Prevalence of gastrointestinal nematodes in donkeys and Mule's species in Anseba Region, Eritrea

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Gastrointestinal helminths (GIH) are the commonly diagnosed infections since ponies, donkeys, and horses are hosts to a wide range of helminths. It is the first qualitative and quantitative cross-sectional study conducted from February 2 to May 20, 2018, to estimate the prevalence of the major gastrointestinal nematodes in donkeys and mules in the Anseba region. For this purpose, 300 donkeys and 30 mules were examined. Fresh fecal samples were collected and subjected to standardized parasitological protocols viz; floatation and McMaster techniques to identify the parasites. Results showed that 300 (90.9%) samples from both species were positive for nematodes of different Genera. The gastrointestinal (GIT) nematodes prevalence in donkeys and mules was 96 and 40%, respectively. Strongyles spp. was the most prevalent parasite in the study area, followed by; Parascarisequorum, Oxyurisequi, Trichomena spp., and Triodontophores spp., mixed - infection among the GIT parasite was also seen in the donkey. From the current study, it can be concluded that donkeys seem to be affected more than mules by GIT nematode parasites. Therefore, routine deworming procedures using broad-spectrum anti-nematode drugs should be conducted to control and prevent the diseases, and, where possible, a rotational grazing program should be implemented.

Key word: Gastrointestinal nematodes, donkeys, mules, prevalence, Anseba region, Eritrea.

INTRODUCTION

Equines (donkeys and mules) are among the most economically valuable asset animals in the world. They are closely associated with humans to increase human production while decreasing human drudgery (Stringer, 2014). They have enormous contributions through their involvement in different social and economic sectors. In many developing countries, equines play an essential role by providing cheap energy for ploughing, traction, riding, transport of agricultural produce, water, and other goods. Eritrea has an estimated equine population of 518,459 (Ministry of Agriculture Eritrea (MOA) 2014. Population census pf Equine species in Eritrea. (Unpublished)). As in many other developing countries, equines in Eritrea are vital resources for poverty-stricken
It is well known that enhancing the welfare of working parasite present, as well as the equid's nutritional and infection is determined by the amount and species of immunological state (Mezgebu et al., 2013). In rural areas, fetching water is mainly the responsibility of girls or women. The water source could be 1-3 h away from the homestead (Hamid, 2004; Upjohn and Wells, 2016).

In Anseba Region, the number of equines was estimated to be 3,176 (MOA, 2014). The donkeys were commonly found in mountainous and dry areas under an extensive management system, whereas the mules were found in Keren town. Equines have been considered pack animals (Figure 2), particularly in areas where modern means of conveyance are absent or/and expensive, drums of water are often carried in donkeys in rural areas (Figure 3), farming practices (Figure 4). Some settlement sites are far from any market, making it difficult for the farmers to sell their goods unless they have donkeys. It was observed that donkeys and mules were mistreated and mishandled, and the attention given to donkeys was far below what they deserve (ESCCAP Guideline 08 Second Edition – March 2019). Despite the growing importance of donkeys and mules in the country's economy, there is no/or limited data on health challenges concerning their welfare.

This is explained as a result of poor health and parasite burdens. Much research on the association between animal disease and poverty identified parasitism in the gastrointestinal tract (GIT) as one of the most severe concerns for donkeys and mules in developing nations (Perry et al., 2002; Getachew et al., 2010). Gastrointestinal helminths (GIH) are the commonly diagnosed infections since ponies, donkeys, and horses are hosts to a wide range of helminths, including 28 genera (75 species) of nematodes, two genera (5 species) of trematodes, and three genera (24 species) of cestodes (Imam et al., 2010, Lester, 2015). Endoparasite infections in animals cause decreased reproductive performance, colic, and diarrhea (Belay et al., 2016, Evans and Crane, 2018). The severity of gastrointestinal infection is determined by the amount and species of parasite present, as well as the equid's nutritional and immunological state (Mezgebu et al., 2013). It is well known that enhancing the welfare of working equids benefits their owners because a healthier working animal may work more efficiently and give increased income-earning potential (Lane, 2015). Svendsen (1994) reported that without anthelmintic medication, a donkey's average life ranged from 9 years in Ethiopia to 15 years in Mexico.

However, in the UK, where there is better management and veterinary service, the average life span is 37 years. Drugs commonly used as anthelmintic in equines are demonstrated in the Table 1. This study reports the prevalence and intensity of internal parasites in working donkeys (Equus asinus) and mules in the Anseba region Eritrean.

**MATERIALS AND METHODS**

**Gastrointestinal nematodes survey in donkeys and mules**

Researchers studied the prevalence and burden of nematode infestation in donkeys and mules in the Anseba region of Eritrea from February 2 to May 20, 2018.

**Study area and animals**

Anseba region was chosen as a study area due to its remoteness and lack of public awareness about veterinary services. Anseba region is one of the six administrative regions, located in the North-Western part of Eritrea, at 15° 46' 55" N, 38° 31' 7" E. The altitude of 765 to 2617 m above sea level, with average rainfall and temperature of 508 mm and 24°C, respectively. The number of donkeys in the region was estimated to be 3,176 (MOA, 2014). The research included donkeys from five Anseba sub-zones: Keren, Hamelmalo, Hagaz, Elabered, and Halhal; because the mules were only available in Keren, they were sampled randomly from Keren town (Figure 1). A total of 330 animals from both species were randomly sampled (300 donkeys and 30 mules). Sixty households (herd owners) were chosen from each sub-region with donkeys, with a herd size ranging from one to three donkeys per household. Each herd had one animal sampled and examined under the microscope, both qualitatively and quantitatively parasitological examinations. Donkeys were sampled at both watering points and marketplaces based on convenience, whereas mules were sampled at Keren market. Given that horses are rarely seen in the field, they have not been considered in the study.

**Samples collection and preparations**

Fresh faecal samples were collected in universal bottles directly from the rectum of individual donkeys and mules. Samples were

<table>
<thead>
<tr>
<th>Species</th>
<th>Positive (N%)</th>
<th>Negative (N%)</th>
<th>Total (N%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donkey</td>
<td>288 (87.3)</td>
<td>12 (3.6)</td>
<td>300 (90.9)</td>
</tr>
<tr>
<td>Mule</td>
<td>12 (3.6)</td>
<td>18 (5.5)</td>
<td>30 (9.1)</td>
</tr>
<tr>
<td>Total</td>
<td>300 (90.9)</td>
<td>30 (9.1)</td>
<td>330 (100%)</td>
</tr>
</tbody>
</table>

Chi-square p<0.0001.
Figure 1. The map showing the study area. The source for the Digital Elevation model: NASA SRTM (https://www2.jpl.nasa.gov/srtm/).

Figure 2. Donkeys as pack animals.
labelled with identification of specimens, year, name of the owner, and place of the collection with a marker. The samples were then placed in an icebox and transported to the National Animal and Plant Health Laboratory’s Parasitology Laboratory as soon as possible (NAPHL). Samples were kept in the refrigerator at 4°C in the laboratory until the qualitative and quantitative parasitological examinations were accomplished (Figures 2 to 4).

Parasitological examination techniques

Qualitative determination of nematodes eggs (Flotation methods)

The laboratory procedure described by MAFF (1986) was used to concentrate the helminth eggs using Sodium chloride solution with specific gravity (1.18-1.2) for easy identification and enumeration of the parasitic stage based on egg morphology. During the process, two grams of fecal samples were weighed, and in a baker, 10 ml saturated sodium chloride solution was added, then using the glass rod the feces, and the solution was mixed thoroughly. The mixture was then sieved through a wire mesh into a 10 ml floatation tube. Flotation solution was added until the meniscus forms slightly above the rim of the tube. A coverslip was placed on top of the meniscus and allowed it to stand for 10 -15 min. After 10-15 min, the coverslip was carefully lifted from the flotation tube and was immediately placed on a microscope slide. The slide was focused

Quantitative determination of nematode eggs

The quantification of parasite eggs was done using the modified
Table 2. Classification of infection types as single or mixed infection in donkeys (n=288) and mules (n=12).

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Single infection (N%)</th>
<th>Mixed infection (N%)</th>
<th>Total (N%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donkeys</td>
<td>167 (55.7)</td>
<td>121 (40.3)</td>
<td>288 (96.0)</td>
</tr>
<tr>
<td>Mules</td>
<td>12 (4.0)</td>
<td>0 (0.0)</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>Total</td>
<td>179 (59.7)</td>
<td>121 (40.3)</td>
<td>300 (100)</td>
</tr>
</tbody>
</table>

McMaster method, and the eggs were classified based on their morphology (Soulsby, 1982). Three grams of feces were mixed with 42 ml of tap water, and the fecal suspension was passed through an 80 µm square sieve to remove debris. The filtrate was collected in a clean, dry container. Fifteen ml of this filtrate was taken into a centrifuge tube and centrifuged at 1,500 rpm for 2 min, and the supernatant was then discarded. The sediment was emulsified by gentle agitation, and saturated NaCl was added until the volume became equal to the initial aliquot of the filtrate. The centrifuge tube was inverted several times to obtain an even suspension of the contents. The two chambers of the McMaster slide were filled using a clean Pasteur pipette. The average number of eggs present in the chambers was multiplied by 100 to obtain the number of eggs per gram of feces (EPG).

Intensity of infection

The severity of the infection was obtained from Soulsby (1982) number of eggs per gram of faeces (EPG) as follows: less than 500 eggs/g of faeces= mild infection, 500 – 1000 eggs/g of faeces= moderate infection and more significant than 1000 eggs/g of faeces= severe infection.

Prevalence rate

The prevalence rate was calculated based on Smith (1995).

\[
\text{Prevalence} = \frac{\text{Number of positive samples at a point in time}}{\text{Total number of animal's samples at the same moment}} \times 100
\]

Statistical analysis

Continuous data were expressed as mean ± standard deviation (SD). In contrast, discrete data (counts) were expressed as percentages, and chi-square was used to explore the association between the infectivity. The type of the animal using the statistical program package Graphpad® Prism 7.00 Software (GraphPad Software, Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037, USA). The statistically significant differences are expressed as p < 0.05.

RESULTS AND DISCUSSION

The prevalence rate and the association of infectivity of gastrointestinal nematode in donkeys and mules

The prevalence rate of gastrointestinal nematode parasites in donkeys and mules (90.9%) is shown in Table 2. Donkeys (96%) exhibited a higher prevalence rate when compared with Mules (40%). Donkeys were highly infected than mules with nematode parasites.

Types of parasitic infection

The positively infected donkeys harbouring a single infection were 55.67%, while that with mixed infection represented 40.33% (Table 3). In Mules, all the animals had a single type of infection (100%).

The intensity of the infection

The intensity of infection was severe in 176 (61.11%), moderate in 87 (30.20%), and mild in 25 (8.7%) donkeys, respectively. In Mules, the intensity of infection was considered mild in all the positive cases (n=12;100%).

Mean egg per gram (EPG) of faeces in infected animals

The overall epg count of the infected animals was (1212±703.3), which indicated severe infection, as shown in Table 4.

Identified species of gastrointestinal nematodes parasites in donkeys and mules

The following species (Table 5) were identified in infected animals. Strongyle spp., P. equorum, were highly prevalent, and the presence of Triodontophores spp., Trichonema spp., and Oxyuris spp. was observed in the order of their prevalence. A higher prevalence was recorded in donkeys. During the period of the study, we are not able to detect trematode and cestode eggs (Figure 5). A serious health issue of donkeys in Africa is gastrointestinal nematode parasites, which contribute to deprived body condition, reduced power demand, low reproductive ability, and short lifetime (Yoseph et al., 2005; Tedla et al., 2018).

According to faecal examinations, the overall prevalence of nematode infection in the Anseba region was 90.9% in both donkeys and mules, the higher in Donkeys (96%) than mules (40%). The current report was lower than Yoseph et al. (2001) and Fikru et al. (2005), showing 100% and 98.2%, in equines Wonchi Highlands of Wollo Province and Oromia Western Highlands, respectively.
### Table 3. Intensity of Infection with Gastrointestinal Nematodes Parasites in Donkeys and Mules (N=300).

<table>
<thead>
<tr>
<th>Animals</th>
<th>Intensity of infection</th>
<th>Total (N%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild (N%)</td>
<td>Moderate (N%)</td>
</tr>
<tr>
<td>Donkeys</td>
<td>25 (8.3)</td>
<td>87 (29.0)</td>
</tr>
<tr>
<td>Mules</td>
<td>12 (4.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>37 (12.3)</td>
<td>87 (29.0)</td>
</tr>
</tbody>
</table>

### Table 4. Overall EPG of GIT nematodes in donkeys and Mules in Anseba Region.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean EPG count</td>
<td>250±96.4</td>
<td>725.9±122</td>
<td>1687±514.7</td>
<td>1212±703.3</td>
</tr>
</tbody>
</table>

### Table 5. Nematodes species identified in infected donkeys and mules in five sub-regions.

<table>
<thead>
<tr>
<th>Type of parasite</th>
<th>Species</th>
<th>Infection type</th>
<th>Subregion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyles spp.</td>
<td>Donkeys</td>
<td>Mixed/single</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>Mules</td>
<td>Single</td>
<td>Keren</td>
</tr>
<tr>
<td>Parascaris equorum</td>
<td>Donkeys</td>
<td>Mixed</td>
<td>All</td>
</tr>
<tr>
<td>Triodontophores spp.</td>
<td>Donkeys and mules</td>
<td>Both mixed</td>
<td>All</td>
</tr>
<tr>
<td>Oxyuris spp.</td>
<td>Donkeys</td>
<td>Mixed</td>
<td>All</td>
</tr>
<tr>
<td>Trichomena spp.</td>
<td>Donkeys</td>
<td>Mixed</td>
<td>All</td>
</tr>
</tbody>
</table>

### Figure 5. This shows some parasite eggs from both the Donkey and the mules.

Such variations could be attributed to changes in ecological conditions, like high temperatures, lower elevations, and low humidity in the Anseba area, which is unfavourable for parasite growth. Furthermore, there may be differences in equine husbandry and management approaches.

As we could closely observe, the care and management provided for equines are inadequate, and the attention given to donkeys has been below what it deserves (ESCCAP Guideline 08 Second Edition – March, 2019). The present 96% parasite prevalence in donkeys was in close agreement with Ayele et al. (2006) reports, who reported 100% in Dugda Bora District. Donkeys had a higher prevalence of nematodes than mules; such variation might be because farmers paid particular attention to mules other than donkeys and considered them more valuable animals. Species difference also could be the probable cause of variation.
in the prevalence of the parasites. All of the mules in the present study were sampled in Keren; they were used as cart's mules as means of income to their owners, while most of the donkeys were sampled from rural areas.

The nematode species encountered during the current study viz: Strongylus sp., Parascaris spp., Triodontophores spp., Oxyrius spp., and Trichomena spp. were similar to previous reports of Seri et al. (2004), Sawsan et al. (2008) and Adam et al. (2013). The pattern of co-infection observed in this study revealed that donkeys have a higher risk of exposure to multiple GIT parasites. The severity of infection by nematodes in donkeys was from high (61.11%), moderate (30.2%) to mild (8.7%) in this study. Ayele et al. (2006) registered a comparable result for mild, moderate, severe infections in Ethiopia with 6.2, 3.8, and 81.7%, respectively. Unlike the current study, Adam et al. (2013) confirmed that 69.7% of examined donkeys were mildly infected; both moderate and extreme shared the lower incidence with 15.6 and 14.7%, respectively. Moreover, higher values were reported by Sawsan et al. (2008) for mild, moderate, and severe infections in donkeys (81.25, 7.89 and 10.86%), respectively. Getachew et al. (2009) reported that a mild degree of infection was observed in mules, which was in line with the finding of this research. There is a need to verify the management system’s role, veterinary services, the number of animals inspected, and factors that favour the variation in the parasite prevalence. Infection with a single nematode was common in both donkeys and mules, though higher in donkeys, only donkeys were identified to harbour mixed nematode infections during the study.

In contrast, Wannas et al. (2012) observed higher single nematode infection in mules. The trematodes and cestodes were recorded neither in mules nor in donkeys in this study. These results were comparable to the finding of Pereira and Vianna (2006) and Kuzmina et al. (2006). It suggested that the resistance of donkeys and mules to cestode infection and the arid climatic condition of the study area prevent the vectors that perpetuate trematode infection. Radostits (2000) indicated that the horses were resistant to trematode infection. It overcomes the migration of this worm inside its body's early stages, so a few numbers are reaching the liver.

Donkeys and mules in the Anseba region are subjected to poor nutrition, especially during the dry season. Their resistance is further compromised due to the heavy agricultural workload. They are not given anti-nematode treatment. Most of the anti-helminthic administered indiscriminately by the farmers are listed in Table 6. Better health and nutrition will undoubtedly contribute positively to these animals’ better performance, consequently improving agricultural production.

From the present study, it can be concluded that GIT parasites are highly prevalent among donkeys. Single as well as mixed types of infection, were present among examined animals. The major GIT parasites species identified in all mules and donkeys in the Anseba region were Strongylus spp., Parascaris spp., Triodontophores spp., Oxyriusequi, and Trichomena spp. Therefore, the research could provide information to the populace on the need to combat the parasite in the working animals and help the owner in prevention and treatment against the specific parasites.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The authors are grateful for their support during the

### Table 6. Class of drug, dosage, and method of administration of equine anthelmintic.

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Anthelmintic</th>
<th>Dosage (mg/kg)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple heterocyclic</td>
<td>Piperazine</td>
<td>88 - 110</td>
<td>S</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>Thiabendazole</td>
<td>44 - 88</td>
<td>S, F, O</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>Mebendazole</td>
<td>8.8</td>
<td>S, F, O</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>Fenbendazole</td>
<td>5 - 10</td>
<td>S, F, O</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>Oxfenbendazole</td>
<td>10</td>
<td>S, F, O</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>Oxibendazole</td>
<td>10 - 15</td>
<td>S, O</td>
</tr>
<tr>
<td>Imidathiazole – simple heterocyclic</td>
<td>Levamisole – Piperazine</td>
<td>8/88</td>
<td>S</td>
</tr>
<tr>
<td>Tetrahydropyrimidine</td>
<td>Pyrantel pamoate</td>
<td>6.6</td>
<td>S, F, O</td>
</tr>
<tr>
<td>Organophosphate</td>
<td>Trichlorofon</td>
<td>40</td>
<td>S, O</td>
</tr>
<tr>
<td>Organophosphate</td>
<td>Dichlorvos</td>
<td>35</td>
<td>F</td>
</tr>
<tr>
<td>Probenzimidazole</td>
<td>Febantel</td>
<td>6</td>
<td>S, F, O</td>
</tr>
<tr>
<td>Avermectin</td>
<td>Ivermectin</td>
<td>0.2</td>
<td>S, O</td>
</tr>
</tbody>
</table>

S = stomach tube, F = feed, O = orally as paste or drench (ESCCAP Guideline 08 Second Edition – March 2019).
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