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# Prevalence of Gastrointestinal Nematodes in Horses and Donkeys in and Around Asella, Central Ethiopia

*[1]Birkie Mengesha and [2]Belay Bekele Tola*

[1]College of Veterinary Medicine, Jigjiga University Ethiopia
[2]Ejere Woreda Livestock and Fishery Resource Development Office, Oromia, Ethiopia

**Abstract:** A cross-sectional study was conducted from November, 2016 to April, 2017 in and around Asella town, to assess the prevalence of gastrointestinal nematodes (GIT) in donkeys and horses. A total of 384 Equines (155 donkeys and 229 horses) were randomly selected and examined during the study period. Floatation and McMaster egg counting techniques were employed on feces to identify parasite eggs and determine parasite loads, respectively. The analysis result revealed that the overall prevalence of the GIT nematodes was 82.66% with a prevalence of 83.84 and 81.29% in horses and donkeys respectively. The prevalence of Strongyle, *Parascaris equorum* and Strongyloid was 46.45%, 2.58%, 8.39% in donkeys and 51.97%, 1.31%, 9.61% in horses respectively. Nematode parasite prevalence was significantly influenced by sex, age and body condition risk factors ($p< 0.05$) whereas species and origin did not significantly affect occurrence of the parasites ($p>0.05$). Mixed infection was also observed as two or three of these parasites co-occurred in examined animals. Nematodes infections in both horses and donkeys were recorded from minimum to maximum EPG values of 50-1850 respectively. Based on this EPG counts in the study area about (53.7%), (17.47%), (12.66%) and (52.9%), (18.7%), (9.68%) in horses and donkeys were lightly (<500), moderately (501-1000) and severely (>1000) infected respectively. In conclusion, the current study revealed considerably high prevalence of GIT nematodes in horses and donkeys in the area suggesting a need to organize appropriate control strategies.

**Key words:** Asella · Donkeys · Gastrointestinal Nematodes · Horses · Prevalence

## INTRODUCTION

In the developing countries, there are estimated 110 millions of equines [1]. Ethiopia has a large numbers of equines. It has a total of 9.83 millions equine populations. From those numbers, donkeys accounts 7.04 million while horses and mules are 2.03 and 0.4 million respectively [2].

In Ethiopia, the low level of development of the road transport, network and the rough terrain of the country make the donkeys and the horses the most valuable, appropriate and affordable pack animals under the small holder farming system [3]. It was estimated that working animals including equines, produced 75% of traction energy in the developing world [4] and it has been suggested that more than half of the world's population depends on animal powers as main energy source. They can be used for such applications as riding, driving, flock protection, companion, breeding, training calves [5] and provide urban dwellers with opportunity of income generation.

Parasitic helminthes are one of the most common factors that constrain the health and working performance of donkeys and horses worldwide. They cause various degrees of damage depending on the species and number at present, nutritional and the immune status of eqiuds [6]. 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. A number of studies conducted to detect association between poverty and animal diseases identified gastrointestinal parasitism as one of the most important problems for eqiuds in developing countries.

**Corresponding Author:** Belay Bekele Tola, Ejere Woreda Livestock and Fishery Resource Development Office, Oromia, Ethiopia.

Equine endoparasites may be divided into three categories: nematodes, or roundworms, cestodes or tapeworms, trematodes or flukes. Parasites are assigned to these categories according to their morphology or structure. Growth and life cycles of parasites within each group are generally distinct from those of the other groups. Equines are hosts to great nematodes of the family Strongylidae, commonly called Strongyle nematodes or Strongyles. The most common gastrointestinal nematode parasites of equines include large strongyles, small strongyle, *Ascaris* and *Oxyuris equi* (*O. equi*) [7]. Gastrointestinal nematodes are serious health hazards, contributing to poor body condition, reduced power output, poor productive performance and short life span. Additionally, less important parasitic infection belongs to cestodes, lung worms, trematodes and intestinal thread worms/ *Strongyloides westeri* (*S. westeri*) [8]. The most important factors which available for the existence of internal parasite to be found within the animals is age. Foals are the most susceptible to the thread worms (*S. westeri*) in the first few months of life following infection via mare's milk. Equine ascaris infection /*Parascaris equorum (P. equorum*) occurs mainly in foals. In views of this, there has been no any research done on equine nematodes parasite in and around Asella. The objective of this study was therefore; assess the prevalence of gastrointestinal nematodes (GIT) in donkeys and horses.

## MATERIALS AND METHODS

**Study Area and Period:** This study was conducted from November 2016 to April 2017 in and around Asella town, Tiyo woreda. The Town is a capital of Arsi Zone, Oromia regional state. It is located about 175 km south east of Addis Ababa at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitudes. The altitude of the area ranges from 2500 to 3000 m.a.s.l. Asella and its surrounding is characterized by mid subtropical weather, with minimum and maximum temperature ranging from 8.4 to 22.6°C, and the relative humidity ranging from 43 to 60%. The average rainfall is 2000 mm. The farmers in the area practice mixed crop-livestock farming system.

**Study Design and Population:** The study employed a cross-sectional study design on randomly selected equines for the detection of the prevalence of GIT nematodes. The study animals included 155 donkeys and 229 horses of all age group owned by the individual farmers for different purposes. The study animals were kept under extensive management system and they were not treated with antihelmintic during the study period. Information about species, sex, age body conditions and origin of the study animals was gathered appropriately. The ages were determined using owners' information.

**Sample Size Determination:** The sample size required for this study was determined according to the formula given by Thrusfield [9] with 95% confidence interval at 5% desired absolute precision and by assuming the expected prevalence of 50%. The estimated sample size was calculated by the formula:

$$N = \frac{(1.96)^2 \, P \, (1-P)}{d^2}$$

where;

N = Required sample size
P = Expected prevalence of nematode parasites
d = Desired absolute precision
1.96 = The value of "z" at 95% level of confidence
d = 5% = 0.5

Hence, a total of 384 horses and donkeys were sampled for the study.

**Study Protocol:** Random fecal samples were collected directly from the rectum of the study animals using disposable plastic gloves and placed in into universal bottles. Each sample was labeled with necessary information (origin, age, sex, species and body condition) and immediately transported to Asella Regional Veterinary Laboratory. Samples were kept in refrigerator at 4°C when immediate processing was not possible. But, it was processed within 48 hours. Some samples were held using10% formalin. Fecal sample examination was carried out by floatation techniques and quantitative fecal examination was performed by using McMaster technique to determine the number of egg per gram of feces (EPG) and performed according to the procedure described by urquhart *et al*. [10]. Level of infection was extrapolated from infection severity index where horses/donkeys are said to have mild, moderate and severe nematode infestation if their fecal egg counts are less than 500,500-1000 and more than 1000, respectively.

**Preparation of Feces for Microscopic Examination**

**Fecal Flotation:** The procedure of flotation methods was as described by principle by Presland *et al*. [9]. Approximately 3 g of feces was put in a mortar or a plastic container and crushed by using pestle. Fifty milliliters of flotation fluid was poured to the mortar or a plastic container containing 3 g of crushed feces. The flotation fluid (sodium chloride) was mixed with feces thoroughly. The resulting fecal suspension was poured through a tea strainer or double layer of cheesecloth into another plastic container. The fecal suspension was poured into a test tube from the second container, then placed in a test tube rack, leaving a convex meniscus at the top of the tube and a cover slip was carefully placed on top of test tube. The tube was left to stand for 15-20 minutes. The cover slip was lifted off from the tube vertically together with the drop of fluid adhering to it and immediately placed on microscope slide and examined under the microscope (10X).

**Determination of Eggs per Gram of Feces (EPG):** A quantitative fecal examination was conducted using a modified McMaster egg counting technique to count nematodes parasite eggs selectively on those samples positive for parasitic eggs upon qualitative procedure. A flotation fluid (sodium chloride) was used to separate eggs from fecal material in a counting chamber with two compartments. The procedures were done based on principle of Taylor *et al.* [10]. Three grams of feces were taken from the collected sample and 42 ml of water was added to it and emulsified using mortar and pestle. The solution was strained through a plastic tea strainer and the strained material was poured into 15 ml centrifuge tube and centrifuged at 2000 rpm for 2 minutes. Supernatant was poured off, sediment was agitated and the tube was filled to the previous level with flotation fluid (sodium chloride). Both sides of the McMaster counting chamber were filled with the subsample. The counting chamber was allowed to stand for 5 minutes and the subsample in the counting chamber was examined under a compound microscope at 10X magnification power. All parasitic eggs within the engraved area of both chambers were counted and the number of eggs per gram of feces (EPG) was calculated by adding the egg counts of the two chambers together and then multiplied the total by 50.

**Data Analysis:** The data collected from the study were entered in to Microsoft Excel spread sheets and were coded appropriately and analyzed using SPSS version 17 statistical software. Chi-square test was applied to test if statistical association exists among the risk factor such as species, sex, age, body condition scoring and origin with the presence of the disease. For all the analysis a significance was held at $P<0.05$.

## RESULTS

From a total of 384 examined animals, 192 horses and 126 donkeys were positive with respective prevalence of 83.84% and 81.29% for different GIT nematodes. The overall prevalence of GIT nematode in horses and donkeys in the study area was found to be 82.565% (Table 1).

Chi-square analysis of different risk factors showed that origin and species of animals was not significantly associated with GIT nematodes infection ($p>0.05$) whereas Sex ($\chi^2=6.1104$, $P=0.013$), age ($\chi^2=17.6438$, $P<0.001$) and body condition score ($\chi^2=64.4638$, $P<0.001$) of the animal were found to be significantly associated with GIT nematode infestation (Table 2).

During the laboratory examinations, different GIT nematodes eggs were found. These were Strongyles, *P. equarum*, Strongyloid and mixed infections of Strongyle and *P. equarum*; strongyles and Strongyloid; Strongyloid and *P. equarum* and strongyle, *P. equarum* and Strongyloid each of having a frequency of occurrence of (51.97%, 46.45%); (1.31%, 2.58%); (9.61%, 8.39%); (6.11%, 8.39%), (12.23%, 11.61%); (2.18%, 1.94%), 0.44%, 1.94%) in both horses and donkeys respectively (Figure 1).

In this study the highest prevalence of Strongyle (in horses, females, old age and medium body condition), *P. equorum* (in donkeys, females, young and good body condition) and Strongyloid (in horses, females, young age and good body condition) was observed (Tables 4 and 5).

The McMaster technique was applied to determine the number of GIT nematode parasites egg per gram of feces (EPG). The minimum and maximum EPG values 50-1850 were recorded. Nematodes infestations in both horses and donkeys were classified as mild (<500) EPG, moderate (501 – 1000) and severe (>1001) EPG as described by Upjohn *et al*. [11]. Based on this categories of EPG counts in the study area about (53.7%), (17.47%), (12.66%) and (52.9%), (18.7%), (9.68%) in horses and donkeys were lightly, moderately and severely infected respectively (Table 6).

Table 1: Over all prevalence of GIT nematodes in examined animals

| Animals | Number of animals examined | Numbers of positive animals | Prevalence (percentage) | $\chi^2$ | p-value |
|---|---|---|---|---|---|
| Horses | 229 | 192 | 83.84 | | |
| Donkeys | 155 | 126 | 81.29 | 0.4231 | 0.515 |
| Total | 384 | 318 | 82.565 | | |

Table 2: Prevalence of GIT nematodes in relation to different risk factors

| Risk factors | Number of examined animals | Number positive (prevalence %) | $\chi^2$(p-value) |
|---|---|---|---|
| Origin | | | |
| Asella | 49 | 38(77.55) | 4.5738(0.334) |
| Dosha | 57 | 45(78.95) | |
| Lalocheka | 73 | 64(87.67) | |
| Gonde | 99 | 79(79.80) | |
| Golja | 106 | 92(86.79) | |
| Species | | | |
| Horse | 229 | 192(83.84) | 0.4231(0.515) |
| Donkey | 155 | 126(81.29) | |
| Sex | | | |
| M.ale | 227 | 179(78.85) | 6.1104(0.013) |
| Female | 157 | 139(88.54) | |
| Age | | | |
| Young | 99 | 91(91.92) | 17.6438(<0.001) |
| Adult | 232 | 177(76.29) | |
| Old | 53 | 50(94.34) | |
| BCS | | | |
| Poor | 59 | 58(98.31) | 64.6438(<0.001) |
| Med ium | 196 | 181(92.35) | |
| Good | 129 | 79(61.24) | |

Table 3: The rate of occurrence of the different GIT nematodes according to species, sex and age of animals.

| | Species | | | Sex | | | Age | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Spp of parasite (%) | Horse | Donkey | $\chi^2$ (P) | Male | Female | $\chi^2$ (p) | Young | Adult | Old | $\chi^2$ (p) |
| Strongyle | 119(51.97%) | 72(46.45%) | 4.6142 (0.707) | 107(47.14%) | 84(53.50%) | 7.7842 (0.352) | 17(17.17%) | 134(57.76%) | 40(75.47%) | 137.08 (0.00) |
| *P. equarum.* | 3(1.31%) | 4(2.58%) | | 4 (1.76%) | 3(1.91%) | | 3(1.91%) | 0(0.00%) | 1(1.89%) | |
| Strongyloid | 22(9.61%) | 13(8.39%) | | 18(7.93%) | 17(10.83%) | | 18(18.18%) | 16(6.90%) | 1(1.89%) | |
| Strongyle+p.equarum | 14(6.11%) | 13(8.39%) | | 16(7.05%) | 11(7.01%) | | 19(19.19%) | 7(3.02%) | 1(1.89%) | |
| Strongyle+Strongyloid | 28(12.23%) | 18(11.61%) | | 26(11.45%) | 20(12.74%) | | 20(20.20%) | 20(8.62%) | 6(11.32%) | |
| Strongyloid+p.equarum | 5(2.18%) | 3(1.94%) | | 6(2.64%) | 2(1.27%) | | 7(0.07%) | 0(0.00%) | 1(1.89%) | |
| Strongyle+p.equarum+ Strongyloid | 1(0.44%) | 3(1.94%) | | 2(0.88%) | | 2(1.27%) | | 4(4.04%) | 0(0.00%) | 0(0.00%) |

Table 4: The rate of occurrence of the different GIT nematodes according to origin and body condition scores.

| | Origin | | | | | | Body condition score | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species of parasites | Asella | Dosha | Lalocheka | Gonde | Golja | $\chi^2$ (p) | Poor | Medium | Good | $\chi^2$ (p) |
| Strongyle | 26(53.06%) | 25(43.86%) | 39(53.42%) | 46(46.46%) | 55(51.89%) | 18.763 (0.905) | 23(38.69%) | 117(59.69%) | 51(39.53) | 132.9973(0.000) |
| *P.equarum* | 1(2.04%) | 0(0.00%) | 2(2.74%) | 3(3.03%) | 1(0.94%) | | 0(0.00%) | 2(1.02%) | 5(3.88) | |
| Strongyloid | 4(8.16%) | 4(7.02%) | 5(6.85%) | 10(10.10%) | 12(11.32%) | | 1(1.69%) | 17(8.67%) | 17(13.18) | |
| Strongyle+p.equarum | 2(4.08%) | 4(7.02%) | 8(10.96%) | 4(4.04%) | 9(8.49%) | | 8(13.56%) | 16(8.16%) | 3(2.33) | |
| Strongyle+Strongyloid | 4(8.16%) | 10(17.54%) | 8(10.96%) | 13(13.13%) | 11(10.38%) | | 21(35.59%) | 22(11.22%) | 3(2.33) | |
| Strongyloid+p.equarum | 1(2.04%) | 2(3.51%) | 1(1.37%) | 1(1.01%) | 3(2.83%) | | 2(3.39%) | 3(2.33%) | 0(0.00) | |
| Strongyle+p.equarum+ Strongyloid | 0(0.00%) | 0(0.00%) | 1(1.37%) | 2(2.02%) | 2(2.02%) | | 3(5.08%) | | 1(0.51%) | 0(0.000) |

Table 5: Degree of GIT nematode parasites infestation in relation to different risk factors

| | Degree of infestation | | | | |
|---|---|---|---|---|---|
| Risk factors | Light (%) | Moderate (%) | Severe (%) | $\chi^2$ | p-value |
| Species | | | | | |
| Horse | 123(53.71%) | 40(17.47%) | 29(12.66%) | 1.1605 | 0.762 |
| Donkey | 82(52.90%) | 29(18.71%) | 15(9.68%) | | |
| Sex | | | | | |
| Male | 114(50.22%) | 37(16.30%) | 28(12.33%) | 7.3352 | 0.062 |
| Female | 91(57.96%) | 32(20.38%) | 16(10.19%) | | |
| Bcs | | | | | |
| Poor | 7(11.86%) | 27(45.56%) | 24(40.68%) | .1765979 | <0.001 |
| Medium | 124(63.27%) | 40(20.41%) | 17(8.67%) | | |
| Good | 74(57.36%) | 2(1.55%) | 3(2.33%) | | |
| Age | Light (%) | Moderate (%) | Severe (%) | | |
| Young | 49(49.49) | 22(22.22) | 20(20.20) | 29.03 | <0.001 |
| Adult | 125(53.879) | 33(14.224) | 19(8.189) | | |
| Old | 31(58.49) | 14(26.415) | 5(9,4339) | | |
| Origin | | | | | |
| In Asella | 27(55.1) | 8(16.33) | 3(6.122) | 8.67 | 0.73 |
| Dosha | 28(49.1234) | 8(14.035) | 9(15.789) | | |
| Lalocheka | 43(58.9) | 13(17.808) | 8(10.959) | | |
| Gonde | 51(51.5) | 19(19.19) | 9(9.09) | | |
| Golja | 56(52.83 | 21(19.81) | 15(14.15) | | |

## DISCUSSION

The present study showed that the GIT nematodes were considerably important health problems in the study area with an overall of prevalence of 82.56% which was 83.84% in horses and 81.29% in donkeys. This result is in agreement with the previous report of 84.4% and 80.2% by Gulima [12] in Awi Zone and Gebreyohans *et al.* [13] in donkeys in and around Mekelle respectively. But this was relatively lower than some of the other reports of 100% by Yoseph *et al*. [14] in Wonchi Area, 100% by Mulate *et al*. [15] in highlands of Wollo province, 97.13% by Mezgebu *et al.* [16] in and around Gonder, 96.9% by Nuraddis *et al*. [17] around Hawassa and 98.2% by Ayele *et al.* [18] in Dugda Bora district. On the other hand, the finding is higher than the work of Tesfu *et al*. [19] in Hawassa town, Regassa and Yimer [20] in South Wollo zone, Enigidaw *et al.* [21] in and around Kombolcha town, and Gebreyohans *et al.* [13] in and around Mekelle with their respective results of 72.7%, 70.4%, 76.04% and 80.2%. The discrepancies in the level of GIT nematode prevalence across different studies might be due to the differences in the agro-ecology of the study areas, deworming strategy and accessibility to veterinary clinic and nutritional status of the animals. It may also be due to the variation in sampling time as seasonality affects the occurrence of the parasites.

In this study, similar overall prevalence of GIT nematode parasites was recorded in horses (83.8%) and donkeys (81.29%). The current figure of prevalence of gastrointestinal nematode infestation in horses (83.8%) is higher than the study reported by Worku and Afera [6] who reported overall prevalence of 52% in Kombolcha and Tesfu *et al.* [19] who reported 63.7% in Hawassa. This difference might be due to the differences in the study areas, deworming strategy and accessibility to veterinary clinic, nutritional status of the animal in the study area.

Prevalence of GI nematodes also appeared to significantly differ between female (88.54%) and male (78.85%) (P = 0.013). This is in agreement with Tesfu *et al*. [19] and and Enigidaw *et al.* [21]. Similarly, age was found to affect the prevalence of nematodes (P = 0.000); higher prevalence was observed in old (94.34%) and young (91.92%) ages, while the lowest prevalence (76.29%) was observed in adult animals. This observation is in agreement with the report of Enigidaw *et al*. [21] in and around Kombolcha and Tesfu *et al*. [19] in Hawassa town.

With regards to body condition higher prevalence was observed in poor (98.31%) and medium (92.35%) body condition scores, while the lowest prevalence (61.24%) observed in good body conditioned animals. This difference in prevalence between different body condition scores was statistically significant (p = 0.000).

Several other workers also made similar observations; Tesfu *et al*. [19] in Hawassa town, Regassa and Yimer [20] in South Wollo zone and Enigidaw *et al*. [21] in and around Kombolcha town. Based on origin of animals, high prevalence was observed in animals originated from Lalocheka (87.67%) while the lowest prevalence was observed in animals from Asella town. This is not statistically significant (p=0.334) agreeing with reports by Regassa and Yimer [20] in South Wollo zone and Enigidaw *et al.* [21] in and around Kombolcha.

The risk factors, sex, age, and body conditions with their respective p-value of (0.013, 0.000 and 0.000), were found to be significantly associated with the infections of GI nematode parasites. This agrees with the study by Worku and Asefa [6] for both age and body conditions on the study of GI nematode parasites of horses in Kombolcha town and with the study of Tesfu *et al.* [19] having significance results for age with p-value (p=0.043). The current study disagrees with the study by Hailu *et al*. [3] and Mezgebu *et al.* [16] who studied respectively in Arsi-Bale highlands of Oromiya Region and in and around Gondar Town, which both showed none significant results for age, sex and body conditions under the study of GI helminthes in equine having $p > 0.05$. This result also disagrees with other studies which noted none association for sex and body conditions. The origin and species of animals was not significantly associated with GI nematodes infection ($p>0.05$) in this study. The percentage prevalence of Strongyle parasite was higher in Horses, females, in old age and medium body condition, but statistical significance was observed only on the ages and body condition of animals. The prevalence of Parascaris was 1.31% in horses and 2.58% in donkeys. This result is lower than the prevalence reported by Tesfu *et al*. [19] in Hawassa town and Enigidaw *et al*. [21] in and around Kombolcha town whose result was 26.2% in horses, 4.6% in donkeys and 6.9% in horses, 4.2% in donkeys respectively. The prevalence of Strongyloid parasites in this study was 9.61% in horses and 8.39% in donkeys. This result is higher than the prevalence reported by Gebreyohans *et al*. [13] in and around Mekelle.

Concerning severity of infection in donkeys reported in this study, both moderate and mild infection had the higher incidence with 18.71% and 52.90%, respectively. Severe infestation was observed in only 9.68% of the examined animals. It is very lower than the values obtained with previous works by Regassa and Yimer [20] in South Wollo where they reported 86.31, 46.82, and 46.82% for severe, mild and moderate infections respectively. Similarly Tesfu *et al.* [19] also reported 53.6, 15.9 and 8.6% for severe, moderate and mild infection respectively in Hawassa town. This study was in agreement with the work reported by Adam *et al.* [22] in Sudan in which high incidence (58.6%) for mild infection and low incidence (21.9%) and (19.5%) for moderate and severe infections respectively was reported. This may be attributed to management system and deforming strategy of donkeys.

With regards to risk factors, generally the highest egg count was observed in young and poor body conditioned animals. As age increases egg per gram feces decreases this are in agreement with previous works of Regassa and Yimer [20] and Sheferaw *et al.* [23].

## CONCLUSION

The current study showed high GIT nematode parasites prevalence of a wide range of species of gastro-intestinal helmintic parasites that compromise the health and welfare of horse and donkey in and around Asella. The major GIT nematode detected include strongyles, *P. equarum* and *S. westeri*. Among the identified GIT parasites, the highest relative percentage was recorded for Strongyles while less occurrence rate was observed for *S. westeri* and *P. equarum*. It was noted that GIT parasitosis mostly affect young and old animals having poor and medium body condition scores.

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