Prevalence Of Gastrointestinal Helminthes Of Donkey In And Around Mekelle

DVM Research article

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Abstract: A cross-sectional study was conducted from November 2015 up to March 2016 in and around Mekelle to estimate the prevalence of gastrointestinal helminthes of donkey and their mean egg count and to assess the associated risk factors. Faecal samples were collected from 404 randomly selected donkeys were examined for nematode cestode and trematode infections. The overall prevalence of GIT helminthes in the study area was found to be 80.2% and the relative percentage of the encountered parasites during the study period were 52.0% Strongyle type, 6.4% *Parascaris equorum*, 2.0% *Strongolides westeri*, 2.5% *Gastrodiscus aegyptiacus*, 2.7% *Anaplocephala Spp*, 2.2% *Fasciola* and 12.4% mixed parasites infection. Furthermore ovaculture identification of third stage larvae of strongyles reveals that 34.8% *S vulgaris*, 22.9% *S. edentatus*, 9.5% *S. equinus*, 23.8% *Cyathostomum Spp* and 9.1% mixed strongyles. In the current study the mean EPG count of nematode parasites was found to be 925.25±662.82. Concerning severity of infection in this study 57.6%, 26.4% and 32.5% of donkeys were infected severely, moderately and mildly respectively. There were statistical significant difference both in prevalence and mean EPG count between body condition score, manure removal frequency, type of house and purpose of the animals ($p < 0.05$). However age and feeding systems were only statistically significant difference in prevalence of gastrointestinal parasites. In conclusion the findings of the present study indicated a high prevalence of helminthic parasites compromising the health and welfare of donkey. Sustainable prevention and control methods should be developed to prevent the burden of gastrointestinal helminthes of donkey in and around Mekelle.
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1. Introdaction

The donkey is a domestication member of the family Equidae. The wild ancestor of the donkeys is the Africa wild ass, *Equus asinus africanus* and the Somali wild ass, *Equus asinus somaliensis*. Donkeys were first domesticated around 3000 BC, probably in Egypt or Mesopotamia and have spread around the world. They continue to fill important roles in many places of the world (Ronald, 1999).

There are more than 40 million donkeys distributed throughout the world (CSA, 2013). In Africa the donkey population is estimated to be 13 million. And Ethiopia has about 6.21 million donkeys or 32% of all the donkeys in Africa and 10% of the world population (Fitsum, 2015). In our country donkeys most commonly found in the dry and mountainous area. The low level of development of road transport network and rough terrain of the country make donkeys the most valuable, appropriate and affordable pack animals (Gebrewold *et al*., 2004). Donkeys have reduced the domestic burden of rural people especially for women and have created employment and income generating opportunities for many people. They are kept and often used for pack purposes, riding, providing of manure for both energy and soil fertility (Mengistue, 2003). Also in areas where draft power is a constraint for crop cultivation a pair of well conditioned donkeys could be used as an alternative draft power sources for secondary and tertiary land preparation (Abayneh *et al*., 2002).

However, the contribution of equine power in the agricultural systems and the role in the production is not yet well organized and magnified (Megebu *et al*., 2013). Also the attention given by society to donkeys has been far below to what it deserves. This might be partly due to the wrong perception that the donkey does not require a lot of care, that when donkeys do get sick they are quick to die and the donkey's low traditional status (Marshall and Ali 2004). This mistake was justified by always low number of donkeys presented annually to the clinic compared to other domestic animals, (Yilma *et al*., 1991).

Donkeys are prone to number of infectious and non-infectious diseases. Among these infectious diseases, GIT helminthes are one of the most common factors that constrain the health and welfare of donkeys' worldwide (Zerihun *et al*., 2011). The degrees of damage are varies depending on the species and number of parasites at present, nutritional and the immune status of donkeys. They decrease the

performance, production and productivity in the animals mainly in the reduction of body weight or failure to gain weight or even increase the mortality in acute case (Zerihun *et al*., 2011). The effects of gastrointestinal parasites are more evident in young and under nourished donkeys. As a rule, older donkeys appear to develop immunity against the common gastrointestinal parasites and tend not be affected by parasite related problems as commonly as younger donkeys (Starkey, 2001).

Donkeys are said to have the largest collection of parasites of all domestic livestock. Since donkeys tend to bite, chew or nibble at their surroundings often consuming parasite infected bedding and normally graze closer to the ground than cattle, easily picking up large number of infected larvae while they graze (Starkey, 2001).

There are more than 150 species of helminth parasites that can infect donkeys. Among them, strongyles (large and small strongyles), *Parascaris equorum*, *Strongyloideswesteri,* and *Anoplocephala* species are the most known devastating parasites of equines (Pandit *et al.,* 2008). But the most frequent disorders caused by gastrointestinal parasites in donkeys are related to infection with *parascaries equirum,* the most species of cyathostomes (small strongly), the large strongly (primarily *S.vulgaries),* and *Anoplocephala* species (Reinemeyer *et al*., 2003). Infected donkeys may show signs of weakness, emaciation, restlessness, unthriftiness, diarrhea, anemia and sometimes intestinal obstruction or perforation based on the parasitic burden.

The development and survival of helminthes egg or larvae with faeces and on pasture are depending on temperature and moisture thus forming suitable environment for development of larvae of nematode to infected stage. Inadequate quality of water stored in the dam from which livestock area using directly for drink may also form suitable way for transmission of cestodes and trematode (Bowman *et al*.,2003).

Some works have been done in different parts of the country such as: Endoparasites of donkeys in Sululta and Gefersa Districts by Zerihun *et al*. (2011), strongyles and parascaris parasites population in working donkeys of Central Shoa by Ayele and Dinka (2010), occurrence of lungworm infection in equine, and their risk factors in and around Jimma town by Tihitna *et al*. (2012) and prevalence of gastro-intestinal parasites of donkeys in Dugda Bora District by Ayele *et al*. (2006). Those previous studies and observations conducted have pinpointed helminthic parasites as being a major health hazard, limiting the overall performance of donkeys. However, there was not been any study conducted on the prevalence and level of infestation of GIT helminthes of donkey in and around Mekelle.

Therefore, the objectives of this study are:

- To identify and determine the prevalence of major gastrointestinal helminthes of donkey in and around Mekelle.
- To estimating the level of infestation of nematodes based on the count of eggs per gram of feces.
- To assess the risk factors that influence the prevalence and level of parasite infections.

2. Litrature Review

2.1. Major Gastrointestinal Helminthes of Donkey

The majority of nematodes and other notable internal parasites such as cestodes and trematodes are the major gastrointestinal parasites of donkeys (Murray, 2003). The nematodes are the most numerous and most diverse group having unsegmented, elongated round on both ends, circular in cross section and bilaterally symmetrical bodies (Charles and Robinson, 2006). Trematoda and cestoda, all typically soft body's flattened dorso ventrally and hermaphroditic. The trematoda of important veterinary medicine may be found as adult in the intestine, bile duct, blood vessel or other organ of their final host. Adult cestodes are parasite of the intestine of vertebrate (Charles and Robinson, 2006).

2.1.1. Strongyles

Strongyle nematodes of donkeys are classified into the subfamilies Strongylinae and Cyathostominae, sometimes categorized as large and small strongyles, respectively. The large strongyles also known as blood worms, palisade worms and red worms. They are considered the most dangerous parasite of donkey because 1) adults are voracious blood suckers and cause anemia, weakness, diarrhea, and damage of the intestinal lining, and 2) immature worms (larvae), before they reach maturity and settle in the large intestine, migrate to the branches of the intestinal (mesenteric) arteries where they may cause damage, irritation and parasitic aneurysm (Pandit *et al*., 2008). An aneurysm is a bulging of the blood vessel wall which may hinder the flow of blood or may rupture, causing the donkey's death by internal bleeding (Burden *et al*., 2010).

Epidemiology

These large strongyles are cosmopolitans in distribution. Again, of the three strongylus species, *Strongylus vulgaris* is the most important where, the prevalence of this infection with one or more of these parasites in foals (Lopez-Olvera *et al*., 2006; Kharchenko *et al*., 2009). *S. vulgaris* and *S. edentatus* are relatively common and *S. equinus* seems to have more sporadic distribution. These parasites are important because they migrate in the circulation and vital organs and can cause severe damage that is fatal

in some instance (Ramsey *et al*., 2004; Yanzhen *el al*., 2009).

Life cycle

The life cycle of equine strongylus is direct and does not involve an intermediate host. It alternates between an exogeneous phase of free living stages present in the external environment and an endogenous phase of parasitic stages that develop in the host (Charles, 2010). Eggs which are passed in faeces are hatched in the environment and development to the infective larvae (L3) is take place. The larvae migrate up the blades of grass until ingested by donkeys. Infection is by ingestion of the L_3 and it migrates to the intestine then to mesenteric arteries (*S. vulgaris*), liver (*S. edentatus* and *S.equinus*) where they grow and molt to L_4 then migrates to the large intestine and molt to L_5. The adult (L_5) worm resides in the large intestinal mucosa and start shedding eggs (Hendrix, 2006; Kahn, 2005).

The life cycle of the small strongyle (cyathostomes) is very similar to large strongyles except the larvae do not migrate beyond the wall of the intestines. The larva burrows in or encysts in the wall of the large bowel.

Clinical signs

The clinical picture varies in line with the intensity of parasite burden, the prevalence of certain parasitic species, and to the stage of development of the worms. Moderate infections due to larval stages or adult worms result in sub clinical or chronic diseases with general clinical signs among which weight loss is the most common (Charles, 2010). Grazing donkeys usually carry a mixed burden of large and small strongyles and the major signs associated with heavy infection in animals up to 2-3 years of age are unthriftness, anemia, colic and sometimes diarrhea (Desalegne *et al.,* 2011). Severe infections with *S. vulgaris* can cause colic or abdominal distress, gangrenous enteritis due to obstruction of the cranial mesenteric artery, torsion or rupture of the intestines which lead to death. This artery can be palpated on rectal examination, and a veterinarian may be able to identify changes consistent with *S. vulgaris* infection (Hendrix, 2006). Marked clinical signs are less common in older animals although general performance may be impaired. The effect of Strongylus in more chronic infections result persistent low grade fever, poor appetite, intermittent colic and poor weight gain (Radostits *et al.,* 2007).

2. 1.2. *Parascaris equorum* (Round Worms)

Parascaris equorum is the equine ascarid under the family of ascaridea and it is found in the small intestine of equines (Hendrix, 2006). It is very large, rigid, stout, whitish nematode, 15- 40 cm in length and cannot be confused with any other intestinal parasites of equines. Males measure 15-25 cm and females up to 40 cm. They are not blood suckers. Much of their damage is due to the migration of immature worms throughout the body (Taylor *et al.,* 2007).

Life cycle

The life cycle is direct and migratory involving a hepato-pulmonary route (Hendrix, 2006). The adult worms live in the small intestine and lay very large numbers of thick- shelled eggs. The infective stage is a very thick-walled egg containing the L_2 (Krecek, 2013), these small larvae are ingested by the donkey, hatch in the intestine, burrow into the intestinal wall and migrate to the liver through the blood stream. From the liver, they reach the heart through the blood, enter small air sacs of the lung (alveoli), reach the trachea (wind pipe), are coughed, swallowed again, and finally reach maturity in the intestines. The lung can be damaged extensively, and pneumonia may occur (Radostits *et al.,* 2007). Large numbers of mature ascarids may block the intestines, particularly in foals, and cause severe digestive upset. It takes 10 to 12 weeks for ascarids to complete their life cycle. Since most foals become infested (by immature larvae) soon after birth, most worms are maturing when foals are two and one-half to three months old (Kahn, 2005).

Pathogenesis and clinical signs

During the migratory phase of experimental infections, up to 4 weeks following infection, the major signs are frequent coughing, accompanied in some cases by a greyish nasal discharge, although the foals remain bright and alert (Bowman *et al*., 2003). Light intestinal infections are well tolerated, but moderate to heavy infections will cause unthriftiness in young animals with poor growth rates, dull coats and lassitude. A wide variety of other clinical signs, including fever, nervous disturbances and colic, has been attributed to field cases of parascariosis, but these have not been observed in experimental studies (Taylor *et al.,* 2007).

2.1.3. *Strongyloides westeri* (Thread worms)

Threadworms affect primarily foals; faecal examinations seldom reveal threadworm eggs in adult donkey. Foals may acquire the infection through larvae present in the mare's milk. Threadworm larvae are found in mare's milk from 4 to 40 days after foaling and foals may become severely infected by two to three weeks of age, exhibiting diarrhea, indigestion, and unthriftiness (Charles *et al*., 2010).

Life cycle

Strongyloides is unique among the nematodes of veterinary importance, being capable of both parasitic and free-living reproductive cycles. The parasitic phase is composed entirely of female worms in the small intestine and these produce larvated eggs by parthenogenesis, i.e. development from an unfertilized egg. After hatching, larvae may develop through four

larval infecting the host by skin penetration or ingestion and migrating via the venous system, the lungs and trachea to develop into adult female worms in the small intestine.

Clinical signs

The clinical signs in very young animals, usually within the first few weeks of life, together with the finding of large numbers of the characteristic eggs or larvae in the faeces are suggestive.

2.1.4. Fasciola (Liver fluke)

Members of genus Fasciola are commonly known as liver flukes. They are responsible for wide spread morbidity and mortality in animals and characterised by weight loss, anaemia and hypoproteinaemia. The most important species are *F. hepatica* found in the temperate areas and cooler areas of high altitude in tropics and sub tropics and *F.gigantica* which predominant in tropical areas. Definitive hosts are ruminant, equine and human. Life cycle is indirect and involves intermediate host's snails of the genus *lymnea* (Urquahart *et al*., 2003).

2.1.5. *Gastrodiscus aegypticus* (Intestinal flukes)

Gastrodiscus aegypticus (Amphistome flukes) are common parasites of equine and pigs in the tropic s and sub tropics A heavy infestation are caused collapse in donkey (Edward, 2005) *G.aegypticus* requires fresh water snail intermediate hosts of the genus Blulinus especially *B.forskalii* for the development of cercariae after living the snail the cercariae encysct on the objects or grass blades semi-immersed in water at this stage they develop into metacercariae.

Symptoms are usually very inconspicuous. They appear in the case of severe parasitism when there are thousands of worms attached alone the digestive tract (Mira and Ralphsay, 1989).

2.1.6. *Anoplocephala spp* (Tapeworms)

Several tapeworm species are found in horses, donkeys and other equines. Disease has been associated with *A. perfoliata*. These parasites cluster at the ileo-cecal junction, where heavy infection can cause ulceration leading to perforation or intussusceptions (Zaja and Conbay, 2012).

The life cycle begins with eggs passed in the feces and are ingested by free-living pasture mites. Donkeys become infected during grazing when they ingest mites containing the tapeworm larvae (Taylor *et al.,* 2007).

2.2. Risk Factors

Many factors are known to influence the transmission and prevalence of gastrointestinal infection in grazing animals. Broadly the three influencing factors that can determine the occurrence of gastrointestinal tract infection could be mentioned as environmental host interaction, environmental parasitic interaction and host parasitic interaction (Radostits *et al.,* 2000).

Environmental factor such as rainfall or moisture is the most important factor which influences the survival, development, dissemination and availability of free living stages of helminths. Moisture facilitates horizontal and vertical migration of nematode larvae on the environment. Temperature also influences the development of nematode larvae and the optimal temperature for the development of most Strongyle and other larvae are 22-30°C. No development of larvae occurs below 5°C while temperatures above 40°C are lethal (Bowman *et al*., 2003).

Host factor like age, nutrition, physiological state and presence or absence of co-current infections are influenced. Poor nutrition lowers the resistance of the animal thus enhancing the establishment of worm burdens and increasing the pathogenicity of the parasites. Consequently, worm burdens tend to be higher in poorly-fed than in well-fed animals. And young animals are more susceptible than adults (Starkey, 2001).

Parasite factors: The intrinsic multiplication rate of the nematode species determines the rate of establishment and size of nematode burden in the host. The multiplication rate is determined by the fecundity of the adult worms, the prepatent period and the survival and development rate of the parasite in the environment (Charles, 2010).

2.3. Diagnostic Methods of GIT Helminthes

Helminth parasites can be diagnosed based on clinical signs together with history of the animal. However, confirmatory diagnosis requires special laboratory procedure. The detection techniques of these parasites include faecal examination, culture of larvae, molecular and post mortem examination techniques (Hendrix, 2006).

Faecal examination techniques are qualitative and quantitative techniques. Qualitative faecal examination includes direct smear (for nematode trematod and cestodes), sedimentation (for terematode) and flotation technique (for nematode and cestodes). The major quantitative techniques include modified McMaster technique (Zaja and Conbay, 2012).

2.4. Control and Prevention of GIT Parasites of Donkey

Clinically important equine parasites are ubiquitous in managed donkey populations. It is impossible to eradicate them, but the parasites can be managed with regular deworming, good nutrition, pasture and environmental managements (Bowman *et al*., 2003). All donkeys have internal parasite so donkey owners need to understand that an internal parasite control program is a continual battle (Nielsen, 2012). Management practices include: Feeding hay in

bunks or mangers; avoiding feeding on the ground, Regular cleaning of stables and pad docks., Avoiding overcrowding of pastures, Not spreading manure where donkeys can come in contact with it and Periodical grazing of cattle in donkeys pastures decreases exposure as equine parasites do not mature in cattle and breaks the life cycle (Nielsen, 2012). Any new animals joining a treated group should receive an anthelmintic and be isolated for 48–72 hours before being introduced.

3. Materials And Methods

3.1. Study area

The study was conducted in and around Mekelle town, which is the capital city of Tigray Regional State. It is located 785km north of Addis Ababa at 39° 29`E and 13° 30` N at an altitude of 2000 masl. The mean annual rainfall is 619mm, which is bimodal with short rainy seasons occurring from March to May and from mid-September to February. The annual minimum and maximum temperature is 11.8°C and 29.9°C, respectively (BoFED, 2008).

3.2. Study animals

The study was conducted on 404 donkeys were tried to be included in the study to investigate the prevalence of major gastro intestinal parasites.

3.3. Sampling method and sample size determination

A cross-sectional study was conducted from November 2015 to march 2016 on 404 donkeys that brought to Kalamino vet hospital, Mekelle donkey sanctuary and vet clinics and Quiha donkey sanctuary branch and vet clinics for different purposes and donkeys from their common collection area (from market and mill house) were randomly selected by using simple random sampling of individuals from a population was taken at a point in time. The sample size was calculated using the formula of Thursfield (2005). As there was no previous report on the prevalence of gastrointestinal helminthes of donkeys in and around Mekelle 50% expected prevalence was taken with 5% absolute precision at 95% confidence interval.

$$n=\frac{1.962\ (Pexp)\ (1-Pexp)}{d2}$$

Where;

n = required sample size,

Pexp = Expected prevalence

d = required precision (usually 0.05).

Therefore, by substituted the value of variables in the formula the sample size was determined to be 384, which is used as representative animal on which the study was done to know the prevalence of GIT helminthes, but it was increased by 5% precision(20) head of donkeys.

3.4. Study methodology

3.4.1. Qualitative faecal examination

The faecal samples were collected directly from the rectum of the donkeys by using rectal gloves or from freshly passed droppings. Each sample was labeled with animal identification, owner's name, date and area of collection. After collecting, the sample was transported to Mekelle University Parasitology laboratory for immediate processing and examination of the sample. The observation of parasites eggs and larvae in the faeces of the donkeys was evaluated qualitatively by using the direct smear, flotation and sedimentation techniques.

3.4.2. Quantitative faecal examination

A quantitative faecal examination was conducted to identify level of infestation by using a modified McMaster egg counting technique to count parasite eggs selectively on those samples positive for nematode parasitic eggs upon qualitative procedure. The levels of worm infection in donkeys were determined by using the infection severity index defined by Soulsby (1982) cited by Getachew, (2010) and Upjohn *et al* (2001) where an average total faecal nematode egg count of less than 500 eggs per gram (epg) suggests a mild infection, 500–1000 a moderate infection and > 1000 severe infection.

3.4.3. Faecal culture

As the egg of strongylus species have much in common it is difficult to make identification based on the kind of eggs so for identification of such parasites to species level faecal samples were cultured and the larvae were recovered using Baermann apparatus technique. The larvae were then identified based on the shape and number of gut cells, relative size and shape of larvae's tail (Kaufmann, 1996).

3.4.4. Risk factor assessment

During sample collection various potential risk factors including, age, body condition score, area, purpose of keeping these animals, feeding management, housing management and manure removal time was recorded. The age of the selected donkeys was determined from birth records of owners' information and by dentition. According to Sevendsen (1997) animals were categorized as young (<5 years), adult (5-10 years) and old >10 years. Body condition score (BCS) was subjectively estimated based on the criteria given by NEWC (2005) and categorized as poor, medium, good, fat and obese.

Information regarding following determinants were collected by interview of donkey owner's; Purpose of keeping the animal (Transportation, Water caring, Traction, carts and multipurpose), Feeding System (Grazing/Ground feeding, Trough feeding and mixed), Housing System (Good house, poor house and no house/tying) and manure removal time (Daily Weekly and not at all).

3.5. Data management and analysis

The data collected from the study area were entered in to Microsoft Excel spread sheet and the data were coded appropriately and analyzed using SPSS version 19 statistical software. Chi-square tests were applied to test the statistical association exists among the risk factor such as age, body condition scoring and management factor of the animal with the presence of the infection. The prevalence was calculated by dividing the number of animals harboring a given parasite by the total number of animals examined. A one-way ANOVA was also used to observe the variations of total mean EPG of the parasites with the independent variables. All results were considered statistically significant when the *P*-value <0.05.

4. Results

4.1. Qualitative faecal examination result

During the study period, faecal specimens taken from a total of 404 donkeys were thoroughly observed for the presence of different eggs of gastrointestinal helminthes. From the examined animals, 324 donkeys were positive for one or more different helminthic parasites. Higher prevalence 210 (52.0%) was recorded for strongyle species of helminthes followed by 50 (12.3%) mixed infestation (Table 1).

Table 1: Prevalence of different gastrointestinal helminthes of donkey during the study period

Species of parasites	**No. of animals examined**	**No. of positive animals**	**Prevalence (%)**
Strongyle Spp	404	210	52.0
P. equorum	404	26	6.4
S. westeri	404	8	2.0
Fasciola	404	9	2.2
G. aegypticus	404	10	2.5
Anaplocephala Spp	404	11	2.7
Strongyle Spp + *P. equorum*	404	43	10.6
Strongyle Spp + *S. westeri*	404	4	1.0
Strongyle Spp + Anaplocephala	404	3	0.7
Total	**404**	**324**	**80.2**

As shown in the table 2 below, age and body condition of the animals were considered during examination. And the higher prevalence (88.9%), (87.5%) was seen in young donkeys and poor BCS donkeys respectively while the low prevalence (33.3%) was observed in good BCS donkeys. The prevalence was statistically significant difference among the age group and BCS categories of the donkey ($p < 0.05$).

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Table 2: Prevalence of GIT helminthes based on age and BCS of the donkey

Variables	No. of animals examined	No. of Positives animals	Prevalence (%)	x^2	P-value
Age					
Young	117	104	88.9		
Adult	181	140	77.3	7.981	0.018
Old	106	80	73.4		
BCS					
Poor	160	140	87.9		
Medium	208	172	82.7	55.97	0.000
Good	36	12	33.3		

The recorded prevalence on the three selected areas (kalamino, Quiha and Mekelle) shows that almost similar and there was no statistical significant difference (p=0.621) (Table 3).

Table 3: Prevalence of GIT helminthes based on the study sites

Study area	**No. of animals examined**	**No. of Positives animal**	**Prevalence (%)**	**X^2**	**P-value**
Kalamino	133	103	77.4		
Mekelle	134	109	81.3	0.954	0.621
Quiha	137	112	80.5		
Total	**404**	**324**	**80.2%**		

Prevalence comparison concerning the purposes of the animals were kept was made and higher parasite infections were observed in donkeys used for water caring 86 (96.6%) followed by cart donkeys 27

(96.4%) than donkeys that used for transporting and multi purposes (Table 4). In addition animals fed on the ground and live in poor and not clean house were found to be high risk for the occurrence of parasites (Table 4). The result indicates that management of donkeys was important factor for the prevalence of parasites infections with statistically significant variations were observed in all these considered factors (P< 0.05).

Table 4: Prevalence of GIT helminthes of donkey based management factors

Variables	No. of animal examined	No. of Positive animals	Prevalence (%)	X^2	P-value
Purpose					
Transporting	132	95	71.96		
Water caring	89	86	96.6		
Carts	28	27	96.4	28.206	0.000
Multipurpose	155	116	74.8		
Feeding system					
Ground	138	121	87.6		
Trough	148	113	76.3	7.391	0.025
Mixed	118	90	76.2		
Housing system					
No house	72	65	90.3		
Poor house	161	146	90.7	37.208	0.000
Good house	171	113	66.0		
Manure removal frequency					
Daily	133	84	63.1		
Weekly	79	72	91.1	36.719	0.000
Not at all	192	168	87.5		

During examination of animals deworming history was also considered. Among the 404 donkeys only 86 donkeys were dewormed within three months and the rest 318 donkey were not dewormed previously. Out of the dewormed animal 42 (48.8%) were positive for gastro-intestinal parasites, out of those not dewormed 282 (88.6%) were positive for gastro-intestinal parasites. In this study, deworming of donkey have a significant relationship with the occurrence of gastro-intestinal helminthes (*P*=0.000), as shown in the Table 5 below dewormed animals have lower rate of infestation with GIT parasites as compared with non dewormed donkey.

Table 5: Prevalence of GIT helminthes of donkey based previous deworming history

Deworming history	No. of animal examined	No. of Positives	Prevalence (%)	X^2	P-value
No	318	282	88.6	67.664	0.000
Yes	86	42	48.8		
Total	**404**	**324**	**80.2**		

4.2. Quantitative faecal examination result

The McMaster technique was applied to determine the number of GIT nematode parasites egg per gram of faeces (EPG) revealed that minimum and maximum EPG values 50-2850. The intensities of nematode eggs infections were categorized by the counted egg per gram of feces (EPG). Nematodes infections in donkeys were classified as mild<500 EPG, moderate 500 - 1000 and severe >1000 EPG as described by Upjohn *et al* (2001). Based on this categories of EPG counts in the study area about 121(41.0%), 78(26.4%), and 96(32.5%) donkeys were severely, moderately and mildly infected respectively (Table 6).

Table 6: The overall intensity of nematode parasites infection in donkey

Infestation categories	No of infected animal	Percentage %
Mild	96	32.5
Moderate	78	26.4
Severe	121	41.0
Total	**295**	**100**

The mean EPG count of GIT nematode infestation was significantly associated with body condition score of the study animals. Highest rate of mean EPG (1126.17±752.08) was seen in poor body

condition donkeys while the lowest rate of mean EPG (365.0 ±440.99) was detected from good body condition scored donkeys. But the age of the animal was not significantly difference on mean EPG count (P=0.341) (Table 7).

Table 7: Mean value ± SD of EPG of nematode infection based on age and BCS of the donkey

Variables	No. of infected animals	Mean value ± SD	F	P-value
Age				
Young	98	870.92±665.77	1.080	0.341
Adult	126	913.89±652.09		
Old	71	1020.42±676.84		
BCS				
Poor	128	1126.17±752.08	13.433	0.000
Medium	157	797.13±535.27		
Good	10	365.00±440.99		

Comparison mean EPG count on the three study sites (Table 8) was slightly higher in Kalamino (964.28±579.67) followed by Mekelle (953.43±691.94) than Quiha (862.25±703.19) but the difference is not statistically significant (P=0.493).

Table 8: Mean value ± SD of EPG of nematode infection based on the study sites

Study site	No of infected animals	Mean value± SD	F	p -value
Kalamino	91	964.28±579.67		
Mekelle	102	953.43±691.94	0.709	0.493
Quiha	102	862.25±703.19		
Total	**295**	**925.25±662.82**		

There was statistically significant association (P<0.05) between purpose of the animal, housing type and farm hygiene (regular manure removal time) of donkey with EPG of GIT nematodes in that high (1331.5±631.57, 1044.0±678.12 and 1245.8±660.64) mean EPG count was detected in donkeys used for carts, donkeys that live in poor and not clean house respectively (Table 9).

Table 9: Mean value ± SD of EPG of nematode infection based on management factors

Variables	No. of infected animals	Mean value± SD	F	P-value
Purpose				
Transporting	90	897.78±617.43		
Water caring	76	907.89±662.57	3.948	0.009
Carts	27	1331.48±631.57		
Multipurpose	102	854.90±681.35		
Housing system				
No house	60	991.67±721.82		
Poor house	133	1043.98±678.12	7.080	0.001
Good house	102	731.37±560.67		
Manure removal frequency				
Daily	73	441.19±385.08		
Weekly	65	694.61±462.59	57.965	0.000
Not at all	157	1245.86±660.64		
Feeding system				
Ground	110	1006.81±672.85		
Trough	108	884.72±655.69	1.350	0.261
Mixed	77	865.58±655.25		

Comparison of mean EPG count between dewormed and none dewormed animals (Table 10) was slightly higher in the non dewormed donkey but the difference was not statistically significant (P=0.537).

Table 10: Mean value ± SD of EPG of nematode infection based on previous deworming history

Deworming history	No of infected animals	Mean value ± SD	F	P-value
No	256	934.57±674.08	0.382	0.537
Yes	39	864.10±94.12		
Total	**295**	**925.25±662.82**		

4.3. Fecal culture result

During the study period 210 fecal samples which were positive for strongyle type egg were processed by faecal culture. Examination of ova culture enabled for the identification 3 species of large strongyles and the genera of small strongyles. Of which 73% *S. vulgaris*, 22.9%, *S. edentatus* 9.5% *S. equinus* 23.8% cyathostomins, 4.3% mixed *S. vulgaris* with *Cythostomine,* 1.9% *S. vulgaris* with *S. edentates,* 2.9% *S. edentatus* with *Cyathostomine* larvae were recovered from the cultured faecal samples (Table 11).

Table 11: Relative percentage of larvae of Strongyles recovered from faecal culture

Species of parasites	No. of positive (%)	Prevalence
S. vulgaris	73	34.8
S. edentatus	48	22.9
S. equinus	20	9.5
Cyathostomine	50	23.8
S. vulgaris + Cythostomine	9	4.3
S. vulgaris + S.edentatus	4	1.9
S.edentates + Cyathostomine	6	2.9
Total	**210**	**100**

5. Discussion

The current study showed that, donkeys from the study area were infected with a wide variety of gastrointestinal helminthes including nematodes, cestodes, and trematodes. The overall prevalence of parasitic infections in the study area was 80.2% and this prevalence was in agreement with the early report of 84.4%, and 77.3% by Gulima (2006) in Awi Zone, and Alemayehu and Etaferahu (2013) in south wollo zone respectively. But this was relatively lower than some of the other reports of 100% by Yoseph *et al*. (2001) in Wonchi Area, 100% by Mulate *et al.* (2005) in highlands of Wollo province, 97.13% by Mezgebu *et al*. (2013) in and around Gonder, 96.9% by Nuraddis *et al*. (2011) around Hawassa Town and 98.2% by Ayele *et al.* (2006) in Dugda Bora District.

The reasons of variable results from previous reports may include: (a) sampling methods; the prevalence could be higher in purposive sampling (b) use of anthelmintic in the selected population; ignoring previous history of anthelmintic therapy during samples selection can provide false negative results, (c) season of surveillance; if limited to only winter season cannot provide true picture of parasitic distribution in the population(Tahir *et al*., 2016), and (d) limited targeted species of parasite; e.g. if focused on only one or two kinds of parasitic, the true cumulative worm burden cannot be attained.

The relative percentage of donkey GIT parasitism reported in this study indicated that strongyle was observed to have higher occurrence rate (52.0%) than other GIT parasites which found to be lower than the previous reported prevalence of 100%, in Dugda Bora district by Ayele *et al*. (2006), 100% in highlands of Wollo province by Mulate *et al*. (2005), 98.2% in Western highlands of Oromia by Fikru *et al*. (2005) and 100% Wonchi Area by Yoseph *et al*. (2001). Cultural identification of larvae of strongyle indicates that 34% *S. vulgaris*, which is in contrast with the study of Yoseph *et al*. (2001), and Ayele *et al*. (2006) in which both have reported prevalence of *S. vulgaris* was 100% in Wonchi Area and Dugda Bora District respectively. Similarly the prevalence, 22.9% *S. edentatus* 9.5% *S. equinus*, 23.8% *Cyathostomines* and 9.1% mixed strongyles were in contrast with Nuraddis *et al.* (2011) in and around Hawassa who reported *S. edentatus* (30.8%), *S. equines* (12.3%) and *Cyathostomines* (29.7%). The difference might be associated with the rate of development and survival of the free-living stages of the larvae is depends on the surrounding temperature and humidity or rainfall, i.e. this parasite is well develop in area with high humidity, low temperature and high altitude areas (Bowman *et al*., 2003).

The prevalence of *Parascaris equorum* (6.4%) in the current study was comparable with Alemayehu and Etaferahu, (2013) who have reported 10.4% in south wollo zone. Our finding lower than the other reported prevalence of 20.8 % by Tsegay and chala (2015) in Haramaya town, 17.3% by Fikru *et al*. (2005) in western highlands of Oromia, 42.29% by Mezgebu *et al*. (2013) in and around Gondar and, 43% by Ayele *et al*. (2006) in Dugda Bora District and 50%, by Tesfu *et al*. (2014) in Hawasa town. In general the relative

low occurrence in this species and other species of parasites in this study might be due to increased awareness and sometimes regular deworming programs provided by the Tigray Donkey Sanctuary Project and accessibility to veterinary clinics in the study area.

S. westeri was one of the least prevalent (2.0%) parasite based on eggs detected in the current study. This is in contrast with the work of Ayele *et al.* (2006) in Dugda Bora District, Nuraddis *et al*. (2011) in and around Hawassa and Desalegne *et al*. (2011) in central region of Ethiopia who have reported 32%, 20%, and 44% respectively. This variation might be due to *S. westeri* infections is most common in foals usually from two to three weeks age (Taylor *et al.,* 2007). But in our study area the most susceptible group of age was absent because of lack of female donkeys i.e. all donkeys in that areas bought from market at least greater than one years old.

The prevalence of *G. aegypticus* in the present study was 2.5%. which is in agreement with the report of Mezgebu *et al*. (2013) who reported 3.5% in and around Gondar. The finding of the current study was lower than the report of Getachew *et al.* (2010) who reported 30% in donkeys and Ayele *et al*. (2006) who reported 6% prevalence in donkeys of Dugda Bora district. This variation observed in this study could be due to the variation in the length of the study period, the season of the study period and ecology of the study area.

The present prevalence (2.7%) of *Anoplocephala spp* was similar with 2.2% in Kurfa Chale District, East Hararghe, reported by Sultan *et al*. (2014). However the result was lower when compared with reports of Ayele *et al*. (2006) who reported 7.6% in Donkeys of Dugda Bora and the 5.7% reported by Adem and Mengesha (2010) in Equine at Asela. This variation could be due to the variation in environmental nature of the regions. Most of the time this parasites is common in area which is characterized by year-round moist humid conditions which tend to favor high prevalence of oribatted mites. Soulsby (1982) cited by Sultan *et al*. (2014) indicated that the occurrence *Anoplocephala spp* is associated with the vector prevalence.

The prevalence for Fasciola 2.2% was lower than the previous report of 17.92% by Bewketu and Endalkachew (2013) in and around Bahir Dar. The lower prevalence of Fasciola eggs in the current study could be due to the geographical location of the area which is not comfortable for the snail pupation which is the intermediate host of Fasciola.

Mixed gastrointestinal helminthes infections of donkeys in the current study was observed with prevalence of 12.4% which is in agreement with that of Mulate (2005) who observed that poly parasitism was common and reported as 10.4 % prevalence in South and North Wollo zones.

Comparison was made in the prevalence parasites with origin of the animal, age, BCS and management factors like feeding, housing, hygienic (regular manure removal) and the work purpose for which the animal was kept. This study confirmed presence of statistical significance difference between all of the above factors except origin of the animal does not bring statistically significant difference in the prevalence of gastrointestinal parasites of the animal. This was in contrast with the studies in other parts of Ethiopia who indicated statistically insignificant difference among the age groups and BCS reported by Meagebu *et al*. (2013) in and around Gondar.

There was a decrease in the prevalence of GIT helminthosis as the animal get older and the prevalence was higher in young donkey (87.9 %) while the prevalence in adult and old donkeys are 77.3% and 73.4% respectively. The observed difference could be due to a lack of immunity in the young donkey and frequency exposure increase immunity of the animal (Urqhuart *et al*., 1996). For instance equine commonly develop marked resistance to *P. eqourum* after 6 months of age (Mulate, 2005).

Likewise, donkeys with poor body condition had higher chance of harboring the parasites. This could be due to the fact that animals with poor body condition might be immuno-compromised probably due to malnourishment and higher workload and as a result exposed to parasitism. On the other hand, poor body condition score could also be due to the parasitism. For example, blood sucking nature of the gastrointestinal parasites of donkeys, most of the time they lead to decreased body condition of the animals (Pandit *et al*., 2008) and this is why they have significant relation with body condition of the animals.

Donkeys that feed on ground/grazing and live in poor and not clean housing were at higher risk of acquiring gastrointestinal parasites than those feed on the trough and live in good and clean house, respectively. It is due to the fact that contamination is one among the major contributing factors in donkey parasites which increase the risk of infestation.

Concerning the purposes for which the animals were kept, donkeys that were used for water caring and donkeys that use for carts was found to be with higher prevalence of parasitism than animals used for transporting other items like wood and grain sacks. This could be associated with the more workload in donkeys which create stress and consequent immuno-suppression and this may facilitate the parasitism (Adam, *et al*., 2013). Further more water caring animals more exposed for parasites because intermediate hosts of some parasites are common in around moist such as river area so water caring donkey

may acquire such parasites during bring water when they graze on this marsh area.

Prevalence was also compared with the history of deworming and it was significantly higher (P<0.05) in animals without the history deworming. However out of the dewormed (86) donkeys 42(48.8%) was positive for different parasites. This was might be due to drug resistance capacity of some parasites. For example small strongyles are highly resistance for common anti helminthes (Murray, 2003). There was no significant association (P>0.05) between origins (Study site) and parasitic prevalence. This might be associated with the similarity of the agro-ecological climate of the peasant associations.

Concerning severity of infection in donkeys reported in this study, higher incidence 121(57.6%), for severe infection and lower incidence 78(26.4%), and 96(32.5%) for moderate and mild infected respectively. This is in agreement with the work of Nuraddis *et al*. (2011) in and around Hawassa who reported 53.6% of donkeys were infected severely while moderate and mild infection had the lower incidence 15.9% and 8.6% respectively. But in Sudan there is a contradictory report in which high incidence (58.6%) for mild infection and low incidence (21.9%) and (19.5%) for moderate and severe infections respectively as reported by Adam *et al.* (2013). This may be attributed to management system and as well as lack of veterinary services.

In the current study the mean EPG count of nematode parasites was found to be 925.25±662.82. The finding was lower than the previous studies conducted by Seri *et al*. (2004) in Sudan and Ayele and Dinka (2010) in Ethiopia of central Shoa, who have reported mean EPG count of 1016.6±363.6 and 2893 respectively. The mean EPG count was significantly higher in poor body conditioned animals. This result agrees with the report of Dessie and Melese (2013) in Ethiopia. The reason might be associated with the fact that animals with poor BCS have waning immunity and as a result they could not resist the parasites burden when compared with animals of good BCS (Sapkota, 2009).

Even though there was no significant variation, higher mean EPG was recorded in older donkey than adult and young donkey. This is in disagreement with the work of Zerihun *et al*. (2011) and Dessie and Melese (2012) who documented higher mean EPG in younger than older donkeys in Ethiopia. This higher mean EPG in older donkeys in our report might be attributed to the compromised immune responses relating to aged animal (Pawelec, 2007).

There was also significant association (P<0.05) between purpose of the animal, housing type and manure cleaning frequency of the premises with EPG of GIT nematodes. High (1331.5±631.57, 1044.0±678.12 and 1245.8±660.64) mean EPG count was detected in donkeys used for carts, donkeys that live in poor and not frequently cleaned premises respectively. Therefore, poor management, high work load and low plane of nutrition could reduce the immune status, which could create a favorable condition to heavy parasitic infestation (Adam *et al*., 2013).

6. Conculision And Recommendations

The study revealed a high prevalence of a wide range of species of gastro-intestinal helmintic parasites that compromise the health and welfare of donkey in and around Mekelle. The identified parasite eggs include *Strongyle, Parascaris equorum, Strongolides westeri, Gastrodiscus aegyptiacus, Anaplocephala spp, Fasciola* and mixed parasites eggs were common in the area of study. Among the identified GIT parasites, the highest relative percentage was recorded for Strongyles while less occurrence rate was observed for *Strongolides westeri,* followed by Fasciola and *Gastrodiscus aegyptiacus.* Body conditions related to feed and work overload, feeding system and type of house were the important risk factors for occurrence of gastrointestinal parasites in the donkeys which were assessed by their prevalence and mean eggs count. Among this poor housing and hygienic conditions were found to be the major contributing factor in the donkeys which increased the risk of infestation.

Based on the above conclusion, the following recommendations are forwarded:

- ✓ The field veterinarian should aware the donkey owners on improving the housing and feeding management system and to providing sufficient food and shelter, minimizing overworking and extensive open grazing of their donkeys.
- ✓ Regular deworming program should be implemented using broad spectrum anthelmintics,
- ✓ Further research should be needed to investigate the clear epidemiology, pathogenicity, and the anthelminitc resistance status of prevailing parasites.

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List Of Abbreviations

BCS	Body condition score
cm	Centimeter
EPG	Egg per gram
GIT	Gastro-Intestinal Tract
kg	Kilogram
L_3	Third stage larvae
masl	Meters above sea level
mg	Milligram
mm	Millimeters
^{O}C	Degree Celsius
SPSS	Statistical Package for Social Sciences
χ^2	Chi-square

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8. ANNEXS

Annex 1; Daily data recording sheet

Animal information													**Laboratory result**			
No	Date	Owner name	Study area	Site	Sex	Age	BCS	Purpose	Feeding	*Housing*	*Manure*	*DH*	+ve *SPP*	EPG	L_3	-ve
1	10/02/08	Berihu G/kidan	Kalamino	Clinic	male	<5	1	Water caring	Ground	poor	Daily	No	Strongyle Spp	1500	S.vulgaris	

Annex 2. Body condition scoring in donkey

BCS	Descriptions
Poor 1	Neck thin and meets shoulder abruptly neck and shoulder bones easily felt. Dorsal spine of withers prominent and easily felt. Ribs can be seen from a distance and felt with ease. Back bone prominent, Dorsal and transverse processes can be felt easily. Hip bones visible and felt easily. May be cavity under tail.
Moderate 2	Some muscle development overlying bones of neck. Shoulder some muscles cover over dorsal withers. Spinous processes of withers felt but not prominent. Ribs not visible but can felt with ease. Dorsal and transverse processes of back bone felt with light pressure. Poor muscle development on either side midline. Poor muscle cover on hind quarter. Hip bones felt with ease.
Good 3	Good muscle development on neck and shoulders, bones felt under light cover of muscle (fat). Neck flows smoothly in to shoulder, which flows smoothly in to neck. Muscle development on either side of mid line is good. Good muscle covers in hind quarter. Hip bone rounded in appearance and can be felt with light pressure.
Fat 4	Neck thick, crest hard, shoulder covered in even fat layer. Withers broad, bones felt with firm pressure. Dorsal and transverse processes of back bone can be felt with firm pressure.
Obese 5	Shoulder rounded and bulging with fat. Ribs not palpable. Hip bones cannot be felt.

Source:(NEWC: 2005)

Annex 3. Estimation of age of donkey by dentition

Age	Description
3 years old	First pair of adult teeth has grown and is in wear
4 years old	2nd pair of adult teeth is up and in wear. One pair of baby teeth is left.
5years old	3rd (corner) pair of adult teeth is up and is wearing down at the front.
6yearsold	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. At seven years, a hook can be seen on the side of the upper corner front teeth.
8 years old	A dark line at the front of the teeth (called a star) has appeared on each of the central pair of front teeth.
9 years old	Tow no more cups, only marks. Stars have appeared on the next teeth. A groove begins to grow down the upper corner front tooth.
10 years old	The biting surfaces are more triangular. The star has appeared on corner front teeth. Stars are becoming more round and neater the middle of the tooth. Marks are less distinct. The seven year hook has worn away.
12 years old	The mark has gone from the centrals. Stars are now round. The groove in the upper corner teeth is about one centimeter long.
15 years old	Only stars on the teeth. The groove is now half way down the upper corner teeth.
19-20 years old	Seen from the side, the teeth have a forward slope. The groove extends down the whole tooth.
20-25 years old	The teeth have an even more forward pointing angle and the groove is growing out (it disappears at about 30 years old). The tops of the now have a more triangular shape.

Source: Sevendsen (1997).

Annex 4: Laboratory procedures for faecal examination

Flotation technique

1. Weigh approximately 3 grams of well mixed faecal sample into a beaker or plastic container 1. If the faeces are pelleted grind it using mortar & pestle.
2. Add approximately 50 ml of flotation fluid and stir/mix thoroughly until all the faecal material is broken down.
3. Pour the faecal suspension through a tea strainer or sieve /a double layer of gauze into container 2 to remove large faecal debris.
4. Place the tube (15ml capacity approximately) in a test tube rack and gently topped off with the suspension leaving a convex meniscus at the top of the tube.
5. Carefully place a coverslip on top of the test tube).
6. Leave the test tube to stand for 20 minutes.
7. Carefully lift the coverslip and place the coverslip on a clean slide.
8. Examine using a compound microscope at x 10 magnification (Taylor *et al*., 2007).

Sedimentation technique

1. Weigh or measure about 3 grams of faeces into a mortar/container 1,
2. Pour 42ml of tap water into mortar/container 1,
3. Mix thoroughly with a pestle/stirring rod,
4. Filter the mixture through Sieve into a beaker/container 2,
5. Pour the filtered material into a test tube,
6. Centrifuge the filtrate for 3 minutes at 1500 rpm,
7. Discarded the supernatant very carefully,
8. Add a drop of 1% methylene blue to the sediment and mix,
9. Take a drop of the sediment on the slide,
10. Cover the smear with a cover slip and examine it under 10x magnification power (Taylor *et al*., 2007).

McMaster Egg Counting Technique for nematode eggs

1. Weigh 4 grams of faeces in a container 1 (mortal/beaker).
2. Add 56 ml of flotation fluid and mix (stir) the contents of the container 1 thoroughly.
3. Filter the faecal suspension through sieve into the second container 2.
4. Using the Pasteur pipette withdraw a sub-sample and fill both sides of the McMaster counting chamber.
5. Allow the counting chamber to stand for 5 minutes.
6. Examine the subsample of the filtrate under the compound microscope at x10 magnification.
7. Count the number of eggs within the grid of each chamber under.
8. Add the counted egg of the two chambers and multiply by 50 this gives e.p.g. (Zajac and Conbay, 2012).

Faecal culture

1. Take a certain amount of faeces(10gm) from the rectum of animal.
2. Break up the collected faeces in a container (Pestle & mortar).
3. Moisten samples with water if too dry and add charcoal or sterile bovine faeces if the faeces are too wet, until the correct consistency is obtained.
4. Transfer the faecal material in to petridish.
5. Leave the culture at room temperature for 14-21 days.
6. Add water to cultures regularly if mixture is getting too dry, every 1-2 days.
7. Recover the larvae using Baermann technique (Bayou, 2005).

Baerman technique

1. Take a funnel fitted to stund and attach arubber tube to the funnle with clump on the lower end.
2. Fill the funnl with luke warm water.
3. Warp the caltured faeces in double layred gaue.
4. Hang or suspend faeces enclosed in gauze in the filled with water by using supporting stick (glass rod or clip wire), till completely immersed in the water.
5. Leave the apparatus in place for, during which time larvae actively.
6. 24 hours later open the clump and collecte the aliquit in test tube.
7. Allow the larvae to settle at the bottom.
8. Discared the supernant and examine the sediment for larvae (Bayou, 2005).

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