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GASTRO-INTESTINAL NEMATODES OF GOATS REARED UNDER COMMUNAL SMALL SCALE FARMING CONDITIONS IN BOTSWANA

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Abstract

The study was carried out in goats reared under communal small scale farming conditions near Gaborone, Botswana, to evaluate two sample preparation methods for McMaster egg counting technique, determine worm egg counts (WEC), identify nematode genera by larval culture, and determine abomasal and intestinal worm counts. Goat faecal samples (n = 30) were collected, split, and paired for preparation by either the simple method or the standard method and WEC was performed using the McMaster technique. The simple method resulted in significantly higher WEC than the standard method (p =0.04). The WEC of a cohort of goats from Modipane communal small scale farmers (n = 30) was found to be 995±229 eggs per gram (EPG). Larval culture of Modipane herds identified Trichostrongylus spp at 86 percent and Haemonchus spp, Ostertagia spp, Oesophagostomum spp, and Chabertia spp all occurring at less than 5 % each. Abomasal worm counts of two separate groups of goats at slaughter were found to be 110 ± 28 Haemonchus spp (n = 21) and 277 ± 129 Haemonchus spp (n = 30) in goats with WEC of 836 ± 236 EPG and 642 ± 120 EPG respectively. Intestinal worms were not found in the group (n = 30) that was tested for them. It was concluded that there is a significant worm burden among goats reared under communal small scale farming conditions in Botswana.

Key words: Nematodes, McMaster technique, goats, abomasal worm count, worm burden

NÉMATODES GASTRO-INTESTINAUX DES CHÈVRES ÉLEVÉES EN SYSTÈME COMMUNAUTAIRE ARTISANAL AU BOTSWANA

Résumé

L'étude a été réalisée sur des caprins élevés en système communautaire artisanal près de Gaborone (Botswana), dans le but d'évaluer deux méthodes de préparation d'échantillons pour la technique McMaster de comptage des œufs, de déterminer la numération des œufs de vers (WEC), d'identifier les genres de nématodes par la culture larvaire, et déterminer la numération des vers de caillette et intestinaux. Des échantillons d'excréments de chèvres (n = 30) ont été prélevés, divisés et jumelés pour la préparation soit par la méthode simple ou par la méthode standard, et la WEC a été réalisée en utilisant la technique McMaster. La méthode simple a révélé une WEC significativement plus élevée par rapport à la méthode standard (p = 0,04). La WEC d'une cohorte de chèvres des petites exploitations communautaires de Modipane (n = 30) a été de 995 ± 229 œufs par gramme (EPG). La culture larvaire dans les troupeaux de Modipane a identifié Trichostrongylus spp à 86 pour cent et Haemonchus spp, Ostertagia spp, Oesophagostomum spp, et Chabertia spp à un taux de moins de 5% chacun. Les nombres de vers de caillette de deux groupes distincts de chèvres à l'abattage ont été établis à 110 ± 28 Haemonchus spp (n = 21) et 277 ± 129 Haemonchus spp (n = 30) chez les chèvres avec respectivement une WEC de 836 ± 236 EPG et 642 ± 120 EPG. Les vers intestinaux n'ont pas été trouvés dans le groupe (n = 30) testé pour leur détection. Il a été conclu que les caprins élevés en système communautaire artisanal au Botswana ont une charge parasitaire importante.

Mots-clés : nématodes, technique McMaster, chèvres, nombre de vers de caillette, charge parasitaire

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Introduction

Infection with gastro-intestinal nematodes is common in domestic ruminants world-wide and has a significant economic impact particularly in the tropics and sub-tropics. While heavy nematode burdens can cause mortality, these parasites largely limit productivity (Sweeny *et al.,* 2012). The control of nematode infection in livestock is limited by the high cost of anthelmintics. Also, increasingly, the parasites develop anthelmintic resistance (Stafford *et al.,* 2009; Morgan and Coles, 2010). The nature of gastro-intestinal nematode infection in goats among small scale rural farming communities in Botswana is undocumented and hence unknown. Anecdotal evidence suggests these communities hardly control the parasites in their herds for various reasons including cost limitations and lack of awareness of the extent to which nematode infection limit productivity.

To address the needs of small scale goat farmers in rural areas it is necessary to carry out empirical studies to determine the nature of gastro-intestinal nematodes among livestock including worm burdens, determination of the prevalent genera and their faecal egg output (Ratanapob *et al.,* 2012). In the current study the nature of gastro-intestinal nematode infection in goats reared under small scale farming conditions was investigated. Detection and quantitation of nematode infection in goats is still widely dependent on the McMaster technique owing to the expense required for molecular diagnostic techniques (Rinaldi *et al.,* 2011; Vadlejch *et al.,* 2011). Firstly, a simple sample preparation method that requires less time and equipment and is increasingly being used in laboratories (http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e05.htm#TopOfPage), was evaluated against the standard method for preparing samples for McMacster worm egg counting technique. Secondly, consistent with the necessity to determine the level of nematode infection to inform intervention measures (Tsotetsi *et al.,* 2013), the worm burden among goats under communal small scale farming conditions was estimated using the selected faecal egg counting technique. Thirdly, larval culture was used to identify the nematode genera constituting the strongyle eggs in the goat faeces. The identity of nematode genera is important owing to the variation in the pathogenicity between different genera (van Wyk *et al.,* 2004; http://www.fao.org/Wairdocs/ILRI/x5492E/x5492e05.htm#TopOfPage). Lastly, abomasal and intestinal worm counts were carried out on goats at slaughter to determine the nematode genera most common in goats from small scale rural communities. The empirical findings from this study are discussed in the context of the role of gastro-intestinal nematode in the productivity of small scale goat herds in rural communities.

Materials and Methods

Comparison of two sample preparation methods for McMaster worm egg counting technique. To compare two sample preparation methods for McMaster worm egg counting thirty (30) goats were randomly selected from a herd known to be infected with nematodes. Faecal material was collected from the rectum of each goat. Each sample was split into two equal amounts and paired so that one part was prepared using a simple method and the other sample prepared by the standard method. Briefly, in the simple method, a small bowl containing a sieve was placed on a balance and used to weigh two grams of faecal material. Twenty eight millilitres of saturated saline was added to the bowl and a spatula was used to homogenise the faecal matter. Any material remaining in the sieve was discarded. The homogenate was loaded into chambers of a McMaster slide for worm egg counting. The standard method was carried out as previously described (Nsoso *et al.,* 2000). Briefly, the faeces were crushed first with a mortar and pestle and then two grams was put in a bottle containing forty-five glass beads and 28 mL of tap water. The bottle was tightly closed and shaken and the contents strained through a sieve. The fluid was centrifuged for two minutes at 1500 revolutions per minute. The supernatant was removed and replaced volume per volume with saturated salt solution. The contents were

mixed with a wooden applicator followed by inverting the tube five times. The mixture was loaded into chambers of a McMaster slide for worm egg counting. For samples prepared by both methods, worm egg counting was carried out as previously described (Nsoso *et al.,* 2000). The mean (± standard error) was determined for each method and the two methods were compared using a paired t-test and statistical significance was determined at 5% level.

Determination of nematode egg counts among goats in small scale farming communities. We identified a community of subsistence farmers at Modipane village in Botswana (24 0 37' S 270 23'E). The farmers were rearing goats in a setup typical of most small scale farmers in Botswana. The goat population per kraal averaged 30 animals. The goats were indigenous breeds reared with little or no anthelmintic treatment. We randomly selected 30 adult goats of mixed sex from three kraals (ten goats per kraal) and collected faecal materials from the rectum of each goat. Using the simple sample preparation method described above, we carried out worm egg counts and determined the mean strongylate egg count for the area.

Identification of nematode genera. To identify the nematode genera represented in the strongylate eggs, larval culture was performed using faecal material from Modipane herds described above and larvae recovered were identified to genus level morphologically and enumerated. Faecal matermlal collected from goat recta were pooled together for culture. Cultures were achieved by homogenising a mixture of faeces and 20% vermiculite (weight per weight), moistened with distilled water in a honey jar bottle. The bottles were closed loosely and had a large air space at the top. Incubation was at 27 degrees Celsius for 7 days with periodic checking to ensure samples do not dry up.

The larvae were recovered using the Baermann technique as previously described (www.rvc.ac.uk). Briefly, a plastic funnel with a rubber tube was clamped on a stand. The tube was closed with a clip. Ten grams of cultured faeces in a gauze was put in a tea strainer and placed in the funnel. Distilled water was poured into the funnel until the faeces were submerged. The set up was left overnight. The first 15 mL run off was collected into a tube and centrifuged at 1500 rpm for 2 minutes to sediment the larvae. All but 0.5 mL of the supernatant was removed. An equal volume (0.5 mL) of 5% formalin was added to the larvae as a preservative and the sample was stored in a refrigerator.

To identify larvae, a drop of larvae suspension was placed on a slide. The larvae were stained with Lugol's iodine. Larvae were identified based on characteristic features including total length, oesophagus length, tail sheath length and the number of intestinal cells as previously described by different workers (Lyndal-Murphy, 1987; Van Wyk *et al.,* 2004; Indre *et al.,*2009; Van Wyk and Mayhew, 2013; www.rvc.ac.uk). Free-living larvae were recognised by absence of the sheath. A key described in Massey University MVS 16.602 Diagnostic Pathology study guide was used to place larvae in the correct genus. A micrometer was used to separate the short tailed larvae (< 40 µm); Trichostrongylus (less than 720 µm in total length) from Ostertagia (over 720 µm in total length). Haemonchus spp were identified on the basis of medium-tailed larvae (50 – 60 µm) and not having a muscular band across the anterior part of the oesophagus, a feature that is characteristic of Cooperia. Also Haemonchus larvae were identified by their tapered bullet-shaped heads. A100 larvae were counted and tallied into different genera.

Abomasal and small intestinal worm counts. Two groups of goats were subjected to worm counts. The goats were slaughter age or older and of mixed sex. They came from small scale communal farmers in the catchment areas of Gaborone city council abattoir (24039'S 25054'E). The first group (n =21) were subjected to abomasal worm count. The second group (n =30) were subjected to both abomasal and intestinal worm counts. The worm egg count for both groups was determined by the simple method described above. To carry out worm counts the entire gastrointestinal tract was removed from the carcass. Two string ligatures were tied around the small intestine not more than 2 cm distal

to the pylorus. To remove the abomasum, a cut was made between the 2 ligatures and a second cut made through the distal part of the omasum. The abomasum was placed in a bucket and opened along its length. A small flow of water was used to wash abomasal contents into a bucket. The mucosa was washed thoroughly to remove all material adhering to the folds. The bucket contents were made up to 2 litres. While mixing vigorously with a stick, a tenth (200 mL) of the bucket contents was ladled out into a beaker. The sample was passed through a sieve and a steady flow of water was used to wash the sieve clear. The sample was made up to 500 mL with water. While mixing, 50 mL amounts were removed into a smaller beaker. This sub-sample representing 1/100th of the original contents, was examined on a white tray. The sample was stained with Lugol's iodine for 3 minutes then decolourised with 5% sodium thiosulphate leaving the worms dark brown. A quick count and identification of the worms was done with a hand lens and a more detailed identification using a dissecting microscope. The worms were identified to genus level. To determine the total worm burden the results of the subsample was multiplied by 100.

For small intestinal worms, the first 10 m of the small intestine was stripped from the mesentery starting from the pylorus and cut off. Scissors were used to open this section of the small intestine along its length. Under a trickle of water the contents of the mucosa was squeezed with fingers into a bucket. The small intestinal contents were processed as described above for abomasal contents to determine the total worm count and identify nematode genera.

Results

Comparison of two sample preparation methods for McMaster faecal egg counting technique. The faecal worm egg count of goats infested with nematodes was found to be significantly higher ($p = 0.04$) when samples were prepared using the simple method (958.7±159 EPG) compared to the standard method (528.7±122 EPG) (Fig. 1).

Determination of nematode egg counts among goats in small scale farming communities. The mean worm egg count (n = 30) was found to be 995±229 EPG. All the goats were positive for strongylate eggs.

Identification of nematode genera. To determine the nematode genera infecting the goats we carried out larval culture and identification of larvae to genus level using physical and morphological features. Enumeration of the larvae revealed that Trichostrongylus spp was the most prevalent at 86% and that the other nematodes detected being Ostertagia spp, Haemonchus spp, Oesophagostomum spp, and Chabertia spp occurred at less than 5% each (Fig. 2).

Abomasal and small intestinal worm counts. Goats (n = 21) subjected to abomasal worm counts only, were found to have 110 ± 28 Haemonchus spp worms and a worm egg count of 836 ± 236 EPG. The individual prevalence of worms was 57 percent (12/21). All the goats were positive for strongylate eggs.

Goats tested for both abomasal and intestinal worms (n = 30) were found to have 277 ± 129 Haemonchus spp worms in the abomasum and no worms in the intestines. The worm egg count of this group was 642 ± 120 EPG. The individual prevalence of worms in this

Text box 1

The most common infection of goats is round worms. They significantly reduce productivity and may even cause deaths in a herd. There is need to develop tools to detect goats that have roundworms and such tools ought to be simple and inexpensive. This study establishes a technique that simplifies the detection of round worm eggs in faeces of goats. The technique is then used to determine worm burden in goats that are reared under communal small scale farming conditions in Botswana. The study also hatches eggs in the faeces to get larvae and then uses the characteristics of the larvae to identify the type of roundworms infecting the goats. The presence of wireworms and their quantity in the stomach of goats at the time of slaughter was also determined. The study highlights the need to establish worm burden in goats reared under traditional farming conditions. The findings can be used to inform intervention measures targeted at improving the productivity of goats among small scale farmers.

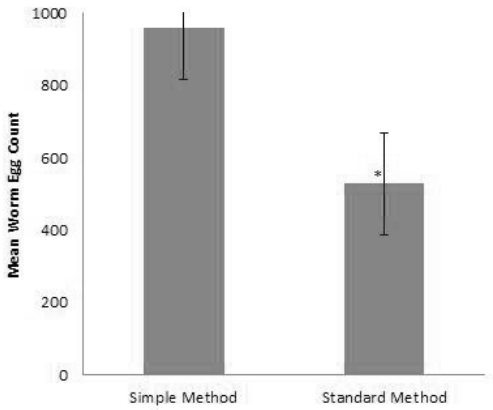

Figure 1: A comparison of two sample preparation methods for McMaster faecal egg counting technique. Goat faecal samples (n = 30) were split and paired samples were prepared by either the simple method or the standard method and faecal egg count was performed using the McMaster technique. Mean egg counts (mean ±SE) were calculated and compared using the paired t-test. * p = 0.04

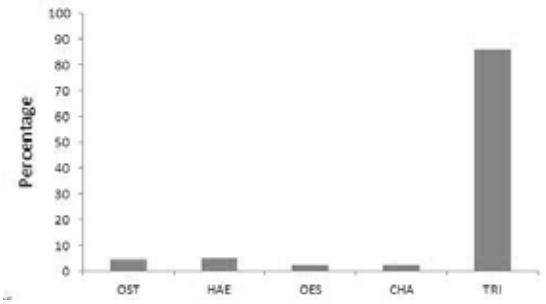

Figure 2: Percentage of larvae identified from cultured goat faeces. Pooled faecal material from nematode infected goats were cultured and larvae recovered by the modified Baermann funnel procedure. Larvae were microscopically examined and enumerated into different genera using their morphological features. OST: Ostertagia spp, HAE: Haemonchus spp, OES: Oesphagostomum spp, CHA: Chabertia spp and TRI; Trichostrongylus spp.

group was 43 percent (13/30).

Discussion

Based on the data from the current study we report that the simple method is more effective and yields higher worm egg counts than the standard sample preparation method for McMaster worm egg counting technique. The McMaster worm egg counting technique is the most common method used for the diagnosis of nematode infestation and to estimate worm burden in livestock including goats (Sweeny *et al.,* 2012). As previously described (Nsoso *et al.,* 2000), the standard sample preparation method for the technique requires a lot of materials that in the simple method are replaced by a sieve and a spoon. In addition, the simple method is quicker both advantages making this method attractive both as a diagnostic and a research tool. The findings of the current study suggest that the simple method is most suitable to use in less equipped laboratories such as in developing countries without compromising the accuracy of both research results and disease diagnosis. In deed even where equipment is abundant laboratories appear to prefer the simple sample preparation method over the standard one.

Decreased productivity of small ruminants due to infection with nematodes is very common across the world including the sub-tropical regions (Tsotetsi *et al.,* 2013). While it is widely recognised that goats reared under communal grazing areas are endemically infected with nematodes, there is a paucity of information on their worm burden as they are not regularly tested for worm egg counts. The current study determined the worm burden in goats under communal small scale farming conditions to be 995±229 eggs per gram. This finding is consistent with that of Totetsi *et al.,* (2013) who reported that most sheep and goats belonging to small scale farmers in South Africa had 100 – 1000 eggs per gram and most herds had a nematode prevalence above 50 percent. Ratanapop *et al.,* (2012) reported a slightly higher strongyle worm egg count (1176 EPG) among goats in Thailand. Worm burden above 1000 EPG in goats and sheep at herd level as compared to individual animal level are considered significant and should necessitate anthelmintic treatment. Segwagwe and Ramabu (1999) reported that helminthiasis and coccidiosis accounted for 43% and 40% of sheep and goat deaths respectfully in Botswana. Thus at flock level the worm burden reported here could be causing mortalities.

Nematodes cannot easily be identified at genus level by examination of eggs. In fact several genera are often lumped together as

strongyles when worm burden is expressed using faecal egg counts. Larval identification revealed that Trichostrongylus spp was the most prevalent nematode in contrast with the finding by Totetsi *et al.,* (2013) who reported Haemonchus spp to be more common than Trichostrongylus spp in sheep and goats. Interpretation of larval culture results as is that of faecal egg counts should consider variability resulting from culture conditions which may increase the hatchability of one species over others.

The presence of worms in goats at slaughter was determined by abomasal worm counts and only Haemonchus spp were recovered in the current study. Similarly, Haemonchus spp were found in the abomasa of sheep and goats in Ethiopia (Kumsa and Wossene, 2006). In contrast, abomasal worm counts in the goats in Ethiopia also recovered Trichostrongylus spp. Also, the Haemonchus prevalence in our study at 57 % and 43% is far less than the over 90% prevalence reported in studies in Ethiopia (Kumsa and Wossene, 2006). In agreement with the current study, Gatongi *et al.,* (1998) found Haemonchus spp to contribute over 80% of the worm burden in sheep and goats raised in semi-arid areas of Kenya. A Haemonchus worm burden of 500 -1000 in sheep less than 12 months of age is considered likely to be affecting health sufficiently to warrant treatment. Therefore the worm burden recorded in this study at less than 500 likely did not severely affect productivity in goats at slaughter age. Nevertheless, Haemonchus females are capable of laying large quantities of eggs resulting in heavy contamination of pasture, a significant risk factor for young goats. It is essential to carry out worm counts both in the abomasum and intestine in livestock since estimation of worm burden by faecal egg count techniques may be misleading by underestimating worm burden during the season when female worms are not laying eggs (Fritsche *et al.,* 1993). On the other hand, as was found out in the current study, some goats with a worm burden below detection, both in the abomasum and small intestine were positive for infection by the faecal egg counting technique suggesting that these goats were infected by few worms with a high egg output.

Conclusion

A simple method for preparing faecal samples for worm egg counting by McMaster technique achieves significantly higher egg counts per gram of faeces compared to the standard method. The worm egg count of goats reared under small scale communal set up is significant enough to impact productivity. Trichostrongylus spp of worms is most predominant in goats compared to other species of nemadotes based on larval culture. Haemonchus spp of worms is most predominant in the abomasum of goats.

Impact

Laboratories may use a simple method to prepare faecal samples for egg counting and expect reliable results. Small scale farmers should implement nematode control measures to increase productivity of their goat herds. And particular attention should be directed to Trichostrongylus spp and Haemonchus spp in the control of gastro-intestinal nematodes of goats in Botswana.

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