14 and 5.73, 95% CI = 1.42, 18.68 and 2.31, 14.21), adult age (OR=1.54 and 2.85, 95% CI = 0.45, 5.29 and 1.2, 6.78) and animals with poor body conditions (OR=10.03 and 6.51, 95% CI=1.31, 76.47 and 3.34, 12.7) and moderate body conditions (OR=8.38 and 2.06, 95% CI = 1.13, 62.24 and 1.26, 3.3) in intervention area and control areas, respectively (Table 9 and 10).

Table 9: The prevalence of *Pequorum* and *O.equis* with respective categories of the risk factors in the intervention area.

Risk factor	Sub variables	Number examined	Number of (Prevalence) positive in intervention area					
			<i>P. equorum</i>	χ^2 (p-value)	Odd ratio	<i>O.equis</i>	χ^2 (p-value)	Odd ratio
Age	Young	79	28(35.4%)	26.57(0.0001)	1	19(24%)	35.53(0.000)	1
	Adult	469	61(13%)		5.14(1.42,18.68)	24(5%)		10.3(1.25,84.98)
	Old	32	3(9.4%)		1.54(0.45,5.29)	1(3.2)		
Body condition	Poor	145	28(19.3%)	8.366(0.015)	1	25(17.%)	26.134(0.000)	1
	Moderate	386	63(16.3%)		10.03(1.31,76.47)	18(4.7%)		8.84(1.13,68.97)
	Good	49	1(2%)		8.38(1.13,62.24)	1(2%)		1.43(0.772,2.66)
Season	Dry	280	42(15.0%)	0.301(0.583)		18(6.4%)	1.04(0.309)	1.79(0.23,14.1)
	Rainy	300	50 (16.7%)			26(8.7%)		
Sex	Male	276	48(17.4%)	0.923(0.337)	1	26(9.8%)	2.53(0.112)	1
	Female	304	44(14.5%)		1.19(0.75,1.89)	18(5.6%)		1.39(0.71,2.73)

Table 10: The prevalence of *P. equorum* and *O. equis* with respective categories of the risk factors in the control area.

Risk factor	Sub variables	Number examined	Number of (Prevalence) positive in control area					
			<i>P. equorum</i>	x2 (p-value)	Odd ratio	<i>O. equis</i>	x2 (p-value)	Odd ratio
Age	Young	190	67(35.3%)	20.978(0.000)	1	49(25.8%)	64.64(0.1)	12.4(0.54,10.69)
	Adult	524	108(20.6)		5.73(2.31,14.2)	29(5.5%)		14.35(3.18,64.69)
	Old	57	7(12.3 %)		2.85(1.2,6.78)	2(3.5%)		
Body condition	Poor	77	33(42.9%)	27.045(0.000)	1	14(18.2%)	11.967(0.003)	1
	Moderate	517	126(24.4%)		6.51(3.34,12.7)	58(11.2%)		5.87(2.2,15.69)
	Good	177	23(13%)		2.06(1.26,3.38)	8(4.5%)		2.12(0.97,1.65)
Season	Dry	370	86(23.2%)	0.052(0.82)	1.14(0.8,1.61)	36(9.7%)	0.32(0.572)	1.001(0.97,1.65)
	Rainy	401	96(23.9)		1	44(11.0%)		1
Sex	Male	293	79(27%)	2.953(0.086)	1	36(12.3%)	1.86(0.17)	
	Female	478	103(21.5%)		1.16(0.81,1.66)	44(9.2%)		

Strongylus

Qualitative faecal analysis by using simple floatation parasitological technique also revealed the overall prevalence of Strongylus to be 545(94.0%) and 771(100%) in intervention and control areas. There was a statistical significance difference ($p < 0.05$) in prevalence of Strongylus in working donkeys between intervention and control areas (Table 11). On the contrary, there was no a statistical significance ($p > 0.05$) in infection between long dry and beginning of rainy seasons in both intervention and control areas (Table 12).

4.1.2. Larvascopy

Species of strongylus

The current study of larvascopy revealed over all prevalence of *S. vulgars* (68.3% and 97%), *S.edentates* (19.5% a and 91%) ,*S. equines* (22.6% and 45.79%), Tridontophorus spp (8.4% and 18.2%), Cyathostomum (81.7% and 100%) and *T. axei* (62.2% and 92.5%) in intervention and control areas, respectively (Table 11). There was a statistically significant difference ($p < 0.05$) in prevalence between intervention and control areas (Table 11). On the contrary, there was not a statistically significance difference ($p > 0.05$) in prevalence between the seasons of both intervention and control areas (Table 12).

T. axei

The current study of larvascopy revealed over all prevalence of *T. axei* (62.2% and 92.5%) in intervention and control areas, respectively (Table 11). There was a statistically significant difference ($p < 0.05$) in prevalence between intervention and control areas (Table 11). On the contrary there was no a statistically significance difference ($p > 0.05$) in prevalence between seasons in intervention and control areas (Table 12).

Table 11: Coprological comparison of nematode parasites in both intervention and control areas.

Parasite species	Prevalence (%)		X ² (p-value)	Odds ratio (95%CI)
	Intervention	control		
<i>S.vulgaris</i>	399(68.3%)	748(97%)	205.67(0.01)	14.75(9.402, 23.15)
<i>S.edntatus</i>	113(19.5%)	702(91%)	707.27(0.01)	41.96(30.42,57.87)
<i>S.equinus</i>	131(22.6%)	353(45.79%)	78.47(0.01)	2.92(2.29, 3.71)
Cyathostomum	474(81.7%)	771(100%)	152.9(0.001)	-
<i>T.axei</i>	361(62.2%)	713(92.5%)	185.65 (0.01)	7.46(5.44, 10.23)
Triodontoporus	49 (8.4%)	140(18.2%)	25.94(0.001)	2.407(1.7, 3.4)
<i>S.westeri</i>	155(26.7%)	362(47%)	57.33(0.01)	2.427(1.924, 3.06)
<i>D.arnfeldi</i>	123(21.2%)	381(49.4%)	112.62 (0.01)	3.6(2.84,4.63)
<i>O.equi</i>	44(7.6%)	80(10.4%)	3.09(0.08)	1.41(0.96, 2.07)
Strongylus	545(94%)	771(100%)	47.76(0.0001)	-----
<i>P.equorum</i>	92(15.9%)	182(23.6)	12.27 (0.01)	1.639 (1.241, 2.07)

Table 12: Seasonal comparisons of larvescopy result of strongylus and *T.axei* in control area and intervention areas.

Parasite species	Prevalence (%) in control area		x2 (p-value)	Prevalence (%) in intervention area		x2 (p-value)
	Dry	Rainy		Dry	Rainy	
<i>S.vulgaris</i>	358(96.8%)	390(97.3%)	0.17(0.83)	190(67.9%)	209(69.7%)	0.221(0.638)
<i>S.edntatus</i>	332(89.7%)	370(92.3%)	1.523(0.21)	51(18.3%)	62(20.7%)	0.524(0.47)
<i>S.equinus</i>	160(43.5%)	193(48.2%)	1.76(0.19)	54(19.3%)	77(25.7%)	3.373(0.066)
Cyathostomum	370(100%)	401(100%)	-	225(80.4%)	249(83%)	0.677(0.411)
<i>T.axei</i>	336(90.8%)	377(94%)	2.84(0.09)	173(61.8%)	188(62.7%)	0.048(0.82)
Triodontoporus	64(17.3%)	76(19%)	0.35(0.55)	22(7.9%)	27(9%)	0.245(0.62)

D. arnfieldi

The result of the present study indicated an overall prevalence of 21 , 49.4% of lungworm in donkeys in intervention (580) and control areas (771), respectively, which showed a statistical significance difference ($p < 0.05$) between intervention and control areas (Table 13 and 14). There was no a statistical significance difference ($p > 0.05$) in prevalence with regard to seasons and sexes in both intervention and control areas (Table 13 and 14). The analysis of logistic regression in both intervention and control areas revealed age and body condition scores to be significant risk factors ($p < 0.05$) for the occurrence of the disease (Table 13 and 14). Hence, the likelihood of occurrence of infection of *D. arnfieldi* among different risk factors by the post hoc Bonferroni multiple comparisons test were young age (OR =5.01 and 7.96 , 95% CI =1.85, 13.51 and 3.9, 16.23), adult (OR =0.7 and 1.43, 95% CI=0.28, 1.74 and 0.77, 2.66) and animals with poor (OR=3.56 and 20.21, 95%CI =1.36, 9.29 and 10.03, 40.74) and moderate (OR=1.05 and 5.581, 95% CI=0.41, 2.68 and 3.57, 8.72) in intervention area and control area ,respectively (Table 13 and 14).

S. westeri

The overall prevalence of *S. westeri* was found to be 26.7, 47 % in intervention and control areas, respectively with a statistical significance difference ($p < 0.05$) in the infection between intervention and control areas (Table 13 and 14). It was also found that age and body condition status were significantly ($p < 0.05$) affecting the prevalence of *S. westeri* in both study areas. On the contrary, sex and seasons did not show a statistically significant difference ($p > 0.05$) in the prevalence of the infection of *S. westeri* in intervention and control areas. The Analysis of logistic regression in both intervention and control areas revealed age and body condition scores to be significant risk factors for *S. westeri* ($p < 0.05$) (Table 13 and 14). The likely odd ratio of ages by Bonferroni multiple comparisons test were found to be (OR = 7.96 and 7.96, 95% CI= 3.9, 16.23 and 3.9, 16.23) for young age and (OR=1.43 and 1.43, 95%CI = 0.772, 2.66 and 0.77, 2.66) for adult age in intervention and control areas, respectively (Table 13 and 14). Animals with poor (OR= 20.21 and 20.21, 95%CI =10.03, 40.74 and 10.03, 40.74) and moderate (OR=5.58 and 5.581, 95% CI =3.57, 8.72 and 3.57, 8.72) body condition scores were also found to be highly significant ($p < 0.05$) in intervention and control areas, respectively (Table 13 and 14).

Table 13: Prevalence of *S.westeri* and *D.arnfieldi* with their risk factors intervention area.

Risk factor	Number of (Prevalence) positive in intervention area						Odd ratio
	N.of examine d	<i>S.westeri</i>	x2 (p)		<i>D.arnfieldi</i>	x2 (p)	Odd ratio
Age							
Young	79	56(70.9%)	93.39(0.000)	1	44(55.7%)	65.87(0.000)	1
Adult	469	89(19%)		7.96(3.9,16.23)	72(15.4%)		5.01(1.85-13.51)
Old	32	10(31.2%)		1.43(0.772, 2.66)	7(21.9%)		0.7(0.28-1.74)
Body condition							
poor	145	70(48.3%)	52.6(0.000)	1	53(36.6%)	27.73(0.000)	1
moderate	386	83(21.5%)		20.21(10.03,40.74)	64(16.6%)		3.56(1.36, 9.29)
Good	49	2(4.1%)		5.58(3.57,8.72)	6(12.2%)		1.05(0.41-2.68)
season							
Dry	280	72(24.6%)	1.4(0.237)	1.325(0.96, 1.84)	56(19.1%)	1.55(0.213)	
rainy	300	83(28.9%)		1	67(23.3%)		
Sex							
Male	276	79(28.6%)	0.97(0.325)		59(21.4%)	0.01(0.92)	
Female	304	76(25%)			64(21.1%)		

Table 14: Prevalence of *S.westeri* and *D.arnfeldi* with their risk factors in control area.

Risk factor	Number of (Prevalence) positive in control area						
	N.exami ned	<i>S.westeri</i>	x ² (p)	Odd ratio	<i>D.arnfeldi</i>	x ² (p)	Odd ratio
Age							
Young(n =)	190	158(83.2%)	133.02(0.000	1	148(77.9%)	82.16(0. 0001)	1
Adult(n=)	524	186(35.5%)		31.056 (13.36, 72.08)	208(39.7%)		7.96(3.9,1 6.23)
Old	57	18(31.6%)		2.97(1. 415,6. 216)	25(43.9%)		1.43(0.77, 2.66)
BCS							
poor	77	64(83.1%)	84.39(0. 001)	1	59(76.6%)	95.6(0.0 001)	1
moderate	517	258(49.9%)		32.975 (14.89, 72.9)	288(55.7%)		20.21(10. 03,40.74)
Good	177	40(22.6%)			34(19.2)		5.581(3.5 7,8.72)
season							
Dry	370	165(44.6%)	1.587(0. 208%)	1.002(0.712, 1.41)	182(49.2)		1.325(0.9 95,1.403)
rainy	401	197(49.1%)		1	199(49.6)	0.015(0. 904)	1
Sex							
Male	293	148(50.5%)	2.405(0. 121)		157(53.6%)	3.283(0. 07)	
Female	478	214(44.8%)			224(46.9%)		

4.1.3. MacMaster egg counting Technique

The mean eggs counts of strongylus detected by the MacMaster egg counting technique analyzed by analysis of variance revealed (304.6 and 1293.00) during dry season and (307.32 and 1456.53) during the rainy season in intervention and control areas, respectively (Table15). There was a statistically significant difference ($p < 0.05$) in epg counts of Strongylus between intervention and control areas during both seasons. The mean epg counts of Strongylus in intervention area did not show a statistically significant difference ($p > 0.05$) during long dry (304.60) and beginning of rainy seasons (307.32) (Table15). On the contrary, the mean epg counts of the Strongylus in control area showed statistical significant difference ($p < 0.05$) between dry season (1293.00) and beginning of rainy season (1456.53) (Table15 and Table 16).

Table 15: Comparative epg assessment of both intervention and control areas during dry and rainy seasons.

Variation of epg assessment on the study areas					
Risk factor	Number of animals	Mean	SE	F	p-value
epg of intervention area					
Season					
Dry	250	304.60	248.830	0.02	0.888
Rainy	280	307.32	195.720		
Epg of control area					
Season					
Dry	370	1293.00	36.368	8.695	0.003
Rainy	401	1456.53	41.389		
Epg comparison of intervention and control area during dry season					
area					
Control	370	1293.00	36.368	459.587	0.000
intervention	250	304.60	15.737		
Epg comparison of intervention and control area during rainy season					
area					
Control	400	1456.53	41.389	519.122	0.0001
intervention	280	307.32	11.697		

Table 16: The comparison of level of severity of infection of Strongylus based on epg count from both study areas.

Level of severity of Strongylus	Season	Study areas	
		Intervention	Control
Dry seasons			
Low		219(87.3)	47(12.7)
Moderate		29(11.6)	133(35.9)
Severe		3(1.2)	190(51.4)
Rainy seasons			
Low		250(89.3)	60(15.0)
Moderate		29(10.4)	110(27.5)
Severe		1(.4)	230(57.5)

4.2. Physical Examination

The analysis of the present physical examination pinpointed overall prevalence of non-nematode parasitic health related problems of working donkeys including sarcoid (1.4% and 1.1%), wound (19.7% and 14.3), dental abnormalities (3% and 3.2%), lameness (7.6% and 1.1%), knits of *Gastrophilus* (31.9% and 32.7%) , mixed diseases (27.6 % and 17.1%) and abnormal visible mucus membrane (31.9% and 32.7%) in control and intervention areas, respectively(Table 17). Chi-square analysis on sarcoid, wound, dental abnormalities, knits of *Gastrophilus* and abnormal mucus membrane did not show a statistical significance difference ($P>0.05$) in prevalence between control and intervention areas On the contrary, there was a statistical significance difference ($P<0.05$) in prevalence with regard to lameness and mixed diseases between control and intervention areas (Table17). The mean body condition score of the working donkeys were found to be 1.98 and 2.01 in control and intervention areas that were not statistically significant ($p>0.05$)

Table 17: Parasitic and non nematode parasitic health related problems of working donkeys identified by physical examination.

S. No.	Variable	Description	Study Areas		Total (%)	Chi Square (P Value)
			Control (%)	Intervention (%)		
1	Wound	Positive	73 (19.7)	40 (14.3)	113 (17.4)	3.289 (0.07)
		Negative	297 (80.3)	240 (85.7)	537 (82.6)	
		Total	370 (100)	280 (100)	650 (100)	
2	Lameness	Positive	28 (7.6)	3 (1.1)	31 (4.8)	14.809 (0.001)
		Negative	342 (92.4)	277 (98.9)	619 (95.2)	
		Total	370 (100)	280 (100)	650 (100)	
3	GIT Parasite	Positive	370 (100)	271 (96.8)	641 (98.6)	12.06 (0.001)
		Negative	0 (0.0)	9 (3.2)	9 (1.4)	
		Total	370 (100)	280 (100)	650 (100)	
4	Dental Abnormalities	Positive	11 (3.0)	9 (3.2)	20 (3.1)	0.031 (0.86)
		Negative	359 (97.0)	271 (96.8)	630 (96.9)	
		Total	370 (100)	280 (100)	650 (100)	
5	Other Diseases	Negative	364 (98.4)	276 (98.6)	640 (98.5)	0.141 (0.932)
		Sarcoide	5 (1.4)	3 (1.1)	8 (1.2)	
		Total	370 (100)	280 (100)	650 (100)	
6	Mixed Diseases	Positive	102 (27.6)	48 (17.1)	150 (23.1)	9.758 (0.002)
		Negative	268 (72.4)	232 (82.9)	500 (76.9)	
		Total	370 (100)	280 (100)	650 (100)	
7	Mucus membrane	pink	366(47.5%)	265(45.7%)	631(46.7%)	0.422(0.516)
		Pale	405(52.5)	315(54.3%)	720(53.3%)	
		Total	370 (100)	280 (100)	650 (100)	
8	Knits of Gastrophilus	Positive	128(31.9%)	98(32.7%)	226(32.2%)	0.044(0.834)
		Negative	273(68.1%)	202(67.3%)	475(67.8%)	
		Total	370 (100)	280 (100)	650 (100)	

There was no statistical significance difference ($P>0.05$) in body condition score of donkey between the two study areas (Table 18)

Table 18: The result of working conditions and body condition score in donkeys.

Variables	Study area	Number	Mean	95% Confidence Interval for Mean		F value	P - value
				Lower Bound	Upper Bound		
					Control area		
Effect of load	Intervention area	280	2.18	2.10	2.25		
	Total	650	2.07	2.03	2.11		
	Control area	370	1.01	1.00	1.02		0.01
Effect of hours	Intervention area	280	1.21	1.17	1.26	23.625	
	Total	650	1.10	1.08	1.12		
	Control area	771	1.98	1.93	2.02	1.134	
BCS	Intervention area	580	2.01	1.97	2.05		
	Total	1351	1.99	1.96	2.02		.287

4.3. Questionnaire Survey

A total of 650 donkey's owners (280) from intervention and (370) from control) area were interviewed for knowledge assessment on strategic mass deworming of donkeys with ivermectin from intervention area as opposed to the control area. Questions were translated into Afan Oromifa. Analysis of the current questionnaire survey on the general information revealed donkey type as home heard (48.9%, 48.2%) and bought (51.1%, 51.8%) , number of donkeys owned 1-4 (93.5%, 95.7%) and 5-8 (6.5%, 4.3%), on how long they kept their donkeys those who kept for 1-3 years (62.6%, 56.1%) and 4-10 years (35.5%, 43.6%, greater than 10 years (2%, 0.4%), working

days per week 1-4 (98.9%,78.6%) from both control and intervention areas, respectively (Table18). There was no a statistical significance difference ($p>0.05$) among respondents on their responses on these general type of questions as opposed to other types of questions (Table19). On the contrary, the proportion of respondents on the fattening ability of deworming was found to be 182 (49.2%) and 280 (100%) in both control and intervention areas, respectively (Table19).

The proportion of the respondents to the working hour(s) of their donkeys per day which was categorized into 1-4 hr (98.9%, 78.6%) and 5-8hr (1.1%, 21.4%) from control and intervention areas, respectively (Table 19). The questionnaire survey analysis also showed a statistically significant difference ($p<0.05$) on the working load of their donkeys among the respondents, the working load was categorized according to the work of Girma *et al.*, (2014) who classified the working load of donkeys in Yilmana Densa into three classes. Analysis of the current questionnaire survey also revealed a statistical significant difference ($p<0.05$) as to the working load of donkeys among respondents. Based on owner's responses 40Kg (43% and 12.9%), 50kg -60kg (85.1% and 53.4%), (7.6% and 16.1%) and 80kg (3% and 35.7%) control and intervention areas, respectively (Table19) (Girma *et al.*, 2014).

There was a statistical significance difference ($p<0.05$) among respondents from intervention area as opposed to control area to questions related to deworming other animals, diseases not prevented by deworming, fattening importance of deworming, cares used for diseases not prevented by deworming (Table 19).

Table 19: Summary of the major focus points as perceived by donkey owners during questionnaire survey from both control and interventions areas.

S. No.	Variable	Description	Study areas		Total (%)	Chi Square and (P Value)
			control	interventions		
1	Deworming other animals	Yes	181(48.9%)	197(70.4%)	378(58.2)	30.102(0.01)
		No	189(51.1%)	83(29.6%)	272(41.8)	
		Total	370(100%)	280(100%)	650(100%)	
2	Diseases not prevented by deworming	Yes	84(22.8%)	129(48.9%)	213(33.6)	46.953(.0.01)
		No	285(77.2%)	135(51.1%)	420(66.4%)	
		Total	370(100%)	280(100%)	650(100%)	
3	Fattening importance of deworming	Yes	182(49.2%)	280(100%)	462(71.1%)	200.164(0.001)
		No	188(50.8%)	0(0%)	188(28.9%)	
		Total	370(100%)	280(100%)	650(100%)	
4	Cares used for diseases not prevented deworming	Antibiotics	6(10.2%)	23(30.7%)	29(21.6%)	13.382(0.01)
		Antibiotics and vaccinations	37(62.7%)	25(33.3%)	62(46.3%)	
		Vaccinations	16(27.1%)	27(36%)	43(32.1%)	
		Total	59(100%)	75(100%)	134(100%)	
5	How long you kept your donkeys	1-3 years	224(62.6%)	157(56.1%)	381(59.7%)	6.950(0.031)
		4-10 years	127(35.5%)	122(43.6%)	249(39.0%)	
		Greater than 11 years	7(2%)	1(0.4%)	8(1.3%)	
		Total	358(100%)	280(100%)	638(100%)	
6	Donkey Type	Home	181 (48.9)	135 (48.2)	316 (48.6)	0.032

	Bought	189 (51.1)	145 (51.8)	334 (51.4)	(0.859)
	Total	370 (100)	280 (100)	650 (100)	
7	Number of donkey owned by the owner				
	1-4	346 (93.5%)	268 (95.7)	614 (94.5)	1.476
	5-8	24 (6.5)	12 (4.3)	36 (5.5)	(0.224)
	Total	370 (100)	280 (100)	650 (100)	
8	Working hours per day				
	1-4	366 (98.9)	220 (78.6)	586 (90.2)	74.339 (0.001)
	5-8	4 (1.1)	60 (21.4)	64 (9.8)	
	Total	370 (100)	280 (100)	650 (100)	
9	Working days per week				
	2-4	49 (13.2)	29 (10.4)	78 (12.0)	1.257 (0.262)
	5-7	321 (86.8)	251 (89.6)	572 (88.0)	
	Total	370 (100)	280 (100)	650 (100)	
10	Working load				
	40	16(4.3%)	36(12.9%)	52(8%)	150.8(0.001)
	50-60	343(92.7%)	144(51.4%)	487(74.9%)	
	80	11(3%)	100(35.7%)	111(17.1%)	
	Total	370(100%)	280(100%)	650(100%)	

The outcome of questionnaire survey revealed that 100% of owners from intervention and control areas had no awareness on questions related to donkey parasites such as signs on internal parasites, their means of transmission and time or months they commonly occur in donkeys. According to the current questionnaire survey, 100% of respondents from control area had never been given any anthelmintics and had no knowledge on these drugs to be given to donkeys. In addition to this, 100% of respondents from both intervention and control areas also kept their animals outside mixed with other animals without any protection. On the contrary, 100% of respondents from intervention area had brought their donkeys for strategic mass deworming and owner perception in changes on their donkeys they observed after strategic deworming was presented (Table 20).

Table 20: The responses of the donkey owner for changes observed on their donkeys after strategic mass deworming in intervention area.

Responses	Frequency	Percent (%)
Active	7	2.5
Good conditions	220	78.6
Active, good conditions	16	5.7
Increased appetite	19	6.8
Active, good conditions and increased appetite	16	5.7
Good conditions and no coughing	1	.4
Active and increased appetite	1	.4

Analysis of the current questionnaire survey on frequency of deworming in intervention area was found to be for 1-3 years (61.5% and 67.6%), 3.5-6 years (36.3% and 31%) and greater than 6 years (2.2% and 1.4%) in home and bought type of donkeys, respectively (Table 20). There was no a statistical significance difference ($P>0.05$) in the frequency of deworming between home and bought type of donkeys among the 3 years of categories) (Table 21).

Table 21: Total frequency of deworming versus donkeys type in intervention area.

Donkey type	Frequency			Total	Chi square (p-value)
	1-3 year	3.5-6 year	6-10year		
Home	83(61.5%)	49(36.3%)	3(2.2%)	135(100%)	1.258(0.533)
Bought	98(67.6%)	45(31%)	2(1.4%)	145(100%)	
Total	181(64.6%)	94(33.6%)	5(1.8%)	280(100%)	

5. DISCUSSION

5.1 Laboratory

5.1.1 Simple floatation

Donkeys sampled from control areas were 100% positive for Strongyles eggs where as donkeys sampled from intervention were 94% positive for eggs of strongylus. A chi-square analysis indicated a statistical significance difference ($P < 0.05$) in prevalence between intervention and control areas. The prevalence in control area was in consistent with the findings of), 100 % in Dugda Bora districts (Ayele *et al.*, 2006) , 100% in project and control areas (Abebew *et al.*, 2011) and 99.5% in Sululta and Gefersa (Zerihun *et al.*, 2011. On the contrary , prevalence result disagreed with 83% in Gambia (Mattioli *et al.*, 1994) and 94.1% in and around Bahir Dar (Bewketo *et al.*, 2013). The current prevalence in intervention area was consistent with the work of Bewketo *et al.*, (2013) who has reported 94.1% in and around Bahir Dar. Lower infection of strongyles in the intervention area could be related to the impact of strategic mass deworming in intervention area as opposed to control area. The other possible reasons attributable to the variation in the occurrence of Strongylus might be due to husbandry practices, agro ecology and general veterinary activities including the application of anthelmintics.

Larval cyathostomes encyst in the caecal and colonic mucosa, where they induce a mild inflammatory enteropathy that causes a subclinical alteration in gastrointestinal function (Lind *et al.*, 2007). Ivermectin does not have effect on the larval cyathostomes where as it has effect all stages of large Strongylus. Consequently, the prevalence of *S.vulgaris* became reduced markedly by using ivermectin for controlling nematode parasites (Chapman *et al.*, 2002; Love, 2003; Boxell *et al.*, 2004; Traversa *et al.*, 2007).

The overall prevalence of *O.equi* in the current study appeared to be (7.6% and 10.4%) in the intervention and control areas, respectively. There was no a statistically significance difference in prevalence between intervention and control areas ($p > 0.05$). It was not in line with previous findings of 17.7 % in South Africa (Well *et al.*, 1998), 16.2% in Berehi (Getachew, 2008) and 31.8% (Nuraddis *et al.*, 2011) in Hawassa .The current result of *O.equi* from both study areas was in agreement with previous reports of 6% Dugda Bora districts

(Ayele *et al.*, 2006), 6.45% Turkey (Sinasi and Mustafa, 2009), 9.8% in Sululta and Gefersa (Zerihun *et al.*, 2011) and 4.5% in South Wollo (Alemayehu and Etaferahu, 2013).

Because of the egg-laying behaviour of the adult female around the perianal region, acetate tape preparations are primarily used to diagnose oxyuris infection, and recovery of eggs through faecal flotation is rare (Soulsby, 1982). The possible capture of eggs from the perianal region during faecal sampling could be one explanation for such a low recovery rate of *O. equi* in donkeys from both areas as simple floatation was used to detect their eggs.

Lack of statistical significance between sexes was in agreement with the work of (Getachew, 2006) in Ada, Akaki and Berehi. But, this finding disagreed with the work of (Ayele *et al.*, 2006) in Dugda Bora districts and (Alemayehu and Etaferahu, 2013) in South Wollo whom reported more prevalence in males than females. The current study also showed higher occurrence of parasitism in young and adult ages than older donkeys in both control and intervention areas ($p < 0.05$). This finding agreed with the work of Alemayehu and Etaferahu, (2013) in South Wollo, Zerihun *et al.*, (2011) in Sululta and Gefersa. On the contrary, this finding disagreed with the work of (Getachew, 2006) in Ada, Akaki and Bereh and (Nuraddis *et al.*, 2011) in Hawassa. This could be attributed to the fact that young animals do not have well organized immune system which can result in the higher chance of parasitism than in older equine (Radostits *et al.*, 2010). Other possible source of variation could also be related to difference in methodology, statistical analysis and agro ecological variations.

O. equi was the only nematode parasites which did not show a statistical significance ($p > 0.05$) in prevalence between intervention and control areas in prevalence despite strategic mass deworming parasites control methods are being implemented in intervention area by DHWP as opposed to control area. Similar result on the lack of a statistical significance difference in prevalence between the two study areas was also reported across different countries. There was no a statistic significance difference ($p > 0.05$) in prevalence between control and intervention areas during the long dry and the beginning of the rainy seasons. It could be related to long prepatent period which may take 4½–5 months (Hasslinger, 1990; Urquhart *et al.*, 1996).

This result coincided with the previous finding of Wolf *et al.*, (2014) who detected recurrence of *O. equi* egg shedding in three out of six horses within 1–4 weeks after treatment with moxidectin and ivermectin in Germany. Other reports of treatment failures using ivermectin

have recently been published (Durham and Coles, 2010; Reinemeyer *et al.*, 2010), as well as two documented records from the America and New Zealand (Reinemeyer, 2012; Rock *et al.*, 2013). Other reports of treatment failures of ivermectin from the United States, New Zealand and United Kingdom have recently been published (Durham and Coles, 2010; Reinemeyer *et al.*, 2010; Reinemeyer, 2012; Rock *et al.*, 2013). Additionally, Reinemeyer (2012) also reported on survival of *O. equi* after ivermectin medication in three horses in Tennessee. These horses were treated with ivermectin followed by pyrantel pamoate two weeks later. This resulted in adult worm findings in the faeces, indicating survival of the first treatment. Similar cases were also reported from two horses from different locations in New Zealand. One animal was treated with ivermectin and the other with abamectin and both received a consecutive treatment with oxfendazol after 8 days leading likewise to the expulsion of several adult worms (Rock *et al.*, 2013).

The overall prevalence of *P. equorum* was found to be 15.9%, 23.6% in donkeys in intervention and control areas, respectively. This result indicated a significant difference ($P < 0.05$) in prevalence between intervention and control areas. The result of current study was higher than earlier reports of 2.8% in Germany (Gothe and Heil, 1984), 8.4% in India (Kotwal *et al.*, 2000) and 10.4% in South Wollo (Alemayehu and Etaferahu, 2013). However, the current prevalence of *P. equorum* from both study areas are lower than the previous 72% in Chad (Graber, 1970), 43% in B. Faso (Vercruysse *et al.*, 1986), 50% in Zimbabwe (Pandey and Eysker, 1990), 46% in Boset (Feseha *et al.*, 1991, Getachew, 1999), 25% in South Africa (Matthee *et al.*, 2000), 48.8% in South and North Wollo (Mulate *et al.*, 2005), 50% in Dugda Bora (Ayele *et al.*, 2006), 61.3% in Ada (Getachew, 2006), 49.7% in control area (Abebew *et al.*, 2011), 52.8% Hawassa (Nuraddis *et al.*, 2011), 53.2% in Sululta and Gefer sa Zerihun *et al.*, 2011) and 42.29% in Gonder (Mezgebu *et al.*, 2013).

The present 15.9% prevalence from intervention area was in agreement with former reports of 13% in USA (Tolliver *et al.*, 1985), 17% in Kenya (Lewa *et al.*, 1998), 15.7% in central Ethiopia (Yoseph *et al.*, 2001). 23.6% prevalence result from control area was consistent with previous finding of 20% in Turkey (Burgu *et al.*, 1995) 22.58% in Turkey (Sinasi and Mustafa, 2009) and 22.2% in Boset (Abebew *et al.*, 2011). Significance lower of prevalence of *P. equorum* in intervention area could be explained by fact that due to the impacts of strategic mass deworming of donkeys as opposed to the control area.

There was no a statistical significance difference($p<0.05$) between sexes It was in agreement with the work of Getachew, (2006) in Ada, Akaki and Bereh and Mezgebu *et al.*, (2013) in Gonder whom reported lack of significance between sexes. This finding disagreed with the work of (Bewketu *et al.*,2013) in Bairdar and Alemayehu and Etaferahu, (2013) in South Wollo. The current study also showed a higher rate occurrence of parasitism in young and adult ages than in older donkeys in both control and intervention areas. This finding agreed with work of Zerihun *et al.*, (2011) in Sululta and Gefersa, Bewketu *et al.*, (2013) in Bairdar and Alemayehu and Etaferahu, (2013) in South, Mezgebu *et al.*, (2013) in Gonder and Ayele *et al.*, (2006) in Dugda Bora District. On the contrary, this finding disagreed with the work of Getachew, (2006) in Ada, Akaki and Bereh nd Nuraddis *et al.*, (2011) in Hawassa. More *P.eqorum* were found in animals with poor body condition than well condition ones and similar findings with past reports of (Ayele *et al.*, 2006),(Zerihun *et al.*, 2011).

There was no statistical significance difference in prevalence between seasons in both control and intervention areas ($p>0.05$) .This lack of significance difference associated with high fecundity of female ascarids and the extreme resistance of their eggs to adverse environmental conditions ensure their persistence for several years. The adhesive property of the eggs facilitates passive spread through stables, paddocks and pastures (Austin *et al.*, 1990; Uhlinger, 1993; Ihler, 1995; Southwood *et al.*, 1998). A as a result, environment may remain a source of infection for a long period. Thus the biology of this parasite makes it unlikely that a change of season would greatly affect the prevalence of ascarid infection in equids since they can also be transmitted by mechanical means of transmission.

The current result was in agreement with this in that almost similar proportion of prevalence was obtained both during the wet and dry seasons of the years .This current finding was in line with the work done by Gethachew *et al.*, (2006). Observed variation in infection prevalence among the different areas could be attributed to the impact of strategic mass deworming strategy in intervention as opposed to control area, frequency of the deworming and appropriate dosage administered in case of intervention area and methodology, management, environmental conditions such as soil types or temperature, which may play a significant role in development of ascarid eggs for all areas where the disease have occurred. Ivermectin and moxidectin can also all parasitic life cycle stages within the host, including migrating larvae (Reinemeyer, 2012).

5.1.2 Larvascopy

The current study of larvascopy revealed over all prevalence of *S. vulgaris* to be (68.3% and 97%) in intervention and control areas, respectively. There was a statistically significance difference ($p < 0.05$) in prevalence between the study areas. The *S. vulgaris* in intervention area has lowered. significantly than previously reported prevalence in different areas 83% (Mattioli *et al.*, 1994) in Gambia, 100 (Matthee *et al.*, 2000) in South Africa ,100 (Ayele *et al.*, 2006) Dugda Bora, (Abebew *et al.*, 2011) in project area and 100% (Zerihun *et al.*, 2011) in Sululta and Gefersa. The overall current prevalence of *S.vulgaris* from control areawas consistent with previous reports who have reported similar result in Gambia (Mattioli *et al.*, 1994), in South Africa (Wells *et al.*, 1998; Matthee *et al.*, 2000), Dugda Bora (Ayele *et al.*, 2006) in control area (Abebew *et al.*, 2011). The high prevalence from control area was consistent with the results of the studies by (Pandey and Eysker,1989; Feseha, 1998; Getachew, 1999). However, a decreasing *S.vulgaris* prevalence (68%) from intervention area was consistent with results in horses after the introduction of the highly efficacious ivermectin formulation in the early 1980s (Herd, 1990) and lower than previously reported. This parasite has become rare in horses in most of the developed world due to regular and strategic mass deworming application in different farms as well on individual animals.

Even though higher prevalence was recorded during the beginning of the rainy seasons, there was no a statistical significance in prevalence of species of *S.vulgaris* during dry and beginning of rainy seasons in both intervention and control areas ($P > 0.05$). It could be due to longer prepatent period of species of *S.vulgaris*. The variation in species prevalence could be impact of strategic mass deworming activities that were conducted for nematode parasites control in intervention areas for the last 10 years by DHWP which indicated a statistically significance lowering of this most pathogenic species of Strongylus as opposed to control area.

The current study of larvascopy also revealed thatthe prevalence of *S.edentatus* to be (19.5% and 91%) in intervention area and control area, respectively. There was a statistically significant difference ($p < 0.05$) in prevalence between study areas. The current prevalence of *S.edentatus* in intervention area was lower than any previous reports,66.6% in Dugda Bora(Ayele *et al.*, 2006)89.9% in Project (Abebew *et al.*, 2011), 89.9% in control area (Abebew *et al.*, 2011), 30.8% in Hawassa (Nuraddis *et al.*, 2011), 73.8% in Sululta and

Gefersa (Zerihun *et al.*, 2011). The current prevalence of control area was in agreement with previous reports of 89.9% in Boset (Abebew *et al.*, 2011) but disagreed with 30.8 % in Hawassa (Nuraddis *et al.*, 2011), 66.6% Dugda Bora 73.8% in Sululta and Gefersa (Zerihun *et al.*, 2011).

The current study of larvascopy revealed that the prevalence of *S.equinus* to be (22.6% and 47.79%) in intervention area and control area, respectively. The current study of larvascopy also revealed that the species of *S.equinus* in intervention area has decreased significantly in comparison to the control area and previously reported prevalence of 44.4% in Project areas (Abebew *et al.*, 2011) but it was greater than 12.3% in Hawassa (Nuraddis *et al.*, 2011). The current prevalence of control area agreed to previous report of 44.4% in Project areas (Abebew *et al.*, 2011) but it disagreed with 33.3 in Control area (Abebew *et al.*, 2011) and 12.3% in Hawassa (Nuraddis *et al.*, 2011).

The current study of larvascopy also revealed 8.4% and 18.2%) in intervention area and control area, respectively (statistically significant ($p < 0.05$). The present larvascopy revealed that *Tridontophorus* spp in the intervention area has reduced significantly in comparison to the control area similar to other species of *Strongylus*. It was the least species of large strongylus identified. The result of species of *Tridontophorus* from the two study areas was lower than previous reports of 50% in Dugda Bora (Ayele *et al.*, 2006), 44.45% in Project area (Abebew *et al.*, 2011), 55.5% in Control area (Nuraddis *et al.*, 2011), 29.7% in Hawassa (Nuraddis *et al.*, 2011), 52.8 in Sululta and Gefersa (Zerihun *et al.*, 2011) (Table 11). The sources of variations might be impact of strategic mass deforming in intervention area.

The current study of larvascopy revealed that the prevalence of *Cyathostomum* to be (81.7% and 100%) in intervention area and control area, respectively (statistically significant, $P < 0.05$). *Cyathostomum* was found to be the predominant species of *Strongylus* from intervention area and control area. The current study of larvascopy revealed that the species of *Cyathostomum* from intervention area has reduced significantly in comparison to the control area. The prevalence of *Cyathostomum* in intervention area was lower than previously reported 100% in Control area (Abebew *et al.*, 2011), 100% in Boset (Getachew *et al.*, 2010), 94.9% in Hawassa (Nuraddis *et al.*, 2011) and 100% in Sululta and Gefersa (Zerihun *et al.*, 2011) but the current prevalence of control area was in agreement with previous reports of 100% in Sululta and Gefersa (Zerihun *et al.*, 2011), 100% in Project area Abebew *et al.*, 2011), 100%

in Boset (Getachew *et al.*, 2010), 100% Control area (Abebew *et al.*, 2011), 94.9% in Hawassa (Nuraddis *et al.*, 2011) The impact of strategic mass deworming has also been seen in this species similar to other species of large Strongylus. The lowering of this species of small strongylus was small as compared to the species of large Strongylus in intervention area as it is related to lack of effect of ivermectin on the encysted stages of small Strongylus. The variations could be due to husbandry practices, sampling methods and other veterinary health services which may vary from one area to another. There was no a statistical significance difference in prevalence of species during dry and beginning of rainy seasons in both intervention and control areas ($P < 0.05$). Again it could be due to longer prepatent period of species of strongylus.

The results of present study revealed an overall prevalence of 21% , 49.4% of lungworm in donkeys in intervention(580) and control areas(771) which showed statistical significance difference between them ($p < 0.05$), respectively. These differences could be due to strategic mass deworming activities in intervention area as compared to the control area. The present study 49.4% prevalence of *D. arnefeldi* in donkeys from control area agreed with previous researchers results who have reported 48% in Morocco (Pandey., 1980), 54% in Kentucky, America (Lyons *et al.*, 1985), 50-80% in North America (Klei, 1986) and 42.8% in Sululta and Gefersa (Zerihun *et al.*, 2011). But it disagreed with those who have reported ,83% in Ethiopia (Feseha *et al.*, 1991), 70.5% in Sudan (Hassan *et al.*, 2004), 20% (Esheta, 2000) in and around Bahir Dar, 32% in Dugdabora districts (Ayele *et al.*, 2006), 9.67% in Turkey (Sinasi and Mustafa, 2009), 87.5% in Denmark (Andersen and Fogh, 2010), 3.6% in Hawassa Town (Nuraddis *et al.*, 2011), 35.3% in and around Jimma town (Basaznew *et al.*, 2012), 66.6% in Mekei (Abebew *et al.*, 2011 in and around Bahir Dar.

There was no a statistical significance difference ($p > 0.05$) in male donkeys than in females. It was in agreement with the work of (Getachew, 2006) in Ada, Akaki and Bereh and (Mezgebu *et al.*, 2013) in Gonder. This finding disagreed with the work of (Alemayehu and Etaferahu, 2013) in South wollo and (Bewketu *et al.*, 2013) in Bairdar.

The age of animals was found as a major risk factor for the variation in the prevalence of lungworm infection (55.7% and 77.9%) for young, (15.4 and 39.7%) in adult, (21.9% and 43.9%) in old in intervention and control areas, respectively ($p < 0.05$). The highest infection rates were recorded in old and young age groups from both study areas. This result was not

consistent with results of previous work (Nuraddis *et al.*, 2011). On the contrary, it was consistent with previous work (Basaznew *et al.*, 2012). These variations in the prevalence of lung worm infection among ages might be related to the condition that older and younger animals are taught to have decreased immunity (Radotitis *et al.*, 2010).

In this study, different levels of prevalence were observed in different body condition scores. A prevalence of (36.6% and 76.6%) in poor, (16.6% and 55.7%) in medium and (12.2% and 19.2%) in good were recorded in intervention and control areas, respectively ($p < 0.05$). This result was not consistent with results of previous work (Nuraddis *et al.*, 2011). But it was consistent with previous work (Basaznew *et al.*, 2012) and (Bewketu *et al.*, 2013). Poorly nourished animals appear to be less competent in getting rid of infection although it is not unusual for well fed animals to succumb to the disease (Kimberling, 1988).

There was no statistical significance on the prevalence *D. arnfeldi* during long dry and beginning of rainy seasons in both intervention and control areas ($P > 0.05$). Again it may be due to the longer prepatent period of the lung worm. Only small proportion of the equine lung worm undergo hypobiotic process (Urquort *et al.*, 1996).

The over all prevalence of 26.7%) *S. westeri* in intervention agreed with 17.5% in Boset (Getachew *et al.*, 2010) and 20% in Hawassa Town (Nuraddis *et al.*, 2011). But it disagreed with 42.8% in Sululta and Gefersa (Zerihun *et al.*, 2011), 77.8% (Abebew *et al.*, 2011) control area, 44.4% (Abebew *et al.*, 2011). The over all prevalence (47%) of *S. westeri* in control area disagreed with 17.5% in Boset (Getachew *et al.*, 2010, 20% Hawassa (Nuraddis *et al.*, 2011) and 77.8% (Abebew *et al.*, 2011) in control area. But it was consistent with 42.8% in Sululta and Gefersa (Zerihun *et al.*, 2011), 44.4 (Abebew *et al.*, 2011) in project area.

Variation was noted in the prevalence of *S. westeri* in infection among different age groups. (70.9% and 83.2%) in young, (19% and 35.5%) in adult, (31.2%) and 31.6%) in old in intervention and control areas, respectively ($p < 0.05$). A different level of prevalence was observed in different body condition scores. A prevalence of (48.3% and 83.1%) in poor, (21.5% and 49.9%) in medium and (4.1% and 22.6%) in good were recorded in intervention and control areas, respectively ($p < 0.05$). The high prevalence in young age and poor body conditions were consistent with previous findings in Sululta and Gefersa (Zerihun *et al.*,

2011), (Abebew *et al.*, 2011) control area and project, (Abebew *et al.*, 2011) , Hawassa Town (Nuraddis *et al.*, 2011). Eventhough higher prevalence was observed in male donkeys than in females, there was no statistical significance difference ($p>0.05$). It was in agreement with the work of (Ayele *et al.*, 2006) in Dugda Bora. This finding disagreed with the work of (Bewketu *et al.*, 2013) in Bairdar and (Alemayehu andEtaferahu, 2013) South Wollo.

The current study revealed that the overall prevalence of *T.axei* to be (62.2% and 92.5%) in intervention area and control area, respectively (statistically significant, ($p>0.05$) and $OR=7.46$) .The species of *T.axei* in intervention area has reduced significantly in comparision to the control area. *T.axei* in intervention area was not consistent with previous reports of 40% in Dugda Bora(Ayele *et al.*,2006), 100% in Sululta and Gefersa (Zerihun *et al.*, 2011),91.3% in Boset (Getachew *et al.*, 2010), in Control area 33.3% (Abebew *e tal.*, 2011), in Gambia 83% (Mattioli *et al.*, 1994), 80.9% in Moroco (Pandey *et al.*, 1980).

The prevalence of *T. axei* in control area was not consistent with previous reports of 40% in DugdaBora (Ayele *et al.*, 2006) an 33.3% in Control area (Abebew *et al.*, 2011), 83% in Gambia (Mattioli *et al.*, 1994), 80.9% in Moroco (Pandey *et al.*, 1980) but it was consistent with 100% in Sululta and Gefersa (Zerihun *et al.*, 2011), 91.3% in Bose t (Getachew *et al.*, 2010). Similar to other species of Strongylus, a significant reduction in *T.axei* has occurred in intervention area into which strategic mass deworming activities have been undertaken by DHWP.

5.1.3 McMaster egg counting Technique

McMaster egg counting was used for quantitative analysis and description of the intensity of Strongylus infection. The mean eggs count analyzed by analysis of variance revealed (304.6 % and1293.00) (307.32, 1456.53) in intervention and control areas during dry and beginning of rainy season, respectively.The mean egg counts between intervention area and control areashowed a statistically significant difference ($p<0.05$) between study areas during dry seasons and rainy seasons. Even though the mean prevalence of the intervention area is only slightly higher during rainy season, there was no statistical significance difference ($P>0.05$) between dry and beginning of the rainy season. On the contrary, the mean prevalence of the control area during the beginning of the rainy season was significantly higher ($P<0.05$) than the mean of the dry season. The current mean prevalence in intervention area is significantly

lower than the work of Abebew *et al.*, (2011) who reported 433.6 egg of *Strongylus* in project area. Getachew *et al.*, (2008) reported 1300 and 1800 egg in project area during the dry and rainy season, respectively. The current result from control area agreed with his work during the dry season but it is lower than his work during the beginning of rainy season and the work conducted by Feseha *et al.*, (1991) who demonstrated an average of 1440 to 2500.

An egg of 42,650 was recorded in donkeys from Morocco (Khallaayoune, 1991). The trend of the current egg count rise was in line with the work of Getachew *et al.*, (2008) who reported low egg count during the dry season and high egg count during the long rainy rainy season. The consequence of lower egg during both long dry and beginning of the rainy season could be attributed to the effect of strategic mass deworming applied in the project areas as opposed to the control area. Higher egg counts at the beginning of the rainy season could be associated with hypobiotic characters of *Cyathostomum* third larval stages (Chapman *et al.*, 2002; Love and Duncan, 1992). In a study that measured body weight, the use of a pre-winter treatment with moxidectin resulted in a 100% reduction in faecal egg counts, improved live weight and body condition score over 16 month period in donkeys kept at a research facility in South Africa (Matthee *et al.*, 2002).

According to Soulsby, (1982), an egg of 500 suggest mild strongyle infection, 500-1000 a moderate infection and above 1000 a severe infection in horses. On this basis, the current study revealed the intensity of low infection (87.3 % and 12.7%), moderate (11.6% and 35.9%) and severe (1.2 and 51.4) during dry seasons in intervention and control areas, respectively. The frequency of level of intensity infection during rainy season was found to be low (89.3% and 15.0%), moderate (10.4% and 27.5) and severe (0.4% and 57.5%) in intervention and control areas, respectively.

The majority of working donkeys in intervention area are mildly infected (87.3% and 89.3%) but only very small proportions (1.2 %and 0.4%) are severely infected during the dry and the beginning of the rainy season .on the contrary, the majority of working donkeys in control area are severely (51.4% and 57.5%) and only a very small proportion are mildly (12.7% and 15.0%) infected during the dry and beginning of the rainy season, respectively. The current egg result of the control area was consistent with work of Abebew *et al.*, (2011) in project and control areas, Zerihun *et al.*, (2011) in Sululta and Gefersa and Getachew *et al.*, (2008) in central low lands.

A significant reduction of faecal egg counts from intervention area is most probably due to deworming strategy employed by DHWP as compared to the very high egg counts in control areas. A similar result of faecal egg count reduction has been observed in different parts of the world after the administration of different anthelmintics in equines. Lower strongyle prevalence has been found in adults treated more frequently while such an effect was not consistently found for the other two age groups on German horse farms (Himmelstjerna *et al.*, 2009). A more frequent treatment requirement has been reported for young horses than adult horses for adequate parasites control (Matthee *et al.*, 2002; Love, 2003)

Dose calculation was done based on visual assessment of donkey weight while they administered ivermectin in intervention area by the DHWP. A similar way of dose calculation has been observed in assessment of worm control practices in Germany, Italy and the UK (Himmelstjerna *et al.*, 2009). This will often lead to under dosing which in turn can propagate anthelmintic resistance (Waller, 1987). Himmelstjerna *et al.*, (2009) reported 90% FECR of strongyles by comparing with their high pre-treatment FEC in their assessment of worm control practices in horses in Germany, Italy and the UK.

Anthelmintic treatment efficacies are generally considered to be suboptimal in diseased or not fully immunocompetent animals. Accordingly, it may be speculated that horses failing to effectively control their worm burden may also possess a less well developed capacity to support the effect of anthelmintic treatment, i.e. to eliminate worms only partially affected by treatment (Himmelstjerna *et al.*, 2009) to variations in conditions and access to worms control program. The faecal worm egg counts recorded amongst the untreated control animals are lower than those reported in other studies on working equids.

The percent efficacy of Ivermectin in Pakistan determined on the basis of reduction in number of eggs per gram of the faeces was 73.21% and 96.42 % on day 7 and 14 post medication, respectively, in a study of nematode parasites control in donkeys. Sipra *et al.*, (1999), who administered Ivermectin at the dose rate of 0.4 mg/kg and 0.2 mg/kg body weight against strongylosis in equines in Faisalabad, Pakistan and found that ivermectin has completely eliminated the egg burden after day 14 post-treatment. Seri *et al.*, (2005) employed ivermectin at the dose rate of 0.2 mg/kg against gastrointestinal nematodes in donkeys and found 100% FECR effective in reducing of nematodes species including *strongylus* spp, small strongyles, *Trichostrongylus axei* in Sudan.

Binev *et al.*, (2005) who administered ivermectin for the control of strongyloids in donkeys in Bulgaria and found that ivermectin was 96% effective in terms of faecal egg count reduction. Aftab *et al.*, (2005) evaluated the ivermectin against ecto and endo-parasites in horses and found the efficacy of ivermectin to be 95.17% against endoparasites. Hassan *et al.*, (2005) who administered ivermectin against ecto and endoparasites in horses in Lahore and found that the efficacy of ivermectin was to be 100% against roundworms.

Crane *et al.*, (2011) conducted randomized triple-blind trial to assess the impact of an anthelmintic treatment programme (using oral ivermectin and fenbendazole) three treatments annually comparing treated and placebo control populations of working donkeys, mules and horses in field conditions in Morocco. Based on his assessment, increased animal body weight did not vary between treated and placebo control group of working equines. On the flip side, he had got increased body condition score and a significantly lower strongyle worm egg count in treated animals as compared to animals in control group. Faecal worm egg count was also measured. Matthee *et al.*, (2002) conducted a study to assess the impacts of deworming on parasites in donkeys and found a 100% reduction in faecal egg counts, improved live weight and body condition score over 16 month period in donkeys kept at a research facility in South Africa.

Sex did not show statistical difference ($P>0.05$) in epg counts between intervention and control areas but higher proportions. It agreed with the work of Getachew *et al.*, (2008) in central low lands but disagreed with the work of Alemayehu and Etaferahu, (2013) in South Wollo. Age and body conditions were statistically significant ($P<0.05$) in both areas. The higher epg proportion was present in younger age and in those donkeys with body conditions in both intervention and control areas which were in agreement with the work of Alemayehu and Etaferahu, (2013) in south wollo and Zerihun *et al.*, (2011) in Sululta and Gefersa but they disagreed with the work of Getachew *et al.*, (2008) in central low lands.

5.2 Physical Examination

The present clinical examination enumerated pale mucus membrane to be 52.5% and 54.3% in control and intervention area, respectively. There was no statistical significance difference between control and intervention areas ($P>0.05$). The current results were in agreement to the report of Solomon and Rahmeto, (2010) who have reported 51.7%. But, they disagreed to the report of Kumar *et al.*, (2014) who has reported 20% abnormal mucus membrane in Mekelle City. This difference could be related to variation in sampling method and other diseases that can cause abnormal mucus membrane in working donkeys. There are miscellaneous causes of abnormal mucus membrane other than nematode parasites reported in donkeys such trypanosomiasis (Bedada *et al.*, 2012) in Ethiopia ,Ghana (Atawalna *et al.*, 2015), Egypt (Badawy *et al.*, 2014), Ethiopia (Getachew *et al.*, 2010), Babesiosis (Radotitis *et al.*, 2010), ticks, lice, fleas(Urquhart *et al.*, 1996), Bacillary haemoglobinuria, nutritional deficiency such as copper, cobalt, iron Babesiosis (Radotitis *et al.*, 2010).

The present study revealed the overall prevalence of knits of *Gastrophilus* to be 31.9% and 32.7% in control and intervention areas, respectively. There was no a statistical significance differences between them during the rainy season ($p>0.05$).The current result was higher than the previous reports of 13% in Mekelle City (Kumar *et al.*, 2014). The difference could be related to the variations sampling methods and the seasons at which samples were collected. The current study on kits of *Gastrophilus* was conducted during June and the beginning of July months which are ideal seasons for the occurrence of ectoparasites in tropical weather conditions. Seasonal occurrence of this disease has been reported (Soulsby,1978; Cogley and congely, 2000).

The prevalence of wound was found to be (19.7% and 14.3%) in working donkeys in control and intervention areas, respectively. There was no a statistical significance difference ($p>0.05$) between intervention and control The current finding from the study areas was markedly lower than the previous reports 79.4% (Biffa and Mogos, 2006) and 77.5% (Curran *et al.*, 2015) in Hawassa, 59% (Burn *et al.*, 2007), 54% in Morocco (Sells *et al.*, 2010), 40% (Pearsons *et al.*, 2002) in Central Ethiopia and 42.2% in Yilmana Densa District (Girma *et al.*, 2014). These differences might be happened due to variation in management and husbandry and the work type of the donkeys in the regions. All the donkeys sampled for the current study was pack donkeys from both intervention and control areas. However, the

results of the intervention area (14.3%) and control area (19.7%) were closer to the report of 16.4% in Dugda Bora (Ayele *et al.*, 2006). This difference could be explained by the fact that longer distance coverage and more heavier load were belonged to the donkeys from the intervention area.

The overall prevalence of lameness in working donkeys in control and intervention areas was 7.6% and 1.1%, respectively. The results from both areas were lower than earlier reports of 40.2% in Hawassa (Amene *et al.*, 2015), 98% in Pakistan and India (Broster *et al.*, 2009) and 98% in Pakistan (Reix *et al.*, 2014). The result of the control area is higher than what has been reported before 3.1% in Debre Zeit (Morgan, 2006), 3.5% in Ghana (Atawalna *et al.*, 2015) but agreed to the reports of 6% in Dugda Bora (Ayele *et al.*, 2006). The result of the intervention area is lower than the report of 6% in Dugda Bora of (Ayele *et al.*, 2006) and 3.5% in Ghana (Atawalna *et al.*, 2015). These differences might be happened due to variation in welfare training in intervention area, management ,husbandry and the work type of the donkeys in both study regions.

The present study pinpointed 1.4% and 1.1% overall prevalence of sarcoid in control and intervention areas, respectively. This result did not show statistical significance difference between the groups ($p < 0.05$). This result was in agreement to the previous finding (1.3%) (Kumar *et al.*, 2014) in Mekelle City. However, this result was lower than the previous reports 3% (Yilma *et al.*, 1991) in Debre Zeit, 3.1% (Ayele *et al.*, 2006) Dugda Bora and 6.2% (Atawalna *et al.*, 2015) in Ghana. This difference may be related to sampling methods and husbandary practices but the lack of statistical difference between might be due to absence of effect of ivermectin for this skin tumour.

The present clinical examination indicated 3% and 3.2% overall prevalence of dental abnormalities in control and intervention areas, respectively. This result did not show statistical significance difference between the two groups ($p > 0.05$). This result was not in agreement to the previous finding of 63.25% (Mengistu *et al.*, 2015) in Ada'a and Dugda Districts), 0.95% in Ghana (Atawalna *et al.*, 2015) , 16% in Mexico (Fernando-Martinez *et al.*, 2006). However, this result was lower than the previous reports of 3% (Yilma *et al.*, 1991) in Debre Zeit, 3.1% (Ayele *et al.*, 2006) Dugda Bora and 6.2% (Atawalna *et al.*, 2015) in Ghana. This difference may be related to sampling methods, husbandary practices and type of the donkeys. Cartpulling donkeys are more vulnerable to the dental abnormalities as

compared to the pack type of donkeys. All the donkeys in the current study belonged to pack type of donkeys.

The present study also indicated 1.4 % and 1.1 % overall prevalence of Sarcoids in control and intervention areas, respectively. This result did not show a statistical significance difference between the two study areas ($p > 0.05$). Sarcoid was the least non-nematode parasitic health related problems identified in both study areas. The current result of sarcoids from both areas was in agreement with previous findings in Debre zeit, Dugda Bora and Mekelle city (Yilma *et al.*, 1991; Ayele *et al.*, 2006; Kumar *et al.*, 2014). On the contrary, this finding was not in line with the finding in Ghana (Atawalna *et al.*, 2015). This difference might be associated with variation in management and methodology.

5.3 Questionnaire Survey

The current semi-structured questionnaire survey on the general information of donkeys (donkey types, number and time of donkeys kept by the owner, working load per week and on the nature of the diseases related to the nematode parasites did not show a statistical significance difference in both control and intervention areas ($P > 0.05$). However, in the current semi-structured questionnaire survey, the higher proportions of respondents for deworming importance related questions (deworming other animals, importance of deworming, diseases not prevented by deworming and cares for other diseases) belonged to intervention area. It showed statistical significance difference in relation to deworming questions among respondents in both control and intervention areas ($P < 0.05$). DHWP gave strategic mass deworming for equines and welfare training activities in their project sites for the donkeys owners. Hence, this awareness differences related to deworming among respondents are most likely linked to it. All respondents from the two study areas could not explain on the parasite related ideas such as means of transmission, season at which it occur and signs of parasite. Hence there was no difference in their knowledge from the two study areas.

There was a statistical significance difference on the working load per hour and working load between intervention and control areas ($P < 0.05$). The proportion of donkeys which travelled 1-4 (98.9 and 78.6) and 5-8 hours (1.1% and 21.4%) and carried 40kg (4.3% and 12.9%) and 80kg (3% and 35.7%) were found in control area and intervention areas, respectively. The

higher proportion of donkeys which travelled the shortest distance (1-4hours) and carried the lowest load (40kg) were found in control area where as the higher proportion of donkeys which travelled the longest distance (5-8hours) and carried the heaviest weight (80kg) were present in intervention area with a significant statistical difference($p<0.05$). This difference could be associated with the scarcity of water in intervention area. The current questionnaire survey was conducted during the longer dry season when water and feed availability were scarce particularly for intervention area. The owners of the donkeys from the intervention area were forced to travel longer distances and to put heavier weight on their animals to bring water from the remote areas where as water was present near to the study area in the control area. There are many diseases which cannot be prevented by deworming but which can be prevented by vaccination and antibiotics and other drugs. For knowledge assessment, the proportions of the respondents on vaccination and antibiotics were higher in intervention area as compared to the control areas. This knowledge difference could be associated with the training on welfare and importance of deworming for donkeys in intervention area. Similarly, the proportions of respondents for deworming of other animals were higher in intervention area which could also be linked with the implementation of strategic deworming and training given by DHWP. Greater than 80% of respondents from the intervention area responded good body conditions of their donkeys for importance of deworming. The response of good body conditions could be linked to one of the clinical signs of nematode parasites (emaciation). Even though they could not associate good body conditions with parasitic diseases.

The frequency of deworming was in 1-3 years (61.5% and 67.6%), 3.5-6 years (36.3% and 31%,) and greater than 6 years (2.2% and 1.4%) in home and bought type of donkeys in intervention area , respectively. There was no a statistical significance difference ($P>0.05$) in the frequency of deworming between home and bought type of donkeys among the 3 years of categories. Again, there was no a statistical significance difference ($P>0.05$) in the proportion of frequency of deworming between home and bought type of donkeys among the 3 years of categories. Since there was no classe of frequency of deworming in the previous studies, years of frequency of deworming were clustered into three categories. Based on this classification, there was no a statistical significance on the frequency of the deworming among the three years of classes ($P>0.05$). Even though there was no a statistical significance differences in home and bought type of donkey, the proportion of bought type of donkeys are higher than the proportion of home reared type of donkeys. This indicated the entry of new donkeys from areas where deworming in general, strategic deworming or tactical deworming in specific are

not practiced. According to the questionnaire, donkeys owner from both areas don't deworm their donkeys by buying anthelmithics from veterinary clinics or any other sources. On the other hand, some owners of the donkeys from both intervention and control areas deworm other animals (source of food) such as ruminants by buying anthehelmitics from the veterinary clinics and veterinary pharmacy. Total frequency proportions also found to be 181 (64.6%), 94 (33.6%) and 5 (1.8%) in 1-3, 3.5-6 and 6-10 year, respectively. Based on the three classes, the highest proportion of frequency is present in the low year class (1-3year) and followed by the middle year class and the least in the highest year class (6-10) year). This also indicated the movement of the donkeys from the intervention sites to other sites.

5. CONCLUSION AND RECOMMENDATIONS

The present study has revealed the presence of a range of nematode parasites which were representative of the important pathogenic nematode parasites of donkeys from both control and intervention areas. The common donkeys nematode parasites recorded in these study areas were Strongyles, *P. equorum*, *O. equi*. Among the identified nematode parasites, the highest relative percentage was recorded for Strongyles while less occurrence rate was observed for *P. equorum* followed by *O. equi*. The prevalence of these parasites was higher in control area as opposed to intervention area as a result of the impact of strategic mass deworming practices done in intervention area. It was also observed that age, body condition scores and study areas were found to be the important risk factors for the occurrence of nematode parasites in donkeys which was assessed by their prevalence and mean eggs count. Another nematode parasites of working donkeys identified from both control and intervention areas were *S. vulgaris*, *S. ednatus*, *S. equinus*, *S. westeri*, *Cyathostomum*, *T. axei*, *Tridontoporus* and *D. arnfieldi* but the prevalence of these nematode parasites in intervention areas were significantly lower than the prevalence of the control areas. *Cyathostomum* was the most predominant species from both intervention and control areas where as *Tridontoporus* was the least nematode parasite in both areas. nematode parasites did not show significant difference between season in both intervention and control areas. This study has also indicated worm egg counts of *Strongylus* from working donkeys from project areas were repressed to low levels as opposed to donkeys from control areas. Detailed Physical examination enumerated other health related problems of working donkeys such as abnormal mucus membrane, knits of *Gastrophilus*, wound, lameness, dental abnormalities and sarcoid in both control and intervention areas from their highest to the lowest in order of importance. The major non-nematode health problems of working donkeys were recorded from both intervention and control areas without a significant difference irrespective of the study areas except lameness and mixed diseases which were lower in intervention area as opposed to control area. The current questionnaire survey on the nature of the parasites did not indicate difference among the donkeys owner from both intervention and control areas. The current questionnaire revealed that the donkey owners from the intervention and control areas did not give any anthelmintics as they did to their ruminants and horses. Majority of respondents from intervention area responded to the importance of strategic mass deworming by relating it with the improved body conditions.

In light of the above concluding remarks, the following recommendations are forwarded:

- As there was a clear indication in decreasing of species large strongylus as opposed to small strongylus and thus it requires efficacy study of the ivermectin against these species of strongyles.
- There is a need and urgency to clearly develop egg cut off values for working donkeys in order to declare the degree of parasitic burden to assess the impact of antihelminthic deworming.
- As egg per gram of faeces did not show significant reduction during long dry period and rainy season in intervention area, egg per gram of faeces count and identification of nematode species study should be conducted at the end of the rainy season
- Correlation study on faecal eggs counts with adult parasites should be conducted by more confirmatory diagnosis.
- Mass deworming of working animals strategically by simply considering seasons of the year without assessment of the parasite load is not economical and scientifically justifiable.
- Moreover, the significance of other helminth parasites such as cestodes and trematodes in working donkeys should also be studied.
- It was noted that owners of the donkeys from both intervention and control areas were not fully aware of the importance of management related animal handling practices Therefore, continuous awareness creations to donkey owners on proper management and handling of donkeys should in place in order to enhance the impact of strategic mass deworming

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7. ANNEXES

ANNEX1: Laboratory materials and procedures

1. Simple Flotation technique

The simple flotation method is a qualitative test for the detection of nematode in the faeces. It is based on the separating of eggs from fecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity. There are differences in the specific gravity of parasite eggs, cysts, and larva and that of fecal debris. Nematode eggs are separated from fecal material and floated by a flotation fluid of an appropriate specific gravity.

Equipment and reagent:

- Mortar and pestle
- Nacl salt
- Distilled water
- Fecal sample
- Aluminum foil
- Balance
- Measuring cylinder
- Beaker
- Sieve
- Centrifuge tubes
- Test tube rack
- Centrifuge
- Microscope
- Glass slide
- Cover slip

Procedure:

- 40g of NaCl was dissolved in 100 ml distilled water
- 3 grams of fecal sample was measured and transferred into mortar
- 45ml of salt solution (flotation fluid) was added to the fecal sample and mixed using a pestle
- The mixture was strained with a sieve into a beaker
- The filtered preparation was filled to test tubes until 2.5 cm was remained to the top
- The test tubes were placed opposite to each other on a centrifuge, and centrifuged at 1500rpm for about 3 minutes
- The tubes were withdrawn and tap water was added to the top of the tube until it had bulged creating a reverse meniscus.
- Cover slip was placed on top of the tubes and allowed to stand for up to 20 minutes.
- Cover slip was carefully lifted up with the adherent drop of fecal flotation solution and placed on a clean microscopic slide.
- The entire cover slip area was examined at 10X objective lens magnification then

2. McMaster Counting Technique

The McMaster counting technique is a quantitative technique to determine the number of eggs present per gram of faeces (epg). A flotation fluid is used to separate eggs from faecal material in a counting chamber (McMaster) with two compartments

Equipment and reagent:

- | | |
|--|-----------------------------|
| - Beakers or plastic containers | - Pasteur pipettes |
| - Balance | - Flotation fluid |
| - A tea strainer or cheesecloth | - McMaster counting chamber |
| - Measuring cylinder | - Microscope |
| - Stirring device (fork, tongue depress) | |

Procedure:

- Weigh 3gm of faeces
- Break up thoroughly in 42ml of water in mortar and pestle
- Pour through a fine mesh sieve (aperture 205µm, or 100 to 1 inch).
- Collect filtrate, agitate, and fill a 15ml test tube.
- Centrifuge at 2000rpm for 2 minutes.
 - Pour off supernatant, agitate sediment and fill tube to previous level with flotation solution.
 - Invert tube six times and remove fluid with pipette to fill both chambers of McMaster slide. Leave no fluid in the pipette or else pipette rapidly, since the eggs will rise quickly in the flotation fluid.
 - Examine one chamber and multiply number of eggs by 100 or two chambers and multiply by 50, to arrive at the number of eggs per gram of faeces (epg).

3. Baermann Technique

The Baermann technique is used to separate larvae from faecal sample. When feces are suspended in water, larvae migrate into the water. They sink to the bottom and can be collected for identification.

Equipment and Regent:

- | | | |
|---------------------|-------------------|---------------------|
| - Baerman apparatus | - Gauze | - Microscope slides |
| - Fecal sample | - Beaker | - Cover slips |
| - Balance | - Centrifuge tube | - Microscope |
| - Warm water | Centrifuge | |
| - Sieve | - Pipette | |

Procedure:

- A sieve was placed in the wide neck of the funnel, which has been partially filled with warm water
- A double layer of gauze was placed on top of the sieve. Cultured feces (7-10 day) or fresh faeces (*Dictyocaulus arnfieldi*) were placed on the gauze and wrapped. The funnel was slowly filled with warm water until the feces are immersed.
- The apparatus was left overnight at room temperature
- The clip on the rubber was then removed and the water in the neck of the funnel collected in a small beaker.
- The fluid was centrifuged at 1500 rpm for 2 minutes to concentrate the larvae in sediment.
- After pouring off the supernatant, a drop of the sediment was transferred with a pipette to a microscopic slide, add drop of iodine to fix the larvae, covered with cover slip. The larvae were then identified under lower power microscope (10 x objectives) based on the shape, relative size and shape of larvae's tail; and under oil emersion (100 x objectives) based on number of gut cell numbers.

ANNEX 1: Questionnaires Survey on Strategic Mass Deworming

Data collection format

Date _____

Semi-structured interview with donkey owner

1. Owner details information

- Sample code _____
- Name of districts _____
- Kebele _____
- Number of donkeys _____
- Type (Pack cart both)
- Work type (Household use commercial use Industrial other)
- workload (hrs/ day _____, days/week _____, load _____, rest _____)
- Bought or home reared (how long did you keep the donkey? _____)
- Feeding practice (grazing, supplement when working and what type? _____, _____)
- Housing practice (indoor outdoor kept with other livestock)

Deworming practice assessment

- When did you start bringing donkeys for mass deworming? month _____, year _____ How frequently? why _____?
-
- How do you tell when a donkey has worm /parasite problems?
- Where do donkeys get parasite from? _____
- What will happen if you deworm your donkeys and your neighbors don't? _____
- When dewormed last? day _____ month _____ year _____
- What change did you see on your donkeys after deworming (Body condition _____, breeding behavior _____, work efficiency _____, feed intake _____, resistance for other diseases _____)
- What other cares do you need to apply so that the deworming brings change in the life of the animal? _____

-
- Do you deworm other animals? _____ how?
_____ cost? _____
 - What other options do you have for deworming animals? _____ (herbal? _____ describe _____)
 - What is the importance of deworming animals? (Benefits of deworming to the animals)
 - Are there any diseases that can't be prevented by deworming? _____

- What do you need to do? _____

2. Environmental factors details information

- Grazing availability(when) _____
_____ mixed grazing (horses, ruminants and others) _____
Swampy (snails and other animals) _____
- Dry or wet area (where the donkey stays or grazes)
- Seasonality of rain (seasonal calendar)
- Altitude _____
- Longitude _____
- Temperature(maximum and minimum) _____

3. Animal observation information details

- Sample code _____
- Sex _____
- Age _____
- Pregnant lactating dry
- dental abnormality lost eruption problems
- BCS _____
- General behavior (social _____, sexual _____, vocalizations _____, rolling on dusty areas _____, ear erecting or not _____, calm _____, aggressive _____)

- Hair coat condition shiny erected dull
- Mucus membrane(pale _____, yellow _____, congested _____)
- Nasal discharge serous mucous purulent muco-purulent
- peri-anal soiling
- diarrhea(mucus _____, blood _____, adult parasites _____)
- Ecto-parasites: ticks mange lice flies
- Itching / Alopecia : absent present , back flank perineum
- neck
- Skin diseases _____

- Lameness forelimb hind limb
- Wound present absent
- Other diseases _____

ANNEX 2: Key for identification of the 3^{ed} stage larvae of common GI parasites

- | | |
|--|--|
| 1. Esophagus rhabdiform
esophagus non rhabdiform | free living |
| 2. Without sheath, esophagus
Nearly 1/2 length of body with gut cell | <i>strongyloides westeri</i>
sheath 3 |
| 3. With eight gut cells
With more than eight cells | <i>Trichonema spp</i> |
| 4. With 16 gut cells
Tail of sheath short and conical | <i>Trichostrongylus axei</i> |
| 5. Very long thin larvae, well defined gut cells
Small trilobed process on larvae | <i>strongylus edentates</i> |
| 6. Broad larvae of medium length with 18-20
defined regular gut cells | <i>triodontophorus tenuicollis</i> |
| 7. Large broad larvae with short esophagus
and 28-30 gut cells | <i>strongylus vulgaris</i> |

ANNEX 3: Determination of the age of donkey by dentation

Age	Identification
6 days old	First pair of milk teeth grown
6 weeks old	Second pair of incisor teeth grown
6 months old	Last pair of incisor teeth grown
3 years old	First pair of incisor teeth is in wear
4 years old	Second pair of incisor teeth is up and in wear
5 years old	Corner pair of incisor teeth is up and in wear
6 years old	The teeth have worn level and all have a central indent called a cup. The corner teeth are now wearing level.
7 years old	The cup is less deep in the central pair of front teeth, where it is now called a mark. There is still a good cup in the other front teeth. A hook can be seen on the side of the upper corner front teeth.
8 years	A dark line at the front of the central pair called a star has appeared on each of the central pair of front teeth
9 years old	Mark of cups, star on next teeth, a groove begins to grow down the upper corner front teeth.
10 years old	Triangular grinding surface, star on corner front teeth. The seven years hook has worn away
12 years old	Mark has gone from the centrals. Round star, upper corner teeth about one centimeter long.
15 years old	Only stars on the teeth. The groove is half way down the upper corner teeth.
19-20 years old	Forward lobe tooth, the groove extends down the whole teeth.
20-25 years old	The teeth have an even more forwarding pointing angle and the groove grown out (disappears at 30 years old)

ANNEX 4: Body Condition Score chart

BCS	Neck and Shoulders	Withers	Ribs and Belly	Back and Loins	Hindquarters
1.poor	Neck thin, all bones easily felt. Shoulder bones felt easily, angular.	Dorsal spine of withers prominent	Ribs can be seen from a distance and felt with ease.	Backbone prominent	Hip bones visible and felt easily
2.moderate	Some muscle development overlying bones.	Some cover over dorsal withers,	Ribs not visible but can be felt with ease.	Dorsal and transverse processes felt with light pressure.	Poor muscle cover on hindquarters, hip bones felt with ease.
3.ideal	Good muscle development, bones felt under light cover of muscle/fat. Neck flows smoothly into shoulder, which is rounded.	Good cover of muscle/fat over dorsal spinous processes	Ribs just covered by light layer of fat/muscle, ribs can be felt with light pressure.	Cannot easily feel individual spinous or transverse processes.	Good muscle cover in hindquarters, hip bones rounded in appearance, can be felt with light pressure.
4.fat	Neck thick, crest hard, shoulder covered in even fat layer.	Withers broad, bones felt with firm pressure.	Ribs only felt with firm pressure, belly overdeveloped	Can only feel dorsal and transverse processes with firm pressure..	Hindquarters rounded, bones felt only with firm pressure. Fat deposits
5.obese	Neck thick, crest bulging with fat and may fall to one side. Shoulder rounded and bulging with fat.	Withers broad, unable to feel bones.	Large, often uneven fat deposits covering dorsal and ventral aspect of ribs.	Back broad, unable to feel spinous or transverse processes.	Cannot feel hip bones, fat may overhang either side of tail head, fat often uneven and bulging.

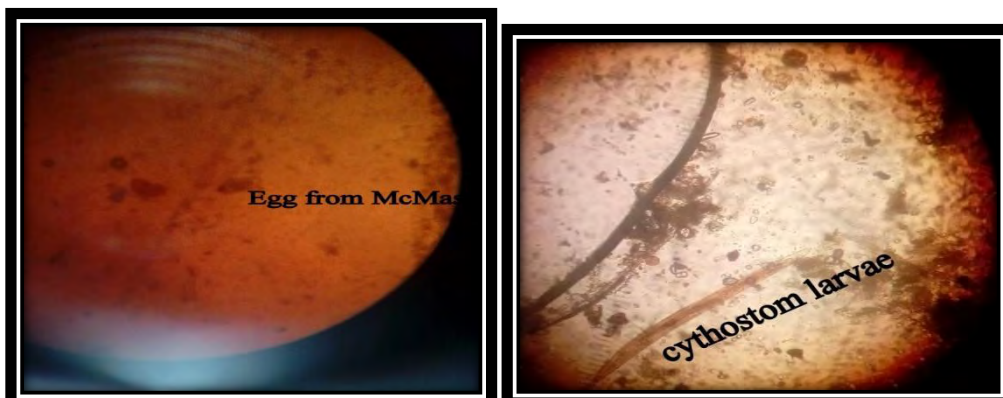
Source: Donkey sanctuary, UK

ANNEX 5. Sample photos taken during the field work and laboratory result

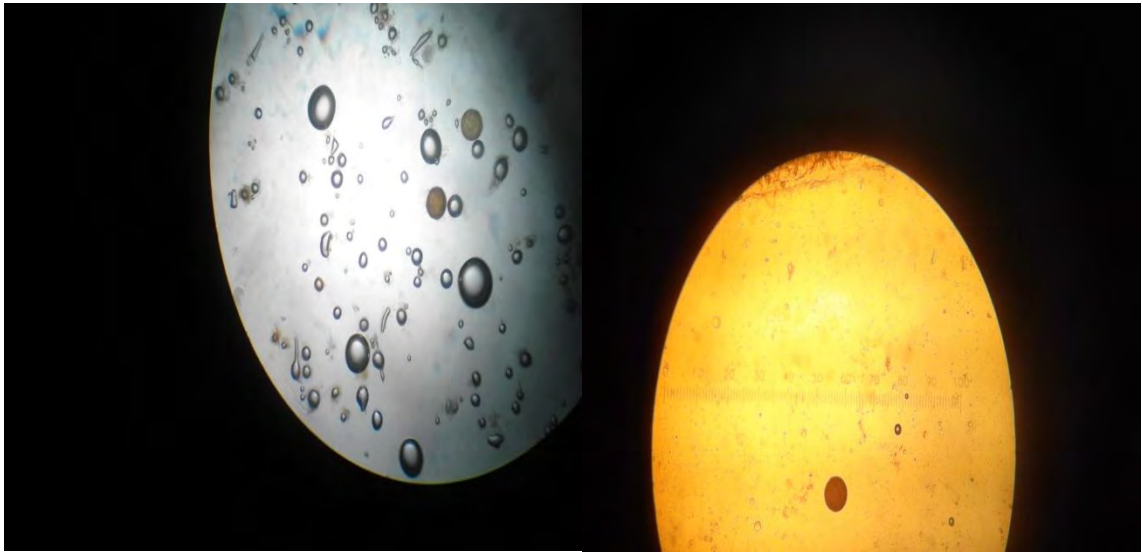
1. Selected photos of Body condition scoring



3. Selected photos of Laboratory results



4. Mixed Parascaris and strongyles egg



5. Kints of Gastrophilus



6. sarcoid



7. Hoof overgrowth



8. Back sore

