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Research article

Prevalence and risk factors associated with donkey gastrointestinal parasites in Shashemane and Suburbs, Oromia Region, Ethiopia

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ABSTRACT

Background: Gastrointestinal parasites are the cause of morbidity and mortality in working donkeys and reduce their working performance.

Methods: A cross-sectional study was conducted from November 2020 to July 2021 to assess gastrointestinal parasite infection in working donkeys in Shashemane and the surrounding district. A total of 395 donkeys were randomly sampled for the study, and an examination was conducted using faecal egg count and the Baermann technique.

Results: Out of the 395 sampled donkeys, different types of gastrointestinal parasites were identified. The parasites identified during the study were *Strongyle* spp. (100%), *Oxyuris equi* (10.1%), *Parascaris equorum* (23.8%), *Fasciola* spp. (0.3%), *Gastrodiscus aegyptiacus* (4.6%), *Strongyloides westeri* (47.8%) and *Anoplocephala perfoliata* (0.5%). Identification of L_3 larvae of GI parasites revealed that *Cyathostomes* spp. (96.2%), *Strongylus vulgaris* (92.9%), *Trichostrongylus axei* (90.4%), *Strongylus edentatus* (89.4%), and *Dictyocaulus arnifieldi* (49.6%) were the most prevalent. Infection with one species of helminth was more common (60.8%).

Conclusions: Thus, working donkeys in the present study area were infected with *Strongyle* spp. (100%), *Strongyloides westeri* (47.8%), *Parascaris equorum* (23.8%), *Oxyuris equi* (10.1%), *Gastrodiscus aegyptiacus* (4.6%), *Anoplocephala perfoliata* (0.5%) and *Fasciola* spp. (0.3%) parasites. Hence, comprehensive donkey health, management, and implementation of appropriate parasite control strategies should be implemented to alleviate these problems.

1. Introduction

The world's equine population is now estimated to be 112.5 million, with 44.3 million donkeys, 58.5 million horses, and 9.7 million mules (FAO, 2015). Ethiopia has the third-largest equine population in the world, with approximately 7.4 million donkeys (Central Statistical Agency, 2016; Mathewos et al., 2021a). Donkeys are vital to the livelihoods of millions of people across the world (Pritchard et al., 2005). Working equines, including donkeys, deliver water, cereals, fuelwood, and agricultural items in rural Ethiopia. They move products and people to metropolitan areas (Gebreab et al., 2004). Despite the fact that donkeys play an important role in the economy, their welfare is very low, particularly in rural, peri-urban, and metropolitan areas of Ethiopia (Geiger et al., 2020). Wounds, eye diseases, lameness, hoof problems, parasites, and poor body health are all common welfare issues (Admassu and Shiferaw, 2011; Getachew et al., 2010).

Gastrointestinal parasite infection is a major health and welfare problem for working donkeys that limits the profitability of the performance of donkeys across the world (Mathewos et al., 2022). Some active bloodsuckers, such as Strongyles, cause varying degrees of injury based on the species and numbers present, as well as the nutritional and immunological health of the equids (Jajere et al., 2016). According to available evidence, gastrointestinal helminths are the primary cause of early death in donkeys (Feseha et al., 1991; Gebreab et al., 2004; Gebrewold et al., 2004; Zerihun et al., 2011).

Donkeys can be infected by more than 150 different helminth parasites. Large and small strongyles, roundworms, tapeworms, lungworms, pinworms, threadworms, and bots are the most prevalent and dangerous (Foster, 1942; Getachew, 1999). Large and small strongyles, roundworms, and tapeworms are probably the most dangerous health hazards (Radostits et al., 2007; Saeed et al., 2019; Sazmand et al., 2020).

The most common intestinal nematodes identified in equids are large strongyles (*Strongyles vulgaris*, *Strongyles equinus*, *Strongyles*

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edentatus and *Triodontophorus* species), *Parascaris equorum, Oxyuris equi* and, to a lesser extent, other small strongyles (Cyathostomins) (Bucknell et al., 1995; Nielsen, 2012). Intestinal nematodes of equids have a similar life cycle (Mathewos et al., 2022; Saeed et al., 2019). Clinical illness occurs not only from the presence of the adult parasite in the intestine but also from larval migration in the intestinal wall and other organs, notably the circulatory system (Wintzer, 1986). Several studies have found that gastrointestinal (GI) parasites are common in poorer nations where diet and hygiene are often poor and donkeys have considerable issues (Enigidaw et al., 2015; Hailu and Ashenafi, 2013; Jajere et al., 2016; Mathewos et al., 2021b; Regassa et al., 2005). The frequency, species composition, and epidemiology of GI parasites affecting donkeys have not been thoroughly researched in Ethiopia, where health care is scarce, especially for equines (Admassu and Shiferaw, 2011; Getachew et al., 2010; Radostits et al., 2007; Sazmand et al., 2020). Previous studies conducted in donkeys of Ethiopia have focused on welfare and gastrointestinal parasites and have been limited to some ecological regions. The current study was therefore conducted to determine the prevalence, potential risk factors, and composition of gastrointestinal parasites in donkeys in Shashemane and Suburbs.

2. Materials and methods

2.1. Study area

The study took place at Shashemane and suburbs, Oromia Regional State, West Arsi Zone, 250 km southeast of Addis Ababa, from November 2020 to July 2021 on a randomly selected donkey. It is located at 7° 11′33″ north latitude and 38° 35′33″ east longitude. The region is located in the Rift Valley, with elevations ranging from 1700 to 2600 m above sea level (AMSL). The yearly rainfall ranges from 700 to 950 mm, while the temperature ranges from 12 to 27 °C (Central Statistical Agency, 2016) (Figure 1).

2.2. Study animals

Working donkeys that had not been dewormed in Shashemane and its suburbs, Oromia regional state, Ethiopia, were the study's target population. Donkeys of different age groups, sex, and body condition groups were included. The age of the study donkeys was determined based on dentition patterns (the twelve front incisors, the shape of the permanent upper corner of the incisors and table of the central incisors, and the disappearance of enamel ring) as described (Du Toit, 2008) and grouped as young (<5 years), adult (5–10 years), and old (>10 years). Body condition scoring (BCS) of the working donkey was estimated based on the deposition of body fat in different areas by separate examination of the neck, back, ribs, pelvis, and rump (Pearson and Ouassat, 2000; Vall et al., 2003).

2.3. Study design and sampling strategy

A cross-sectional study was conducted from November 2020 to July 2021 to assess the prevalence and type of helminths and examine the relationship between helminth load and risk variables in the study area. Donkeys in the research locations were chosen at random for GI parasite investigations, regardless of their age, sex, body condition, or color. On daylight from various villages of the district, the sampling procedure was carried out at the field level, market, homestead, and around water point locations.

2.4. Sample size determination

The sample size of the study was determined using the formula given by Thrusfield (2018). Because there had been no previous report on the prevalence of the parasites in the study area, a 50% predicted prevalence for gastrointestinal helminths was used to determine the sample size. Furthermore, the requisite absolute precision of 5% and a confidence level of 95% were applied.

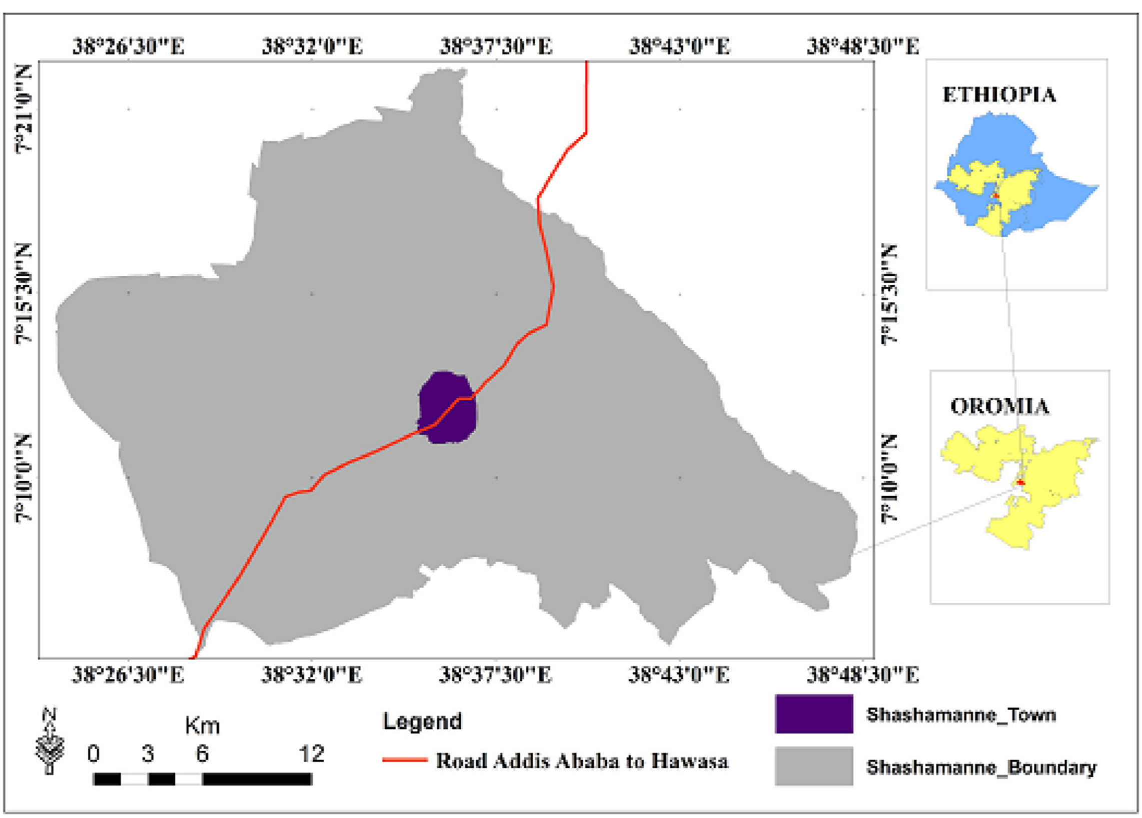

Figure 1. Map of the study area.

$$n = \frac{Z^2 \times Pexp(1 - Pexp)}{d^2}$$

where n = the required sample size, Z = Confidence level (1.96),

Pexp = expected prevalence (50%) and d = desired absolute precision (0.05).

As a result, the sample size computed was 384, which is the minimum sample size required in the target area. The sample size in the area after and during the small rainy season was calculated to be 395.

2.5. Sample collection and examination

Faecal samples were taken from donkeys that had not been dewormed by rectum using plastic rectal gloves. Each sample was given a code number to match against the questionnaire paper, which included the date of collection, age, sex, BCS, number of animals kept, and welfare assessment format. The collected samples were stored in an icebox, which had sufficient ice and could seal tightly, and transported to the parasitology laboratory. The samples were stored in refrigerators at +4 °C when immediate processing was not possible, but most samples were processed within 48 h.

Faecal examination was carried out using sedimentation and flotation methods (Taylor et al., 2015). For identification of parasites to the species level, fecal samples were cultured, and the larvae were recovered using the Baermann technique (Boyle and Houston, 2006). Larvae were identified based on their morphology using a compound microscope (Magnus Binocular Microscope, India, 4x-100x) (10x objectives) based on the shape, relative size, and shape of the larval tail and under oil emersion (100x objectives) based on multiple intestinal cells (Foreyt, 2013; Zajac et al., 2021). The flotation fluid used in the study was a laboratory-made supersaturated solution of sodium chloride (NaCl). Standard parasitological procedures were followed for fecal flotation, sedimentation (Hendrix and Robinson, 2016), and Baermann techniques (Traversa et al., 2010). Egg counts were also performed using the McMaster egg counting technique (Kaplan and Nielsen, 2010; Nielsen et al., 2006).

The severity of helminth infection was defined based on egg count per gram of faeces (EPG). Donkeys with an EPG less than 500 were considered to have a mild infection, and those with a EPG 500–1000, moderate, and over 1000 eggs/gram of feces were considered to have a severe infection (Foreyt, 2013). In addition, clinical symptoms were told by the equine owner and were used to classify the donkeys. Donkeys that were examined with a history of unthriftiness, depression, anorexia, emaciated body, severe anemia, pruritis, severe colic, and diarrhea were categorized into severe, whereas donkeys that were examined with a history of thin body condition, anemia, colic, and intermittent diarrhea were categorized into moderate. Donkeys that were examined with a history of moderate body condition and an apparently good health condition were categorized as mildly infected (Du Toit, 2008; Radostits et al., 2006).

2.6. Data analysis

The laboratory result data were properly encoded and entered into the Microsoft Excel 2019 spreadsheet. The data were filtered for missing values or invalid entries and then transferred to STATA 13 version for Windows for statistical analysis. Descriptive and analytical statistics were used to describe the results. In all analyses, the confidence level was kept at a confidence interval of 95%, and the p value was considered significant if $p < 0.05$. Multivariate logistic regression was also applied with Bonferroni multiple comparison tests, which was very useful for seeing the interaction between the variables. The variables to be tested were age, sex, and body condition scores.

3. Results

3.1. Prevalence of gastrointestinal helminthes

3.1.1. Coprological examination

The study results showed that 143 (36.3%) male donkeys and 252 (63.7%) females were infected with different GI parasites, namely, *Strongyle* spp. (100%), *Strongyloides westeri* (47.8%), *Parascaris equorum* (23.8%), *Oxyuris equi* (10.1%), *Gastrodiscus aegyptiacus* (4.6%), *Anoplocephala perfoliata* (0.5%) and *Fasciola* spp. (0.3%). The findings revealed an overall prevalence of 100% in Shashemane and its surrounding areas (Table 1).

Based on the EPG counts in the study area, all donkeys were infected with different degrees of infection. Furthermore, 53 (13.4%) were mildly infected, 81 (20.5%) were moderately infected and 261 (66.1%) were severely infected (Figure 2).

3.1.2. Ova culture and larvae identification

Helminths were identified to the species level by ova culture. Identification of L_3 GI parasites from copro-cultured faeces revealed the predominance of *Cyathostomes* spp. (96.2%), *Strongylus vulgaris* (92.9%), *Trichostrongylus axei* (90.4%), and *Strongylus edentatus* (89.4%) over the other GI parasites (Table 2).

Infection with one species of helminths occurred in *Cyathostomes* spp., *Strongylus vulgaris*, *Strongylus equinus, Strongyloides westeri, Trichostrongylus axei,* and *Strongylus edentatus,* with an overall prevalence of 60.8% (240/395), whereas infections with two species of GI helminths were recorded in 27.8% (110/395) of donkeys by *Dictyocaulus arnifieldi* + *Strongylus vulgaris, Strongyloides westeri* + *Trichostrongylus axei*, *Cyathostomes* spp + *Trichostrongylus axei,* and infections with more than two species of helminths were seen in 11.4% (45/395) of donkeys by *Strongyloides westeri* + *Trichostrongylus axei* + *Cyathostomes* spp and *Strongylus vulgaris* + *Strongylus equinus* + *Strongyloides westeri* (Figure 3).

3.1.3. Analysis of risk factors

Analysis of different risk factors showed that age and body condition scores were significantly associated with the severity of GI parasite infection ($p < 0.05$), whereas sex of the animal had no significant value with the severity of GI parasite infection ($p > 0.05$) (Table 3).

In the current study, different types of eggs of GI parasites were identified from fecal samples of donkeys, including *Strongyle spp.* (thin shelled and oval-shaped, Figure 4: A), *Parascaris equorum* (a spherical brownish egg, Figure 4: B), *Cyathostomes spp.* (a small red worm, Figure 4: C), *Oxyuris equi* (thick eggshell, with a plug on one end and an elongated form with a slight unilateral depression, Figure 4: D), and *Fasciola spp.* (Opiculated, broadly ellipsoidal, Figure 4: E) (Figure 4).

4. Discussion

Intestinal parasitism has a direct impact on the health and production of draft donkeys, which contributes to the reduction of their draft performance and, ultimately, the income of the owner and the community

Table 1. Prevalence of gastrointestinal parasites in donkeys of the study area (n = 395).

Parasites identified	Number of Positive	Prevalence (%)	Stand. Err	95% CI
Strongyles spp.	395	100	0.000	[0.000]
Oxyuris equi	40	10.1	0.015	[0.07–0.13]
Parascaris equorum	94	23.8	0.021	[0.20–0.28]
Strongyloides spp.	189	47.8	0.025	[0.43–0.53]
Fasciola spp.	1	0.3	0.03	[0.00–0.01]
Gastrodiscus aegyptiacus	18	4.6	0.011	[0.02–0.07]
Anoplocephala perfoliata	2	0.5	0.004	[0.00–0.01]

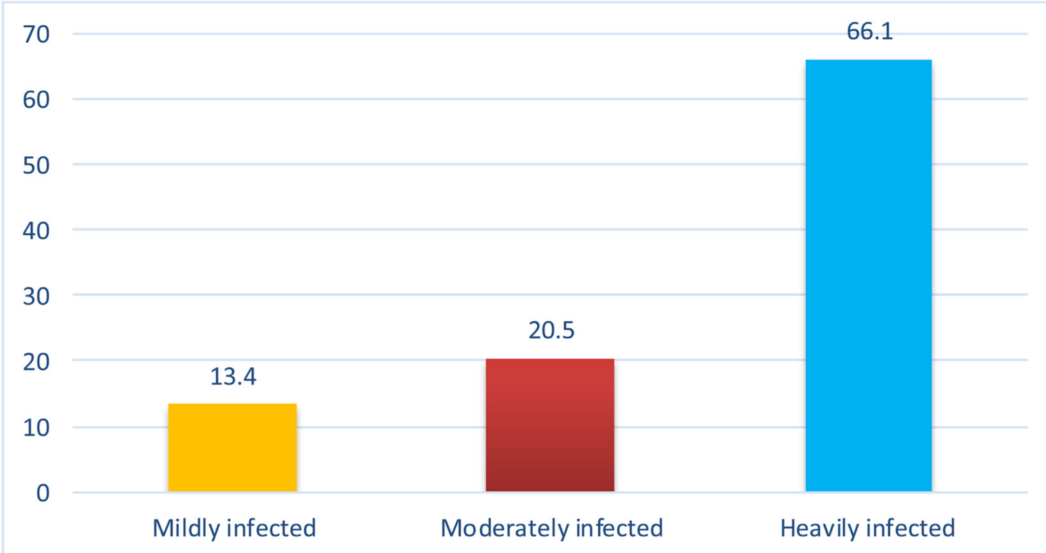

Figure 2. Severity of GI parasites of donkeys on the basis of EPG.

Table 2. Species of GI parasites identified from coprocultured feces of donkeys.

Parasite spp.	No. of Positive	Proportion (%)	Stand. Err	95% CI
Strongylus vulgaris	367	92.9	0.025	[0.55–0.65]
Strongylus edentatus	353	89.4	0.023	[0.66–0.75]
Strongylus equinus	174	44.1	0.025	[0.39–0.48]
Strongyloides westeri	189	47.8	0.025	[0.43–0.53]
Dictyocaulus arnifieldi	196	49.6	0.025	[0.45–0.55]
Trichostrongylus axei	357	90.4	0.015	[0.87–0.93]
Triodontophorus tencollis	70	17.7	0.019	[0.14–0.22]
Cyathostomes spp	380	96.2	0.015	[0.94–0.98]

(Debere et al., 2018; Fesseha et al., 2020; Mathewos et al., 2021b). The overall prevalence of GI parasites using coprological investigation was 100%, which is consistent with earlier reports that reported 100% in Dugda Bora districts (Ayele et al., 2006), 100% in project and control areas in the central region of Ethiopia (Abebew et al., 2011), and 99.5% in Sululta and Gefersa (Zerihun et al., 2011). The similarity ought to be due to the management of the donkeys and the massive incidence of GI parasites.

This finding was relatively higher than previous reports of 88.2–97.2% in different regions of Ethiopia (Ayele et al., 2006; Berhanu et al., 2014; Chemeda et al., 2016; Debere et al., 2018; Gulima, 2006; Ibrahim et al., 2011; Mathewos et al., 2021b; Mezgebu et al., 2013; Seyoum et al., 2015; Takele and Nibret, 2013; Tolossa and Ashenafi, 2013) and elsewhere across the world (Upjohn et al., 2010; Valdéz-Cruz et al., 2013). This difference could be attributed to the variation in the sampling time, as seasonality influences the occurrence of the parasites. In addition, donkeys' accessibility to free-range pastureland increases the chance of ingesting the eggs and larvae of a large number of GI parasites, availability of veterinary service, the donkeys' deworming practice, and the feeding of these animals with supplementary feed have an impact on the occurrence.

Donkeys were also 100% positive for strongyle eggs, which is in line with the findings of Ayele et al. (2006) in Dugda Bora districts, Zerihun et al. (2011) in Sululta and Gefersa, Yoseph et al. (2001) in Mulate (2005) in highlands of Wollo Province, who reported a prevalence of 100%, and Wannas (2012) in Al Diwaniyah Governorate and Hassan et al. (2004) in

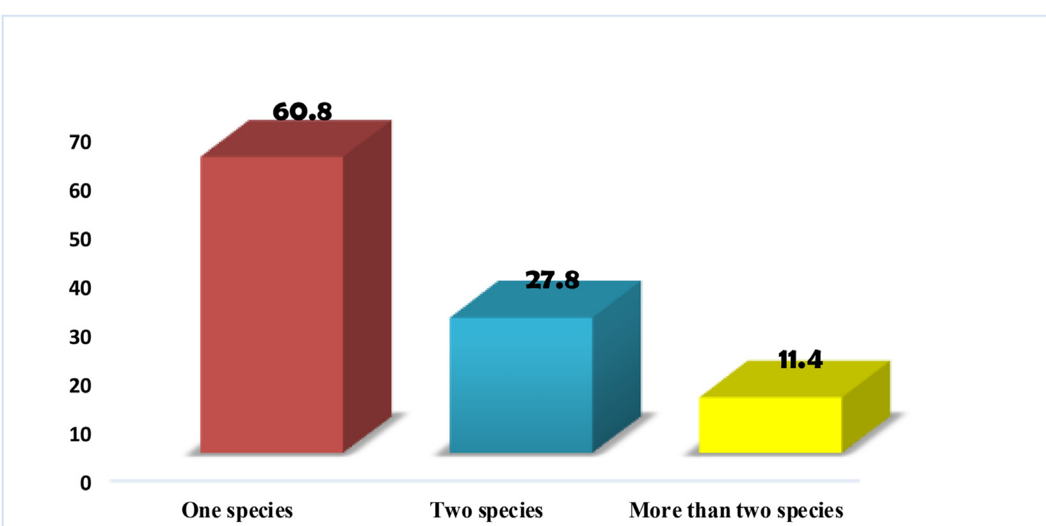

Figure 3. Prevalence of polyparasitism in donkeys of the study area.

Table 3. Level of strongyle infection with risk factors in the study area.

Risk factor level		Number of Examined	EPG count			x^2	p value
			Low	Medium	High		
Age	Young	94 (23.8%)	10 (10.6%)	15 (16.0%)	69 (73.4%)	18.58	0.001
	Adult	259 (65.6%)	42 (16.2%)	63 (24.3%)	154 (59.5%)		
	Old	42 (10.6%)	1 (2.4%)	3 (7.1%)	38 (90.5%)		
BCS	Poor	94 (23.8%)	3 (5.7%)	15 (18.5%)	76 (29.1%)	19.63	≤0.0001
	Moderate	246 (62.3%)	36 (67.9%)	54 (66.7%)	156 (59.8%)		
	Good	55 (13.9%)	14 (26.4%)	12 (14.8%)	29 (11.1%)		
Sex	Male	143 (36.2%)	19 (35.8%)	33 (40.7%)	91 (34.9%)	0.93	0.629
	Female	252 (63.8%)	34 (64.2%)	48 (59.3%)	170 (65.1%)		

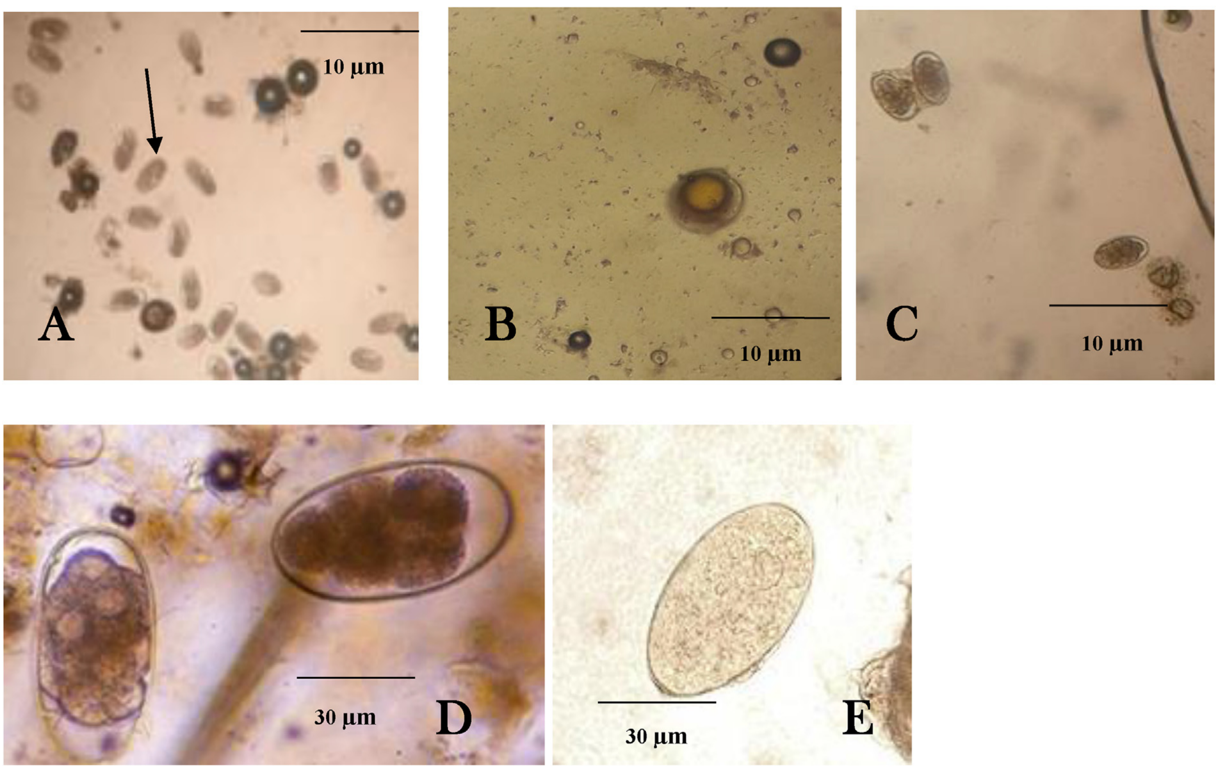

Figure 4. A. Egg of *Strongyle* spp., B. *Parascaris equorum*, C. *Cyathostomes* spp., D. *Oxyuris equi*, E. *Fasciola* spp.

Sudan reported a higher prevalence of 99.15%. However, a lower prevalence of strongyle was reported in various areas, with 87.8% by Mezgebu et al. (2013) in Gondar town, 81% by Uslu and Guclu (2007) in Konya, Turkey, 80.2% by Asefa and Dulo (2017) in Bishoftu, 79.7% by Abdulahi et al. (2017) in Jigjiga, 76% by Tesfu et al. (2014) in Hawassa, 6.07% by Mathewos et al. (2021b) in Hawassa District and 59.1% by Mathewos et al. (2021a) in Hosaena District. These differences might arise from differences in topographical nature and misuse, low coverage of donkey health care, and inappropriate husbandry systems of the donkey.

The eggs of *Strongylus* spp. (100%) were the most prevalent GI parasites in the study area using the McMaster technique. Similarly, Waller and Mfitlidoze (1989) in Australia, Mathewos et al. (2021a) in Hossana and Mathewos et al. (2021b) in Hawassa reported prevalence rates of 80%, 48.2%, and 56.1%, respectively, for *Strongylus* spp. infection. In line with this study, Chapman et al. (2003), Seyoum et al. (2017) in Gondar, and Fesseha et al. (2020) in Hossana reported a higher level of strongyle species per gram of feces in infected hoses. The differences could be explained by egg presence or the possible development of resistance to some type of anthelmintics or the absence of intervention.

The current prevalence of *Strongyloides* spp. in donkeys was 47.8%, which was comparable with the finding of Mathewos et al. (2021b) that reported 50.0% in Hawassa, whereas this was higher than the report of Ibrahim et al. (2011) in Hawassa, Getahun and Kassa (2017) in Tenta woreda Gebreyohans et al. (2017) in Mekelle and suburbs who reported a prevalence of 20%, 9.5%, and 2%, respectively. The prevalence of 23.8% *Parascaris equorum* recorded in the current study is higher than the prevalence reported by Getahun and Kassa (2017) (11.2%) in Tenta Woreda, 6.4% by Gebreyohans et al. (2017) in and around Mekelle, 26.2% by Tesfu et al. (2014) in Hawassa Town, and 15.1% by Asefa and Dulo (2017) in Bishoftu Town. In contrast, it was lower than the reports of Naramo et al. (2016), Ibrahim et al. (2011), Abdulahi et al. (2017), Fikru et al. (2005), Zerihun et al. (2011), Mezgebu et al. (2013) and Mathewos et al. (2021b), who reported prevalence rates of 53.6%, 52.8%, 44.8%, 43%, 42.8%, 42.3%, and 34.8% in donkeys located in different parts of Ethiopia, respectively. Additionally, these figures were higher than other country reports of 9.8 and 13.6% in donkeys by Arslan and Umur (1998) and Gul et al. (2003), respectively. These differences in prevalence might be due to the length and season of the study period, the agroecology of the study area, the relatively low numbers of these parasites in the pasture, and the application of parasite control programs.

In the present study, *Dictyocaulus arnfieldi* in the donkey was 49.6%, which is approximately in line with a report from Morocco at 47.8%. In contrast, the figures were higher than the previous report of Mathewos et al. (2021b), who reported 3.6%, and Ibrahim et al. (2011), who reported a prevalence of 3.6% in Hawassa town, southern Ethiopia. The variation might be the sample size variation, the sampling technique, and the laboratory technique.

Identification of infective larvae of GI parasites showed that *Cyathostomes* spp. (96.2%), *Strongylus vulgaris* (92.9%), *Trichostrongylus axei* (90.4%), and *Strongylus edentates* (89.4%) were the major larvae encountered. In mixed infections, the burden of one or both infectious agents may be increased, one or both may be suppressed or one may be increased and the other suppressed. In this study, infections with one species of helminths were more common, 240 (60.8%), than infections with two, 110 (27.8%), and infections with more than two species, 45 (11.4%), of helminths. This was higher than the previous work of Mathewos et al. (2021b), who reported a prevalence of 43.3% (*Cyathostomins* species), 26.6% (*S. vulgaris*), and 13.3% (*S. edentatus*) from the coproculture study. The differences in the study period, agroecology, and veterinary services, such as the quality of infrastructure, may influence the intensity of parasitic infections.

In the current study, 73.4% of younger donkeys showed a high level of EPG count of strongyle infection. The possible justification for the high levels of EPG in young donkeys could be that young donkeys, in general, have more mucosal larval stages than older horses (Höglund et al., 2001; Love and Duncan, 1992). An experimental study of ponies of various ages revealed that older individuals had fewer worms than younger individuals (Monahan et al., 1997). Lower worm fertility is another marker of immunity, which could explain the age gap in mean EPG levels (Claerebout and Vercruysse, 2000; Vercruysse et al., 2018). Another reason for the increased EPG scores in younger donkeys could be that past anthelmintic treatments were less effective in younger donkeys than in older animals (Höglund et al., 2001; Love and Duncan, 1992).

The study revealed that 59.8% of medium-conditioned donkeys and 29.1% of poorly conditioned donkeys harbor a high EPG count. Similarly, Mathewos, (Mathewos et al., 2021b) in the Hawassa reported that 81% of medium and 40% of poor conditioned working equines harbor a high EPG count. This could be related to malnutrition or other concurrent bacterial and parasitic illnesses, which result in a weak immune response to the parasites' infective stage. The production of eggs by nematodes is also reliant on the immune status of the host, as innate immunity can restrict egg production if the animal is in excellent health. 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 (Ayele et al., 2006). Sex did not show a significant difference ($p > 0.05$) in EPG counts in the study area. This result agrees with the work of Getachew et al. (2008), in central lowlands but disagrees with the work of Regassa and Yimer (2013), in South Wollo.

In the current study, the identified GI parasites of donkeys were *Strongyle spp*. (thin shelled and oval-shaped in outline) (Mulwa et al., 2020), *Parascaris equorum* (a spherical brownish egg) (Mulwa et al., 2020), *Cyathostomes spp*. (a small red worm because it is typically under 2.5 cm long and occasionally has a redder appearance than a white color) (Corning, 2009), *Oxyuris equi* (thick eggshell with a plug on one end and an elongated form with a slight unilateral depression) (Yevstafieva et al., 2020), and *Fasciola spp.* (Opiculated, broadly ellipsoidal) (Tolan, 2011).

5. Conclusion

Generally, GIT nematode infection was prevalent in donkeys, with an overall prevalence of 36.3% in male and 63.7% in female donkeys. Faecal culture and larval identification revealed that gastrointestinal parasites such as *Strongylus vulgaris, Cytostomes, Strongylus edentatus,* and *Parascaris equorum* are common in the study area. Donkeys with poor body conditions had higher parasite EPG than good-conditioned animals. In conclusion, donkey owners should be educated on factors predisposing to parasitic infection and ways of reducing adverse effects of parasitic infection. An appropriate parasite control strategy should be implemented to reduce the burden of parasite infestation in the study area.

Declarations

Author contribution statement

Haben Fesseha, Saliman Aliye, and Mesfin Mathewos: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kebede Nigusie: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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