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Original article

Prevalence of gastrointestinal helminths on equines in and around Debre Markos, Ethiopia

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ABSTRACT

The study was conducted from October-2013 to June-2014 in and around Debre Markos, Ethiopia to assess the major gastrointestinal helminthes of equines (donkeys and horses), to determine their prevalence and find associations between measurable parameters and parasites burden. A total of 384 faecal samples (215 donkeys and 169 horses) were collected randomly for qualitative and quantitative faecal analysis. The overall prevalence of different parasites was found to be 86.51% in donkeys and 78.10% in horses. In the study area, 3.87% of donkeys and 6.13% of horses harbored only one type of parasite (single infection), whereas 96.13% of donkeys and 93.87% of horses harbored two or more types of parasites (mixed infection). The parasites encountered in both donkeys and horses in the study period were Strongyles (75.27% and 59.85%), *T. strongylus axei* (41.94% and 40.15), *T. dontophorus* (43.01% and 34.85) *P. equorum* (18.82 and 18.94) and *Fasciola* (22.58% and 18.94), respectively. Based on sex only the prevalence rate of strongyle was statistically significant ($p<0.05$) in both donkeys and horses. The prevalence of *T. strongylus, T. dontophorus, P. equorom* and Fasciola was statistically significant ($p<0.05$) between age groups of donkeys, but only the prevalence of *T. strongly* was statistically significant ($p<0.05$) between age groups of horses. Parasitism and other health problems were identified affecting the health and welfare of equines. Government or other

development agencies should include donkeys and horses in their priority lists of research and develop sustainable prevention and control methods that would prevent the high transmission of gastrointestinal helminths and other parasites of equines.

1. Introduction

Ethiopia has about 7.9 million equines and possesses approximately half of the Africa's equine population with 37% donkeys, 58% horses and 46% mules (FAO, 1996). There is one equine for every four people in the agricultural sector and for every five per- sons of the total population (Williams, 2000). Equids; donkeys, mules and horses play an important role as working animals in many parts of the world, employed for packing, riding, carting and ploughing (Feseha et al., 1991). They serve as a means of transport for men and materials provide livelihood to a number of rural and semi-urban populations of the world. It is suggested that donkeys can play a great role in the frameworks of food security and social equity of high food insecure countries. In areas away from roads, many people use mules, horses and donkeys to transport food and other supplies to villages (Karki and Mandhar, 2006). Long working hours and difficult conditions are experienced by donkeys and mules working in and around Bahir Dar (personal communication with owners of equines). Animals are often engaged in work for long hours, and when they get free, they are left to browse and feed on garbage. These have the potential to affect negatively their welfare and quality of life (Karki and Mandhar, 2006). Misuse, mistreatment and lack of veterinary care for equines have contributed enormously to early death, majority of which currently have working life expectancy of 4 to 6 years. However, in countries where animal welfare is in practice, the life expectancy of equines reaches up to 30 years (Fred and Pascal, 2006).

Although equines are often described as hardy and resistant animals; they do suffer a number of health problems (Marquardt et al., 2000). Parasitic diseases have an economic impact on donkeys and mules as they cause loss through lowered fertility, reduced work capacity and increased treatment cost (Krecek et al., 2002). Infections of equines with gastrointestinal parasites are recorded from most countries of Africa and few parts of Ethiopia. In Ethiopia, few studies were done in central and eastern parts of the country (Mohammed and Teketel, 1991; Gebreab, 1998; Ayele Gizachew et al., 2006). Among the helminths; *S*trongyles (large and small Strongyles), *Trichostrongylus axi*, *Triodontophorus* species, *Trichonema* species, *Parascaris equorum*, *Anoplocephala, Dictyocoulus arnfieldi*, *Fasciola* and others are most devastating parasites of equines (Pandit et al., 2008). The prevalence of Strongyles in different parts of Ethiopia in working donkeys was reported as 100% in Wonchi, Highlands of Wollo provine, and Dugda Bora district, by Yoseph et al. (2001), Mulate (2005), and Ayele Gizachew et al. (2006), respectively. Fikru et al. *(*2005) also was reported the prevalence of this parasite as 98.2% in Western highlands of Oromia.

The prevalence of *Parascaris equorum* in working donkeys of central Shewoa and Dugda Bora district also reported to be 50% by Ayele Gizachew et al. (2006) and 32% by Ayele and Dinka (2010), respectively. Ayele Gizachew et al. (2006) also reported the prevalence of *Anoplocephala* species to be 7.4% and that of *Fasciola* species to be 1.5% in working donkeys of Dugda Bora district, Ethiopia. Strongyles are the most injurious, whereas others are generally less harmful. A few parasites may be tolerated by the equines without apparent signs of ill effect, but larger numbers are quite likely to be harmful. Acquiring a large number within a few days may overwhelm and kill equines; however, getting the same number over a period of weeks or months is generally much less harmful (Hardin, 1997). Despite being that much significant to the livelihood of the population, equines in the Amhara region have not been given a serious attention they deserve. As any other animals, equines are vulnerable to various diseases of biological origin, nutritional diseases or disorders and miscellaneous causes, among which the most common entities leading to ill-health, suffering and early dimes, and finally death are infectious diseases and parasitism, which resulted in considerably reducing equines work output, reproductive performance and most of all their longevity (Gebreab, 1998). The effects of parasitism on production are well documented. Anorexia and reduced food intake, loss of blood and plasma protein into the gastrointestinal track, alteration in protein metabolism, depressed levels of minerals, depressed activities of some intestinal enzymes and diarrhea, all contributed to depress weight gain and reduction in traction power (Soulsby, 1996).

In and around Debre Markos, much work on helminthosis of equines (horses and donkeys) has not been done. Documents on helminthosis and other related diseases of equines are not also available. The present study was necessitated to determine prevalence of helminthosis in equines in the region, especially in and round Debre Markos.

2. Materials and methods

2.1. Description of the study area

The study was conducted from October-2013 to June-2014 in and around Debre Markos town, the capital city of East Gojam Administrative Zone in Amhara National Regional State. Debre Markos is located in the north west of the capital city of Ethiopia, Addis Ababa at a distance of 300Km and 265Km to the capital of Amhara National Regional State, Bahir Dar. The astronomical location of Debre Markos is 10^{O} 21' longitude to the North and 37^{O}43' latitude to the East. The core city has an estimated area of 6160 hectares. Regarding the climate of the town, it has temperate climate with mean annual rainfall of about 1380mm and mean annual temperature of 18.5^{O}C (Ayen, 2004).

2.2. Study animals

Fresh faecal samples were taken from 384 equines. Among 384 equines; 169 were horses (127 males and 42 females) and 215 were donkeys (125 males and 90 females). The samples were randomly selected from the cart-men and farmers in and around Debre Markos and subjected to quantitative and qualitative coprological examination in Debre Markos University, Biology department laboratory to identify the major gastrointestinal helminths involved. The cart-men and farmers were informed on the importance of the study. The age of the selected donkeys and horses was determined from birth records and by dentition (Crane, 1997) and BCS was subjectively estimated based on the guides published by Sevendsen (1997). Accordingly, donkeys were grouped into three age categories, under two years of age were classified as young (n=30), above two to ten years were considered as adult (n=130) and those beyond ten years were classified as old (n=55). The same system was applied to horses that is horses under two years of age were classified as young (n=45), above two to ten years were considered as adult (n=90) and those beyond ten years were classified as old (n=34). These age classes were based on age of first work, productive age and the life span of Ethiopian donkeys (Sevendsen, 1997; Yosef et al., 2001). Regarding BCS, the studied animals were grouped as poor, medium and good.

2.3. Sampling technique

Faecal samples were taken directly from the rectum or from the ground with strict sanitation when the animals were seen defecating and placed in universal bottles. Each samples were labeled with animal identification (sex, age, BCS and owner's name) and then brought to Debre Markos Biology department laboratory. Samples were kept in refrigerator at 4°C to be examined for coproscopic examination. Sodium chloride solution was used as flotation fluid for this study. Sedimentation and flotation methods were used to identify the eggs of helminthic parasites (Soulsby, 1982; Urquhart et al., 1996).

2.4. Sample size determination

A total of 384 equines (215 donkeys and 169 horses) were collected to examine for the presence of helminth eggs. To determine the sample size, 50% expected prevalence of gastrointestinal helminths was taken into consideration with 95% confidence level and 5% absolute precision as described by Thrusfield (1995). The required sample size was calculated by the formula:

$$n = Z^2 \times P\,(1-P)\,/d^2$$

Where n = the required sample size,

Z =Confidence level (regular value=1.96)
P = expected prevalence (50%) and
d=desired absolute precision (0.05)

$$n= (1.96)^2 \times 0.5(1-0.5)/(0.05)^2 =384$$

2.5. Preparation of faeces for microscopic examination

2.5.1. Sedimentation technique

Sedimentation technique was used to concentrate the helminth eggs as described by Hendrix (1998). Using a tongue depressor, about 2 gram of faeces was mixed with distilled water in a 250 ml conical plastic beaker. The mixture was allowed to sit undisturbed for one hour on a table. The supernatant in the top of the beaker was poured-off without disturbing the sediment at the bottom. Using pasture pipette, small amount of the middle layer of sediment was transferred to a microscope slide. A cover slip was applied to the drop. The slide was examined microscopically as described by Hendrix (1998).

2.5.2. Flotation technique

The procedure of flotation methods was as described by Hendrix (1998). Approximately 3 gram of faeces was put in a beaker or a plastic container. 50 ml of flotation fluid was poured to the beaker or a plastic container containing 3 g of faeces. The flotation fluid (sodium chloride) was mixed with faeces thoroughly with stirring device (tongue blade, fork). The resulting faecal suspension was poured through a tea strainer or double layer of cheesecloth into another beaker or plastic container. The faecal 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 carefully place a cover slip on top of test tube. The tube was standing for 15-20 minutes. The cover slip was lifting 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.

2.6. Microscopic examination of faeces for helminth eggs

This was done as described by Hendrix (1998). Compound microscope with the objective lenses' magnification power of 10X was used to examine the prepared faecal smears. Mechanical microscopic stages were used for smooth and uniform movement of the slides. All the area under the cover slip was thoroughly and uniformly searched for the presence of parasitic eggs. When a parasite egg was observed at low magnification power (10X), high power object (40X) was used to more closely examine it.

2.7. Data analysis

Data collected from the study animals were coded and entered in a Microsoft Excel sheet. Statistical analysis was performed using SPSS version 16 for windows. The association between prevalence of each studied parasite and the study variables (age, sex, and BCS) was analyzed by Chi-square test of independence. In all the analysis, confidence level was held at 95% and P values of at least <0. 05 were considered as significantly different.

3. Results and discussion

3.1. Prevalence of gastrointestinal helminths in donkeys and horses

A total of 384 equines that comprised 215 donkeys and 169 horses were thoroughly observed for the presence of different helminthic parasites. From the observed animals, 186 (86.51%) donkeys and 132 (78.10%) horses were positive for different helminthic parasites. In the study area, 3.87% of donkeys and 6.13% of horses harbored only one type of parasite (single infection) whereas, 96.13% of donkeys and 93.87% of horses harbored two or more types of parasites (mixed infection). The overall prevalence of parasites in equines in the study area was found to be 82.81% (Table 1).

Table 1
Overall prevalence of gastrointestinal helminths during the study period in (horses and donkeys).

Species studied	No of animals examined	No of positive animals	Percentage (%)	χ2 (p value)
Donkeys	215	186	86.51	4.696
Horses	169	132	78.10	(0.030)*
Total	384	318	82.81	

*Statistically significant between horses and donkeys (p<0.05).

The parasite encountered in both donkeys and horses in the study period were Strongyles (75.27% and 59.85), *Trichostrongylus axei* (41.94% and 40.15%), *Triodontophorus* (43.01% and 34.85%), *Parascaris equorum* (18.82% and 18.94%), and *Fasciola* (22.58% and 18.94%), respectively (Figure 8 and 9).

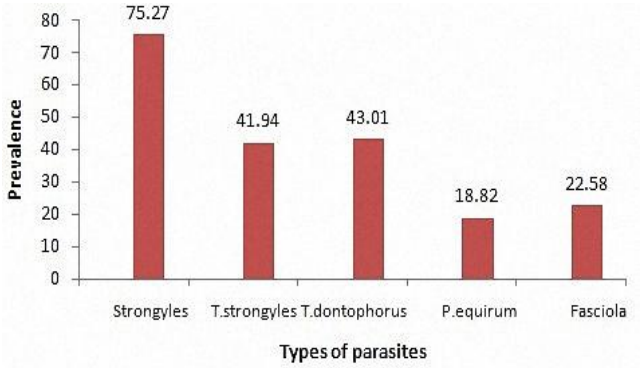

Fig. 1. Types of gastrointestinal helminths and their prevalence in donkeys in the study period.

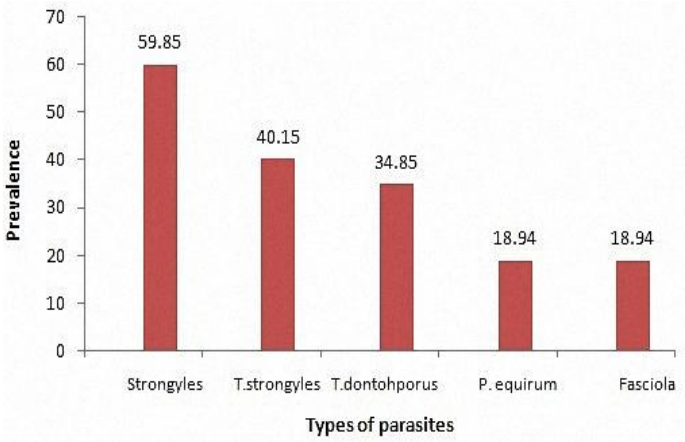

Fig. 2. Types of gastrointestinal helminths and their prevalence in horses in the study period.

Age-wise comparison of studied animals for hosting one or more types of parasites showed varied prevalences. In the study area the prevalence rates of parasites were higher in young donkeys (96.67%) and horses (91.11%) than their adult counterparts (83.85% and 68.89%) in donkeys and horses, respectively. In donkeys the prevalence of *T. strongylus, T. dontophorus, P. equorum* and Fasciola was statstically significant ($p<0.05$), but, the prevalence of *Strongyles* was not statistically significant between age groups ($p>0.05$). In horses, only the prevalence of *T.strongyles* was statistically significant ($p<0.05$), but the prevalence of *Strongyles*, T.dontophorus, *P. equirum* and Fasciola was not statistically significant between age groups of horses ($P>0.05$) (Table 2).

Table 2
Age-wise prevalence of different gastrointestinal helminths in donkeys and horses.

Parasites Identified	Donkeys				Horses			
	Young (%)	Adult (%)	Old (%)	χ2 (P value)	Young (%)	Adult (%)	Old (%)	χ2 (P value)
Strongyle	89.66	69.72	79.17	5.416 (0.67)	70.73	50	65.51	4.911 (0.086)
T. strongylus	68.96	25.68	62.50	28.855 (0.000) *	53.66	29.03	44.83	6.567 (0.038)*
T. dontophorus	75.86	26.61	60.42	30.669 (0.00) *	46.34	29.03	31.03	3.495 (0.174)
P. equorom	37.93	10.09	27.08	14.515 (0.001) *	29.27	12.09	17.24	4.375 (0.112)
Fasciola	44.83	12.84	31.25	16.185 (0.000) *	9.76	20.97	27.58	3.831 (0.147)

*Statistically significant between age groups of donkeys and horses ($p<0.05$).

Comparison of the prevalence rates of sexes of animals revealed the percentage prevalence of gastrointestinal helminthes to be higher in females (90%, 83.33%) than males (84%, 76.37%) in both donkeys and horses, respectively. In donkeys and horses, only the prevalence rates of *Strongyles* was statistically significant between the two sexes (P<0.05). In contrast, all studied parasites were not statistically significant (p>0.05) between males and females of donkeys and horses (table 3).

Table 3
Sex-wise prevalence of different gastrointestinal helminths in horses and donkeys.

Parasites Identified	Donkeys			Horses		
	Male (%)	Female (%)	χ2 (P value)	Male (%)	Female (%)	χ2 (P value)
Strongyle	68.57	83.95	5.810 (0.0.017)*	53.61	77.14	5.928 (0.0.015)*
T. strongylus	40.95	43.21	0.096 (0.0757)	36.05	51.43	2.521 (0.112)
T. dontophorus	45.71	39.51	0.719 (0.396)	30.92	45.71	2.477 (0.116)
P. equorom	19.05	18.52	0.008(0.0927)	17.53	22.85	0.476 (0.490)
Fasciola	21.9	23.46	0.063 (0.802)	15.46	28.53	2.878(0.090)

* Statistically significant between sex groups of donkeys and horses (p<0.05).

In relation to BCS, the percentage prevalence of helminthic parasites was higher in animals with poor BCS and medium than animals with good BCS. All studied parasites except *T. strongyle* were statistically significant in donkeys of different BCS (p<0.05). In horses, *T. strongyles* and *T. dontophorus* are not statistically significant (p>0.05) but other studied parasites were statistically significant (p<0.05) (Table 4).

Table 4
Percentage prevalence of gastrointestinal helminths based on BCS in donkeys and horses.

Parasites Identified	Donkeys				Horses			
	Poor (%)	Medium (%)	Good (%)	χ2 (P value)	Poor (%)	Medium (%)	Good (%)	χ2 (P value)
Strongyles	88.24	72.27	62.71	9.051 (0.011)*	83.33	71.43	46.51	12.814 (0.002)*
T.strongylus	52.94	40.59	29.41	4.807 (0.091)	54.17	45.71	32.87	4.020 (0.134)
T.dontophorus	54.90	44.55	20.59	10.014 (0.007)*	45.83	37.14	30.14	2.070 (0.355)
P.equorom	33.33	14.85	8.82	10.297(0.006)*	29.17	28.57	10.96	6.779 (0.034)*
Fasciola	35.29	19.80	11.76	7.437 (0.014)*	62.50	28.57	0.00	48.835 (0.000)*

*Statistically significant among BCS groups of donkeys and horse (p<0.05)

The results of the present study demonstrated the presence of 6 major different types of helminthic parasites in donkeys and horses in the study area. The overall prevalence of different helminthic parasites was found to be 86.51% and 78.10% in donkeys and horses, respectively. The prevalence was significantly higher in donkeys (p<0.05) than horses. This might be associated with donkeys being neglected animals in the study area, receiving less attention by owners and kept under poor management conditions without any supplementary feed than horses (personal communication with the owners of equines).

The finding of this study was in line with the previous reports in other countries; Mattioli et al. (1993), Paudel (2007), Umur and Acici (2009), and Bewketu and Endalkachew (2011) who have reported 84.4%, 80.48%, 93.5% and 88.21% prevalence rates of parasites in equines of Gambia, Chitwan, Turkey and Bahir Dar, respectively. The current finding, however, was lower than in the findings reported by other workers in Ethiopia, Yosef et al. (2001), Fikru et al. (2005), Mulate (2005) and Ayele Gzachew et al. (2006) who have reported the prevalence of helminthic parasites 100%, 100%, 98.2% and 100% in donkeys of Wonchi, Highlands of Wollo provine, Western high lands of Oromia and Dugda Bora district, respectively. The relative low occurrence of these helminthic parasites in the study area might be associated with the agro-ecological differences, veterinary services provided by Debre Markos Veterinary clinic for equines and the diagnostic capacity of the parasitological technique used and the sampling season (Mekibib et al., 2010).

The current study also demonstrated that among 6 different types of helminthic parasites, Strongyles (65.09% in donkeys and 66.28% in horses) were found to be dominant in the study area. This was in agreement

with the findings of Ayele et al. (2006) who recorded strongyles (100%), *P. equorum* (50%), *A. cephala* (7.4%), *Oxuris equi* (3%) and *Fasciola* (1.5%) that showed the dominance of strongyles parasites. Female donkeys and horses were found to have significantly higher infestation rate of strongyles ($p<0.05$) than males as they might have lower immunity due to gestation, lactation and stresses occurred during this period (Sapkota, 2009). Generally it is assumed that sex is a determinant factor influencing prevalence of parasitism (Pal and Qayyum, 1992). The highest prevalence of strongyles infestation was seen in animals of old age in both donkeys and horses due to waning body conditions and immunity. As the age of the animal increases, the immunity decreases as they are exposed to different parasites when compared with the adults (Sapkota, 2009). The prevalence of this parasite was also higher in animals of poor BCS than good BCS in both donkeys and horses. These may 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 with good BCS (Sapkota, 2009).

The prevalence of *Trichostrongylus* was found to be 41.94% and 40.15% in donkeys and horses, respectively. This was in line with the findings of Krecek et al. (1989), Gebreab et al. (1991), Gebreab (1998), and Ayele Gizachew and Dinka (2010) which ranged from 20% to 58.5% in equines of Ethiopia and South Africa. But the finding was higher than from the reports of Seri et al. (2004) and Umur and Acici (2009) who reported the prevalence of this parasite 12% and 3.31% in donkeys of Sudan and Turkey, respectively. The difference might be due to geographical differences, climatic conditions and management systems of equines such as food, shelter and treatment schedules. The prevalence of this parasite was significantly higher in female donkeys than males and in animals of poor BCS.

The prevalence rate of *T. dontophorus* in this study was found to be 43.01% and 34.85% in donkeys and horses, respectively. This was in agreement with other studies Krecek et al. (2002); Gebreab (1998), Nuredin Ibrahim et al. (2011) and Bewketu Takele and Endalkachew Nibret (2011) reported the prevalence rates of 23%, 35%, 29.7% and 36.32 in equines of Turkey and Ethiopia, respectively. But it was higher than the findings of Reinemeyer et al. (1984); Shrikhande et al. (2009) and Ayele Gizachew and Dinka (2010) who reported the prevalence rates of 3.6%, 2.43% and 15.6%, respectively. The difference might be associated with geographical variations, climatic factors and management systems of equines. The prevalence of this parasite was significantly higher ($P<0.05$) in females and old age groups of donkeys as female donkeys are lactating and olds have waning body conditions and low immunity (Sapkota, 2009) but it was not statistically significant between the two sexes of donkeys and horses ($P>0.05$).

The prevalence of *P. equorum* in this study was found to be 18.82% in donkeys and 18.94% in horses. This is in agreement with other findings (Yosef et al., 2001; Fikru et al., 2005; Uslu and Guclu, 2007; Umur, Acici, 2009) and Bewketu and Endalkachew (2011) who reported prevalence rates of 15.7%, 17.3%, 9.8%, 22.58%, and 13.68 respectively. Ayele Gizachew et al. (2006) and Nuredin Ibrahim (2011) have reported high prevalence rates of 50% and 52.8%, respectively of this parasite. The difference might be due to geographical variations, climatic conditions and management systems of equines. Infection usually takes place through ingestion of the eggs. Heavy infections of *P. equorum* cause impaction and perforation leading to fatal peritonitis (Urquhart et al., 1996). The prevalence of *P. equorum* was significantly higher in young donkeys ($P<0.05$) than olds. This was not unexpected because *P. equorum* is more of a problem of young equine as immunity develops following exposure during adolescence (Gebreab, 1991; Reinemeyer et al., 1994; Hundson et al., 1996; Urquhart et al., 1996; Hendrix, 1998; Gebreab, 2003; Merck, 2003). Infection rate of *P. equorum* was higher in female horses than their counter males. The reason might be due to the fact that females have a close relation to their foals which favors frequent recycling of the parasite between different age groups of equines.

The prevalence of *Fasciola* in this study was found to be 22.58% and 18.94% in donkeys and horses, respectively. This was higher when compared with the previous studies (Ayele Gizachew et al., 2006 and Umur and Acici, 2009) who reported the prevalence rates of 1.5%, and 4.7% in equines of Ethiopia and Turkey, respectively. But the result is in line with other studies (Uslu and Guclu, 2007; Yosef et al., 2001; Mulate, 2005) and Bewketu and Endalkachew (2011) who reported 16.5%, 15.2%, 18.09% and 17.92 in Turkey and Ethiopia, respectively. The relative higher percentage of *Fasciola* in the study area might be associated with suitable ecological conditions for the development of intermediate hosts (snail) and the parasite. Hamami and Ayadi (1999) have reported permanent dampness, suitable luminosity, basic PH of soil and water, and temperature contribute to the multiplicity of snails. A study conducted by Hardy et al. (2002) in Egypt indicated donkeys and horses as potential reservoirs of fasiolosis and recommended donkeys and horses to be considered within the preventive and control measures of zoonotic fasciolosis.

4. Conclusion

Equines suffer from a number of gastrointestinal parasites. Strongyles, *T. strongylus*, *T. dontophors, P. equoro and, Fasciola* are the most important parasites exerting a significant economic impact wherever they are raised. In this study, 6 types of helminthic parasites were found in both donkeys and horses with a higher overall prevalence of 82.81%. Among the infected equines, 3.87% of donkeys and 6.13% of horses harbored only one type of parasite (single infection) whereas, 96.13% of donkeys and 93.87% of horses harbored two or more types of parasites (mixed infection). The prevalence of helminthic parasites becomes relatively lower and lower compared to the previous studies conducted in Ethiopia and other countries due to anthelmintics practiced over equines. Owners of equines should be educated about proper management of equines such as providing sufficient food and shelter, minimizing overworking and extensive open grazing. Therefore, strategic treatments and improved pasture management should be practiced to prevent excessive pasture contamination and continuous education should be given to owners of equines about proper management of equines.

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