

Prevalence and Identification of Gastrointestinal Nematodes of Donkeys and Horses in and Around Alage, Southwestern Ethiopia

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Abstract: Gastrointestinal parasites are one of the most common factors that constrain the health and working performance of donkeys and horses. A cross-sectional study was conducted from November 2018 to May 2019 in and around Alage, South Western, Ethiopia to know the prevalence of gastrointestinal nematode parasites in donkeys and horses. A total of 384 donkeys and horses (289 donkeys and 95 horses) were randomly selected and examined during the study period. During the examination, a coprological study was carried out to investigate the diversity of gastrointestinal nematodes in naturally infected equines in and around Alage. For this, individual fecal samples were collected and examined qualitatively for parasitic infection, using a sodium chloride flotation method; further larval cultures were performed from positive samples for the identification of third-stage larvae (L₃) of strongyle nematodes. The overall prevalence of gastrointestinal nematode parasites was 94.5% (363 from 384) with 95.8% in donkeys and 90.5% in horses. The prevalence of small *Strongyles*, large *Strongyles*, *Parascaris equorum* and *Oxyuris equi* were 88.5%, 82.3%, 30.5% and 5.5% respectively. There were also mixed infections like small Strongyle + large strongyles, small strongyles + *P. equorum*, large strongyle + *P. equorum*, small strongyles + large strongyles + *Parascaris equorum* and small strongyles + large strongyles + *O. equi* with total prevalence of 48.4%, 2.3%, 1.6%, 25.3% and 4.7% respectively. Nematode parasite prevalence was influenced by species and body condition risk factors ($p < 0.05$) whereas sex and age were not influenced ($p > 0.05$). In conclusion, the current study revealed a higher prevalence of gastrointestinal nematode parasites of horses and donkeys in the area. Therefore, regular deworming, improvement of housing and feeding management systems, awareness creation to animal owners and prevention mechanisms should be implemented to reduce the economic burden of the disease in the area.

Key words: Alage • Nematode • Prevalence • Risk Factor

INTRODUCTION

Ethiopia is one of the developing countries in Africa, which is predominantly an agricultural country with over 85% of its population engaged in agricultural activity [1]. The country has the largest livestock population from African countries with an estimated 190.93 million tropical livestock unit, which includes 7.04 million donkeys, 2.03 million horses and 0.4 million mules [2]. Equines play an important role as working animals in many parts of the world, employed for packing, riding, carting and ploughing. Equine power is vital for both rural and urban transport systems which are cheap and provides the best alternatives in places where the road network is insufficiently developed, the terrain is rugged and

mountainous and in the cities where narrow streets prevent easy delivery of merchandise. It is suggested that Donkeys can play a great role in the frameworks of food security and social equity of high food-insecure countries [3].

In areas away from roads, many people use equines to transport food and other supplies to villages. Long working hours and difficult conditions are experienced by Horses and Donkeys. These animals are often engaged in work for long hours and when getting free, they are left to browse and feed on garbage. This has the potential to affect negatively their welfare of life and health [4]. Horses and mules are faster and more powerful animals for work but, one which is more costly to buy and maintain than a donkey [5]. Despite its huge population

size, equine remains marginal due to the high prevalence of malnutrition, management constraints and diseases like parasites. Parasitism represents a major obstacle to the development of the livestock farming system in the country and is characterized by high morbidity and mortality rates are to be mentioned [6].

Parasitic helminths are one of the most common factors that constrain the health and working performance of equines worldwide. They cause various degrees of damage depending on the species and number present, nutritional and the immune status of equines. They decrease the performance and productivity in the animals mainly, in the reduction of body weight or failure to gain weight or even increase the mortality in acute cases [7]. The gastrointestinal parasite has a significant negative relation with health status and working ability, despite the infection rate. That means the equines can be infected and still working, but generate lower-income [8].

Helminthes in working equines have highly prevalent; infection intensities are very high and are the main reasons for early demises of equines in the country [9]. In equines, infection by internal parasites was responsible for problems including poor body condition, reduced power output, diarrhea, colic, emaciation, impaired growth, poor reproductive performances, short lifespan and predisposition to other infectious diseases [3]. Internal parasites in equines can also cause health damage to the host with clinical signs that include reduced growth rate, abdominal distension, poor body condition, weakness, reduced physical performance and fertility and death [10].

Equines are hosts to great nematodes of the family Strongylidae, commonly called Strongyle nematodes or Strongyles. The most common gastrointestinal nematode parasites of equines, which include large strongyles, small strongyles, *parascaris equorum* and *Oxyuris equi* [11]. In Ethiopia including many other African countries, gastrointestinal nematodes being an important constraint to the growth and productivity of equines. However, apart from few studies in other parts of Ethiopia, there was no information on gastrointestinal nematodes of donkeys and horses in and around Alage Agricultural Technical and Vocational Education and Training (ATVET) College [12].

Therefore, the objectives of this study were to estimate the current prevalence of gastrointestinal nematodes infections of horse and donkey in and around alage (ATVET) college, to assess' risk factors associated with the occurrence of gastrointestinal nematodes of

horse and donkey in and around alage (ATVET) college and to identify the major gastrointestinal nematodes of horse and donkey in and around alage (ATVET) college.

MATERIALS AND METHODS

Study Area: The study was conducted in and around Alage Agricultural Technical Vocational Education and Training (ATVET) College, Southwestern Ethiopia. It is located at about 217 km south of Addis Ababa and 32 km west of Bulbla town. It is situated at a longitude of about 38°30' East and latitude of 07°30' North. It lies at an altitude of 1600 m above sea level and agro-ecologically in the dry plateau of the southwestern part of the Ethiopian central rift valley. The area has three distinct seasons, namely main rainy (June to September), short rainy (March to May) and dry (October to February) seasons. The mean annual rainfall of the area is 800 mm, with mean temperatures 20°C. The Predominant farming system is a mixed livestock and crop production system [13].

Study Population: The Equines were kept under an extensive management system and they feed on communal grazing land. These Equines are used by the people to the transport of goods, fetching water and firewood, transport grains and Agricultural products, transport sick animals to animal health clinics and transport people from one area to another area. This study was conducted on a total of 384 Equine of the local breed in and around Alage (ATVET) College, Horse 95 and Donkeys 289 including all age and sex groups managed under smallholder mixed crop-livestock farming system. The age of the study animals was determined based on the owners' information and dentition. The equines were grouped into three age categories as young when the age is <2 years, adult when the age is from 2-10 years old and when the age is beyond 10 years [14]

Study Design: Cross-sectional studies were conducted from November 2018 to May 2019 to estimate the prevalence of equine gastrointestinal parasites and assess the gastrointestinal parasites circulating in the study area. For this study, a total of 384 equines were selected by simple random sampling technique from Donkeys and Horses in and around Alage Agricultural Technical and Vocational Education and Training (ATVET) College. The age of the study animals was determined based on the owners' information and dentition. The equines were

grouped into three age categories as young when the age is <2 years, adult when the age is from 2-10 years and old when the age is beyond 10 years [14].

Sample Size: The sample size was determined based on an expected prevalence of 50% for gastrointestinal nematodes, with a 95% confidence level and 5% absolute precision by using the formula given by Thrusfeld [15].

$$n = \frac{1.96^2 P_{ex}(1 - P_{ex})}{d^2}$$

where P_{ex} = expected prevalence; n = is required sample; d = desired precision; and 1.96 z-value = for 95% confidence level. Accordingly, a total of 384 fecal samples were collected.

Collection and Examination of Fecal Samples: Fecal samples were collected directly from the rectum of the study of animals using disposable arm length gloves. The collected samples were kept in a universal bottle and each sample was labeled with the necessary information and transported to Alage Agricultural Technical Vocational Education and Training (ATVET) College, animal health Parasitology laboratory for examination. Samples were kept in the refrigerator at 4°C if immediate processing was not possible, but it was processed within 24 hours of the collection and examined coproscopically and then positive samples were cultured to identify the eggs in feces and identification of Nematodes larvae, respectively. The age of selected equine was determined by dentition [16] and body condition scores were estimated based on the available guides [17].

The fecal examination was carried out by floatation techniques. Put approximately 3gm of feces into Container 1. Pour 50 ml floatation fluid into Container 1. Mix (stir) feces and floatation fluid thoroughly with a stirring device, Pour the resulting fecal suspension through a tea strainer into Container 2. Pour the fecal suspension into a test tube from Container 2. Place the test tube in a test tube rack or stand. Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a coverslip on top of the test tube. Let the test tube stand for 20 minutes. Carefully lift off the coverslip from the tube, together with the drop of fluid adhering to it and place the coverslip on a microscope slide and examined under the microscope (10x) [18]. Sodium chloride is used for floatation fluid.

Preparation of Flotation Solutions, Add salt to warm tap water until no more salt goes into the solution and the excess settles at the bottom of the container. To ensure that the solution is fully saturated, it should be allowed to stand overnight at room temperature. If remaining salt crystals dissolve overnight, more can be added to ensure that the solution is saturated [19].

Fecal Culture, Larvae Recovery and Identification: Those samples positive to the floatation technique were subjected to fecal culture to identify the Nematodes larvae by observing morphology and the number of gut cells of larvae. Approximately 10 gm moist and crumbly feces were broken up finely using a spatula. Petri-dish were filled with the feces and left in an incubator for 7-10 days at 27°C. Stirring the feces each day to prevent the growth of fungi in the culture and then L₃ (3rd stage larvae) were recovered by using the Baerman technique [20]. But *Parascaris* spp and *Oxyuris* spp were identified based on the morphology or shape of the eggs [19].

Data Analysis: All the information collected throughout the study period was entered into a Microsoft Excel sheet, edited and then coded. The data summarized by using descriptive statistics. To check the relationship of the risk factors with the occurrence of gastrointestinal parasites statistical analysis was done by STATA 14.2 statistical software. The variations between different factors were analyzed using the chi-square (χ^2) test. A P-value <0.05 was considered to be statistically significant.

RESULTS

Coproscopic Prevalence of GIT Nematodes: The overall prevalence of GIT nematode in horses and donkeys in the study area was 94.5%. From 384 examined fecal samples 289 (95.8%) donkeys and 95 (90.5%) horses were found positive for the gastrointestinal nematode parasites (Table 1).

The finding of linear logistic regression analysis of the different putative risk factors considered during this study was shown in (Table 2).

Nematode Genera Identified: Four groups of nematode genera were identified during this study and of these, the proportion of small strongyles was the dominant (Table 3).

Table 1: Overall prevalence of gastrointestinal nematode parasites of donkeys and horses

Animals spp.	Number examined	Number positive (%)	95% CI	χ^2	P-Value
Donkey	289	277 (95.8)	92.8-97.6		
Horse	95	86 (90.5)	82.7-95.0	3.92	0.048
Total	384	363 (94.5)	91.7-96.4		

Table 2: Donkey and horse gastrointestinal nematode parasites prevalence and associated risk factor

Risk factors	Level of risk factors	Number examined	Number positive (%)	95% CI	χ^2	P-Value
Species	Donkey	289	277 (95.8)	92.8-97.6		
	Horse	95	86 (90.5)	82.7-95.0	3.92	0.048
Sex	Male	188	175 (93.1)	88.4-95.9	1.49	0.222
	Female	196	188 (95.9)	92.0-97.9		
*BCS	Good	56	43 (76.8)	63.8-86.1	41.1	<0.001
	Medium	251	243 (96.8)	93.7-98.4		
	Poor	77	77 (100)			
Age	Young	54	54 (100)			
	Adult	247	229 (92.7)	88.7-95.4	5.26	0.072
	Old	83	80 (96.4)	89.3-98.8		

*BCS: Body condition score

Table 3: Proportions of the group of gastrointestinal nematode parasites identified in Algae (n=363 animals were positive for GIT nematodes)

GIN parasites	No of positive	Percentage (%)	95% CI
Small strongyles	340	88.5	84.9 - 91.4
Large strongyles	316	82.3	78.1 - 85.8
<i>Parascaris equorum</i>	117	30.5	26.1 - 35.3
<i>Oxyuris equi</i>	21	5.5	3.6 - 8.3
Small strongyles + large strongyles	186	48.4	43.4 - 53.5
Small strongyles + <i>Parascaris equorum</i>	9	2.3	1.2 - 4.5
Large strongyles + <i>Parascaris equorum</i>	6	1.6	0.7 - 3.4
Small strongyles, large strongyles and <i>Parascaris equorum</i>	97	25.3	21.1 - 29.9
Small strongyles, large strongyles and <i>Oxyuris equi</i>	18	4.7	3.0 - 7.3

DISCUSSION

This study showed that the overall prevalence of gastrointestinal nematode parasites was 94.5% with the prevalence of donkeys and horses was 95.8% and 90.5%, respectively. This result agrees with the works of Wondimu and Sharew [21] in Gonder town, North West Ethiopia, with their result of 95.8%. This present finding is higher than the work of Tesfu *et al.* [22] in Hawassa Town and Enigida *et al.* [23] in and around Kombolcha town with their results of 72.7% and 76.04% respectively. But, it is relatively lower compared with the results of Shiferaw *et al.* [14], Gizachew *et al.* [24] and Mulate [25] who have reported 100%, in donkeys of Dugda bora district, Wonchi and high lands of Wollo province, in North and South Wollo. This difference might be due to the differences in the study areas, deworming strategy and accessibility to a veterinary clinic, the nutritional status of the animal in the respective study area. It may also be due to the variation in sampling time, as seasonality affects the occurrence of the parasites.

The risk factors of species and body conditions with their respective p-value of (0.048 and 0.000) are significantly associated with the infections of gastrointestinal nematode parasites. This agrees with the study of Tesfu *et al.* [22] having significant results for species with p-value (P = 0.001). This could be due to the relatively less attention given to these animals. This also agrees with the study of Worku and Afera [11] having (P<0.001) for body conditions on the study of gastrointestinal nematode parasites of equine in Kombolcha town. This study confirmed that there is a significant difference in the prevalence of the parasite among the different body condition scores and it is shown helminths parasites are more prevalent in animals with poor body condition than well-conditioned animals which are in agreement with the results of Gizachew *et al.* [24] which implies that the body condition score is a good indicator of parasitic burden, which can be used by farmers to identify donkeys with the immediate requirement of anthelmintic remedies. Different prevalence was observed between females (95.9%) and males (93.1%),

but there was no statistically significant difference ($\chi^2=1.49$, $P=0.222$). Age was also considered as a risk factor, higher prevalence (100%) observed in young animals, the lowest prevalence (92.7%) observed in adult animals and (96.4%) prevalence was observed in old animals. The difference between the prevalence among the deferent age groups was not statistically significant ($\chi^2=5.26$, $P=0.072$). Sex and age were not statistically significant associations with infections of GIT nematodes. This is in agreement with Naramo *et al.* [26]. This might be attributed to an augmentation of land cultivation, which restricts equines on small communal grazing land allowing the animals for continuous larvae exposure to infected pasture.

Small strongyles and large strongyles were the highest percentages of 88.5% and 82.3% respectively, compared to other gastrointestinal nematodes. This finding agrees with observations of Naramo *et al.* [26]. Who have reported 97.5% small strongyles and 100% large strongyles compared to (53.6%) *Parascaris equorum* and (4.9%) *Oxyuris equi*. Higher infections of strongyles correspond with the biology and epidemiology of these parasites as they require a longer period to complete the life cycle and their burden under different anthelmintic pressures over the years [11]. The prevalence of *Parascaris equorum* was 30.5%, which disagrees with Regassa *et al.* [3] and Shiferaw *et al.* [14] who have reported 15.7% and 17.1% in the highland of Wollo provinces and western highlands of Oromia, respectively. These differences might be due to the ecological and climatic differences among localities. *Oxyuris equi* with a prevalence rate of 5.5% was very low compared with the work of Shiferaw *et al.* [14] who reported 32.4%. The low prevalence in this study might be the effect of relatively higher temperatures in the present study area which affects the highly susceptible *Oxyuris equi* eggs.

CONCLUSION AND RECOMMENDATION

The present study suggested that donkeys and horses' gastrointestinal nematodes are the major helminths in and around Alage ATVET College with an overall prevalence of 94.5% (88.5%, 82.3%, 30.5% and 5.5% for small strongyles, large strongyles, *P. equorum* and *P. equi* respectively). Species and body condition are the risk factors associated with gastrointestinal nematode infection. Also, the lack of proper management in the study area, mixed crop-livestock farming and cultivation of most land were observed to be contributing factors. The owners of the study area give low priority to equines

especially donkeys. They give the first line to other animals to graze highly pastured grass but equine are allowed to graze behind on overstocked areas which leads them to graze close to the ground and on fecal materials, resulting in the uptake of higher numbers of infective larvae. Based on the above conclusions, the following recommendations are forwarded:

- Regular and strategic deworming programmes with efficacious anthelmintic should be carried out regularly.
- Owners should be educated about proper management of equines to reduce the worm burden and minimize pasture contamination with larvae.

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