Sveriges lantbruksuniversitet
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Faculty of Veterinary Medicine and Animal Science
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Gastrointestinal nematodes in goats in small holder flock around Gaborone, Botswana
Gastrointestinala nematoder hos getter i små besättningar runt Gaborone, Botswana

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SUMMARY

Small ruminants are of great importance in developing countries, where people rely on them for meat, milk and hides. In Botswana, as well as in other African countries, a major health and production problem is internal parasites, such as nematodes. The control of nematodes has for many years relied solely upon anthelmintic treatment, which has led to emergence of resistant worms. This study was conducted to map gastrointestinal nematodes in goats around Gaborone in Botswana and to investigate their response to anthelmintic treatment with ivermectin.

Ten farmers from three different areas around Gaborone were included, each of them keeping approximately 30 goats. A total of 10 animals from each flock were sampled, resulting in a sample size of 100 animals. Each animal was assessed in regards to body condition score (BCS) and color of conjunctivae (FAMACHA®), sampled for faecal material and blood and marked with spray paint. The whole flock was dewormed with ivermectin. Counting of nematode eggs in the faeces was done with a modified McMaster method, and HCT was done with microhematocrit tubes. Between 10-14 days after deworming, the farms were revisited and the animals that were positive the first time were resampled and new egg counts were performed. To assess the efficacy of ivermectin a faecal egg count reduction test (FECRT) was performed. Larval cultures from each positive flock were set and harvested larvae were used for DNA extraction and later PCR and gel electrophoresis to determine presence of *Haemonchus contortus.*

The results showed presence of nematodes in all flocks, but with significant differences between all three areas. The lowest mean EPG was 30±42, and the highest 510±261. The top three highest mean EPG were observed in Kopong and the top three lowest mean EPG in Modipane. In Kopong, one flock had significantly higher faecal egg counts than all the other herds except from one in the same area. After deworming, only one flock (Modipane) was negative at the time of resampling while one flock in Kopong had higher egg counts than before treatment. *Haemonchus contortus* was shown to be present in 4 out of 10 flocks and in all three areas.

Due to low levels of infection and sup-optimal doses of ivermectin, the results of FECRT and the assessment of anthelmintic resistance could not be considered valid. However, the complete lack of egg reduction in one of the flocks coupled with a management of frequent deworming strongly suggests presence of anthelmintic resistance to ivermectin in this particular flock.

SAMMANFATTNING

Små idisslare är viktiga produktionsdjur i utvecklingsländer där människor är beroende av deras kött, mjölk och hudar. I Botswana, och andra länder i Afrika, är inälvsparasiter en stor orsak till nedsatt hälsa och produktion. Dessa har länge kontrollerats framför allt, och många gånger enbart, med hjälp av antiparasitära medel, men denna användning har lett till utvecklingen av resistens hos parasiterna. Den här studien utfördes med mål att kartlägga prevalensen av nematoder hos getter runt Gaborone och hur dessa svarar på behandling med avmaskningsmedel innehållande ivermektin.

Tio djurägare från tre olika områden runt Gaborone deltog i studien. Varje besättning bestod av ungefär 30 getter. Från varje besättning valdes 10 djur ut, vilket resulterade i sammanlagt 100 provtagna djur. Dessa djur bedömdes med avseende på BCS och FAMACHA, det togs träck- och blodprov varefter de märktes med färg. Hela besättningen avmaskades med ivermektin. Därefter utfördes äggräkning med en modifierad McMaster metod och hematokrit mättes med mikrohematokrit, Mellan 10-14 dagar efter första provtagningen återbesöktes besättningarna och ett nytt träckprov togs från de djur som varit positiva vid första besöket. En ny äggräkning utfördes och därefter beräknades reduktionen i äggutskiljning efter avmaskning med hjälp av en reduceringsmodell (FECRT) för att bedöma effekten av ivermektin. Från varje positiv besättning sattes en larvodling där larverna användes till att extrahera DNA som senare användes för att undersöka förekomst av *Haemonchus contortus* efter PCR och med efterföljande elektrofores.

Resultaten visade att det fanns nematoder i samtliga besättningar, även om antalet nematodägg varierade signifikant mellan alla tre områden. Den lägsta beräknade medelmängden var 30±42 EPG och den högsta 510±261 EPG. De tre högsta värdena återfanns alla i området Kopong och de tre lägsta i området Modipane. I Kopong hade en besättning signifikant högre EPG än alla andra besättningar, bortsett från en som fanns i samma område. Efter avmaskningen var en besättning negativ vid den andra provtagningen medan en besättning i Kopong hade högre EPG än vid första provtagningen. *Haemonchus contortus* påvisades endast i 4 av de 10 besättningarna men i samtliga tre områden.

På grund av låga infektionsgrader och en suboptimal dosering av ivermektin kunde resultaten av reduceringsmodellen FECRT och bedömningen av resistens inte anses giltiga. Det obefintliga behandlingssvaret i en besättning kombinerat med de mer frekventa avmaskningarna som utfördes där ger dock en stark misstanke om resistens gentemot ivermektin hos denna djurgrupp.

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INTRODUCTION

Small ruminants are sometimes referred to as “the cow of the poor” due to their ability to produce meat, milk and hides from a relatively low input. Thus, goats and sheep are of great importance in most developing countries, for both economic and social reasons (Vatta et al, 2001). However, production can be reduced directly by internal parasites leading to lower quality of the product, reduced weight gains as well as higher mortality. Indirectly, these parasites are also an economic burden through costs for control and treatment (Hoste et al., 2010).

Botswana is an upper middle economy, but a fifth of the population is regarded as poor (World Bank, 2016). Nearly half of the population lives outside the official economy, mostly relying on livestock such as cattle, sheep and goats (Utrikespolitiska institutet, 2015). A majority of the smallholder farmers in Botswana prefer goats due to their resilience and ability to quickly adapt to environmental conditions as well as the general populations preference for goat meat over mutton (Panin and Mahabile, 1996). Moagabo and Baipoledi (2008) continued to see the dominance of goat production in later years and around the turn of the century.

In the years 1999-2002, 60% of recorded mortalities of goats in Botswana were due to endoparasites (Moagabo and Baipoledi 2008). This is in agreement with earlier studies that claim endoparasites as a major reason of constrained production among small ruminants in Botswana (Nsoso et al., 2010; Walker et al., 2015), as in the rest of southern Africa (Vatta et al., 2002).

The cornerstone in nematode control has globally for many years relied on the use of broad spectrum anthelmintics, often without any other control measures. This extensive use has led to development of resistance against the drugs (Jackson et al., 2012), and due to this rising problem there has been an increase in research into alternative strategies, addressed in greater degree to maintain parasitic infection under an economic threshold. These novel or alternative parasite control strategies are based on a combination of knowledge of the epidemiology of the infection and management practices (Waller, 2006).

To correctly handle decreased productivity due to nematode infection in small ruminants, in commercial as well as small farm holdings, there is a need to have knowledge about the species composition and how the parasite respond to anthelmintic treatment. This will enable farm holders to take appropriate measures, either by anthelmintic treatment in general in combination with other prophylactic measures.

The goal of this study was to investigate gastrointestinal nematodes of goats in 3 different areas surrounding Gaborone and to study how they respond on anthelmintic (ivermectin) treatment.

LITTERATURE

Nematodes in goats

The phylum Nematoda consists of several families. They are commonly called roundworms due to their wormlike appearance (Taylor et al., 2007). Trichostrongyloidea is a superfamily of

nematodes under the order Strongylida, and in this family several genera, such as *Haemochus*, *Oesophagostomum* and *Trichostrongylus* are of major relevance to ruminant production on the African continent (Nginyi et al., 2001; Pandey et al., 1994; Wanyangu et al., 1994; Frietsche et al., 1993). Trichostrongylides are frequently found in the gastrointestinal tract and are monoxenous, e.g. they make use of one host in their life cycle (Andersson, 2000). Thus, the life cycle of nematodes consist both of external stages outside the host and internal stages in host (Taylor et al., 2007).

Nematode eggs are excreted in the faeces from infected animals, and will then develop in the external environment if the conditions regarding moisture and temperature are favorable. The third infective stage of development, (L3), crawl onto vegetation to infect grazing animals by oral uptake. L3 are resistant towards dry conditions and low temperatures, but do not thrive during freezing conditions (Andersson, 2000). For optimal transmission of the parasites by survival of L3 on pasture, temperature, moisture and pasture growth are important factors (Banks et al., 1990; Besier and Dunsmore, 1993). Green pasture can hold enough moisture on its own for eggs to develop into L3 without any substantial rainfall (Besier and Dunsmore, 1993).

Inside the animal the adult worms depending on species inhabit either the abomasum or the anterior part of the small intestine, where they develop from L3 to a fourth stage (L4) before they become L5 or adults (Andersson, 2000). While *Haemonchus* inhabit the abomasum, most species within the genus *Trichostrongylus* are found as adults in the small intestines (Trapani, et al., 2013). All trichostrongylid larvae invade the external mucosa of the gastrointestinal tract before they develop into L4. After that, they either develop into a L4 before they reappear in the lumen where they are completing the final development into the adult stage, or they stay as inhibited larvae in the mucosa, or closely attached to the mucosa without any further development. This is usually called arrested development or hypobiosis and it mainly occurs in temperate climates where the parasites must survive harsh winter conditions (Andersson, 2000), or, as Gatongi et al. (1998) reported from Kenya, during the dry seasons, when the conditions for development and survival of the larvae are unfavorable.

In Botswana, Nsoso et al. (2001) showed that fecal egg counts (FEC) increased during the dry seasons, e.g. spring, summer and autumn, with a significant reduction during the winter months. This pattern was attributed to more conductive environmental conditions such as higher temperature and moisture. A similar pattern of higher FEC during the rainy seasons in Kenya at the same time as it was noted that the worms were present in the gastrointestinal tract the whole year (Nginyi et al. 2001). High egg counts was also observed during wet seasons in Gambia although goats carried significantly lower numbers of eggs compared to sheep (Fritsche et al. 1993).

Thus, large variations within the seasons will occur when hypobiotic larvae suddenly resume their development. This has been seen in goats in Kenya (Gatongi et al, 1998), and Zimbabwe (Pandey et al., 1994). According to Gatongi et al. (1998) it was specifically seen before the onset of the long seasonal rains.

Goats and sheep share the same nematode species, but the distribution of different species may vary depending on the climatic conditions of the host (Torres-Acosta and Hoste, 2008). Furthermore, the susceptibility to gastrointestinal nematodes of goats and sheep are not the same. While sheep usually graze the grounds, goats mainly browse from bushes and trees. This adaption has brought the goats away from the higher concentrations of nematode eggs on the grazing grounds, and it is believed that this strategy has led to a lesser development of the immune system compared to sheep (Hoste et al., 2010). There is also evidence of differences in the metabolism of xenobiotics, including drugs such as anthelmintics (Hennessy et al., 1993).

The different species of nematodes have different pathogenesis even though the life cycles are similar. For example, *Trichostrongylus* spp. invade the mucosa of the small intestine and are found just beneath the intestinal epithelium. When development into L4/L5 occur, the larvae ruptures the mucosa, causing hemorrhages, oedema and loss of proteins. Clinically, this is usually expressed as diarrhea and weight loss. If the animals are malnourished and heavily infected mortalities can occur (Taylor et al., 2007). In comparison, *Haemonchus contortus* is a blood sucking nematode, and in this case the adult worms is the most harmful stage. An adult worm consumes up to 30 microliters of blood per day causing severe anemia and even death (Emery et al., 2016). In contrast, acute infection with *Haemonchus* is not associated with diarrhea (Taylor et al., 2007).

Supplementary feeding can improve the host resilience against nematode infections (for a review, see Torres-Acosta et al., 2012). Thus, while the worm burdens increase during wet seasons (Nginyi et al., 2001; Besier and Dunsmore, 1993 etc.), access to feed during this season can mask the parasites' effect on the animals (Vatta et al., 2002). A similar pattern of reduced effects during periods with greater availability of nutrients in young goats was observed in Kenya, and it was even theorized that there might be a compensatory growth during this period (Githigia et al., 2001). Torres-Acosta et al. (2012) reviewed several studies and came to the conclusion that supplementary feeding can improve resilience against nematodes, especially in tropical countries.

The clinical signs of haemonchosis, are lethargy, pale mucous membranes, submandibular and facial oedema, ascites, progressive weight loss and, in some cases, death (Taylor et al., 2007). Presumably the most common picture of infection in naturally infected animals is a chronic syndrome with anemia/affected hematology but without any obvious signs of clinical disease. It is also believed that these subclinical signs, in a chronic setting can occur even though the worm burden may be relatively low (Allonby and Urquhart, 1975). Chronic haemonchosis can be observed during dry seasons when continuous loss of blood due to a persisting burden of adults occurs. The animals do not get re-infected, but the reduction of nutritious feed combined with the persisting worm burden can cause signs such as weight loss, weakness and loss of appetite (Taylor et al., 2007)).

FAMACHA®

A simple method to identify animals that are not able to cope with *H. contortus* is the FAMACHA® scoring chart (van Wyk and Bath, 2002). The FAMACHA® scoring chart was

developed by Dr Faffa Malan (FAffa Malan CHArt), and is based on sheep losing color in their conjunctivae due to anemia caused by *H. contortus*.

During fatal haemonchosis the color of the conjuntivae will change from red/pinkish to, in severe cases of anemia, almost white. It is important to remember that this method is not appropriate to evaluate nematode parasites such as *Trichostrongylus* spp., which are not feeding on blood (van Wyk and Bath, 2002).

FAMACHA® scores have previously been evaluated in relation to hematocrit/packed cell volume both in goats (Vatta et al., 2002) and sheep (vanWyk and Bath, 2002) in South Africa as well as in goats in Uganda (Nabukenya et al., 2014). Although, results from these studies show a relationship between FAMACHA® scores and hematocrit/packed cell volume, further development of the chart, especially when performed with goats was recommended (Nabukenya et al., 2014). It has also been stressed that the usage of the chart in goats should be combined with other diagnostic measures such as FEC (Vatta et al., 2002). Still, the FAMACHA® chart has been regarded as a valid technique to evaluate clinical haemonchosis and it has been stated that the studies with poor results either had a problem with applying the technique correctly or problems related to the operator (Besier, 2012).

The FAMACHA® chart was suggested as a simple strategy to reduce the use of anhelmintics. By using FAMACHA® the number of anthelmintic treatments can be reduced at the same time as it will leave a population of worms *in refugia* (Besier, 2012). The purpose with keeping a population of nematodes *in refugia* is to dilute resistant genotypes so that the relative contribution of resistant worms to the next generations is kept at a minimum, thereby keeping a proportion of the worm population susceptible to anthelmintics (vanWyk, 2001). The *in refugia* strategy has been considered as one of the most promising for slowing down the development of anthelmintic resistance (Kenyon et al., 2009).

Thus FAMACHA® is a tool that can be included in a targeted selective treatment (TST) strategy, where only the animals most affected/only the animals who have something to gain from deworming are treated (Kenyon et al., 2009). Such a TST program was studied in goats in Botswana and resulted in reduced anemia in the animals. It was concluded that the method showed potential for increasing production as well as improving surveillance and management of the animals (Walker et al., 2015).

In case of other, non-blood sucking, nematodes such as *Trichostrongylus spp, Teladorsagia circumcincta* and *Oesophagostomum columbianum* are present, other methods for clinical evaluation of worm burden are needed. Body condition score is regarded as a promising factor to evaluate, at least in sheep where the tissue over the lumbar vertebrae is easily scored (Bath et al., 2005). However, goats are not as easily assessed with this method due to their tendency to store fat intra abdominally (Vatta et al., 2002). BCS was also used as an indicator in the TST study in Botswana mentioned above (Walker et al., 2015), but could not be properly evaluated due to the study being conducted during the rainy season when the animals had greater access to feed.

Anthelmintic resistance

There are three major groups of broad-spectrum anthelmintics that can be used for small ruminants in Botswana?; 1) benzimidazoles, 2) imidazothiazoles/hydropyrimidines, and 3) macrocyclic lactones. They all have different mechanisms for expelling parasites. In adddtion, there is narrow-spectrum anthelmintics include salicylanilides and nitrophenols, used for specific control of *H. contortus* and in some countries, organophosphates are still used (Coles et al., 2006).

The history and implications of anthelmintic resistance (AR) are reviewed by Kaplan (2004). The author comes to the conclusion that even though anthelmintics may be needed in some life-threatening cases the overall use must be reduced before there are no effective drugs left for treatment.

AR in nematodes in goats towards all major groups of anthelmintics has been noted in Uganda (Nabukenya et al., 2014), in Kenya (Wanyangu, et al., 1996) and South Africa (vanWyk et al., 1997; vanWyk et al., 1999). In Kenya, there is also evidence of resistance against multiple anhelmintic drugs (Mwamachi et al., 1995). In general *H. contortus* has been noted as the dominant species associated with resistance to anthelmintics (Wanyangu et al., 1996; Tsotetsi et al., 2013).

Because of differences in the metabolism between goats and sheep, it has been suggested that goats should be dosed with higher amounts of anthelmintics than sheep (Hennessy et al., 1993). For example, in Australia there are recommendations of 1.5 times the sheep dose when treating goats with levamisole (Barger et al., 1994), whereas the Farm & Animal Health in Sweden recommends to deworm goats with twice the dose advised for sheep. Wanyangu et al. (1996) used Barger's dose recommendation and noted a higher prevalence of AR against levamisole among goats compared to sheep. It was noted that dose recommendations by the manufacturers are seldom adapted for goats, and that is one reason for the higher prevalence of resistance among parasites of goats (Chartier, et al., 1998; Wanyangu et al., 1996)

Nabukenya et al. (2014) showed anthelmintic resistance to albendazole, ivermectin and levamisole, dominantly in *Haemonchus contortus*, in different districts in Uganda, but noted at the same time differences between the districts attributed to variations in scale and management practices. Large scales producers who kept their goats in grazing paddocks had a higher prevalence of AR, while small scale farmers who moved their animals between different grazing areas and did not deworm on a regular basis had less problems with AR. Size of the farms has been reported to be of significance in earlier studies in Kenya with smaller farms being less susceptible to severe infections (Wanyangu, et al., 1996). The same author warns against movements of animals from large scale production units to smaller farms without prior testing of the nematode infection status.

Torres-Acosta and Hoste (2008) recommends alternative methods for nematode control such as lower stocking densities, use of rangelands and use of indigenous breeds. Other methods for control and possible future strategies are reviewed by Torres-Acosta and Hoste (2008) and includes *in refugia* methods, vaccination, genetic selection, nutritional manipulation etc.

MATERIAL AND METHODS

Location and study material

The study was conducted between September 6th 2016 and November 3d 2016 in southern Botswana on 10 farms around the capital Gaborone (district Katlang). These were located in three different areas; Modipane (n=4), Kopong (n=3) and Gakuto (n=3). The whole area is located in a semi-arid region with dominant *Acacia* bush savannah. Temperatures range from 19 °C in the dry winter months up to 33 °C during the summer. The wet season usually occurs between November and April, and the studied region usually gets 300-500 mm/year (Omphile et al., 2004). Extremes such as winter frost and temperatures up to 40 °C are occasionally observed.

The goats were kept at so-called cattle posts in the outskirts of the villages where they are secured during the night in areas fenced with bush material (kraals). In the morning the animals are released to graze freely and look for water in the surrounding areas during the day. There was access to water in their kraals and two of the farmers also fed their goats with hay at night. This way of semi-extensive management of goats in north-central Botswana has previously been outlined by Walker et al. (2015).

The farmers who participated in the study were chosen on flock size and willingness to participate. It was not a completely homogenous group of farms; at Kopong one farmer had almost 100 animals and the production was more commercialized than for the other participants. In Modipane, two farmers had roughly 10-15 animals each, which were combined and considered as one farm unit.

Sampling and laboratory methods

A first contact was established and the studies and procedure of sampling were presented. At day of sampling the flock size was calculated. All adults were caught and dewormed with a subcutaneous dose of 0,2 mg ivermectin per visually assessed kg bodyweight (Ecomectin 1%, Afrivet). Depending on number of animals, every second or every third animal in each flock was assessed in regards to body condition score (BCS) and FAMACHA® on a 5 point scale, respectively. Blood was sampled by Vacutainer tubes (EDTA). Furthermore, rectal fecal samples were taken before deworming and marking each animal with spray paint according to a system for 10 animals, based on 10 anatomical points on different parts of the animal.

Then, the farmers were interviewed about their flock and the management procedures, including questions about contact with other animals, previous medication/treatments and perceived illnesses and problems seen within the flock (see appendix A).

The samples were transported in a cooler box the same day to the lab at Botswana University of Agriculture and Natural Resources where the fecal samples were kept in a refrigerator. HCT was performed with microhematocrit tubes.

The nematode fecal egg counts (FEC) were counted using a modified McMaster method (Coles et al., 2002) where 3 g of feces are mixed with 42 ml of water into a homogenous mix before

sieving and centrifuging. After centrifugation water was replaced by saturated saline. Egg counting was made at 100X magnification in a McMaster chamber with a minimum detection level of 50 eggs per gram (EPG) faeces.

After 10-14 days, a second visit took place. Results from the first round of sampling were reported and a new round of rectal faecal samples were taken from all animals that were egg positive at the first visit, regardless of the EPG. Counting was done again performed with the McMaster method as described above. Three animals could not be found at the second visit; one in flock D and two in flock I.

Larvae were cultured from each positive flock with feces from positive animals. Approximately 6 g of feces from each animal was pooled in a jar, mixed with vermiculite and moisted with water and then kept in room temperature for seven days. During this time the cultures were regularly checked and watered if needed. Harvesting was done after the jars were filled with distilled water and turned upside down in a petri dish with a rim of water. After 24 hours, larvae in the petri dishes were harvested and kept in tubes in a refrigerator.

DNA was extracted with NucleoSpin Tissue kit (Macherey-Nagel) and the extracted products were applied on FTA-cards (Whatman) for storing (n=19). DNA was later extracted from the FTA-cards according to the supplier protocol with rinsing and washing reagents. For the PCR a master mix containing, for each sample, 2,5 µl 10x buffert, 1,5 µl $MgCl_2$ (25 mM), 0,5 µl HcFor (20µM), 0,5 µl HcRev (20 µM), 0,65 µl cNTP (10 mM), 0,13 µl AmpliTaq Gold and 19,2 µl H_2O was added. The PCR was performed with 5 minutes in 94°C followed by 40 cycles with 45 seconds in 94°C, 30 seconds in 50°C and 45 seconds in 72°C, one cycle with 10 minutes in 72°C and finally the samples were kept in 4°C until they were taken out of the thermocycler. Gel electrophoresis was performed shortly after retrieving the samples. An agarose gel was mixed and prepared in a suitable tray. Each sample was dyed before added to the gel tray, and run for 35 minutes in 100 V.

Statistical analysis

Statistical analysis were performed in Excel 2013 spread sheets, GraphPad Prism v.7.02 and MiniTab 17.

To determine the efficacy of ivermectin in these farms a fecal egg count reduction test (FECRT) was performed according to Coles et al. (1992). FECRT is based on the faecal egg counts before and 7-14 days after treatment in naturally infected animals. If both of the following criteria are met, resistance is declared; a) the reduction in egg counts is less than 95% and or b) the 95% confidence interval is less than 90%. If the first criteria is fulfilled, resistance is suspected.

RESULTS

General and basic information are presented in table 1 below, with mean BCS, FAMACHA®, HCT and FEC at first sampling. The FEC values from the first sampling are presented in table 2 as mean together with standard deviation (SD), standard error of mean (SEM) and highest

egg count. The egg counts ranged from no eggs at all to 1200 at the most. There was only one animal with egg counts higher than 1000 EPG.

*Table 1. General information about the ten flocks. Flocks marked with * were kept together with sheep.*

Area	Farm	Total stock (adults + kids)	Sampled animals	Last deworming	BCS	Famacha	HCT	Positive animals at 1st sampling
Modipane	A	29+17	10	Unknown	3	2,2	33	4/10
	B1	15+15	5	March/April 2016	3,1	2,2	34,9	5/10
	B2	9+12	5	May 2016 with Ecomectin				0
	C	35+25	10	None, uses plants	2,9	2,4	33,4	9/10
	D	45+21	10	January 2016 with Ivomec	3,4	2	35,6	4/10
Kopong	E	30+23	10	2014	3,9	2,2	30,9	10/10
	F	25 in total	10	None	3,4	2,8	31,7	10/10
	G	70+30	10	June 2016	3	3,2	26,3	9/10
Gakuto	H	45+15	10	Unknown, not in 2016	3,4	2,4	32,1	10/10
	I*	25+3	10	None	3,3	2,4	34,4	9/10
	J*	25 adults	10	2015	3,5	2,1	37,2	7/10

Table 2. Arithmetic mean EPG at first sampling with standard deviation (SD) and standard error of mean (SEM). The top 3 highest egg counts were all observed in Kopong, and the 3 lowest egg counts were observed in Modipane.

Farm	Mean	SD	SEM	Median	Highest EPG	Total sum of EPG
A	40	56.76	17.95	0	150	400

B	60	90.68	28.67	25	250	600
C	140	115	36.36	125	350	1400
D	30	42.16	13.33	0	100	300
E	380	271	85.7	250	950	3800
F	510	261.2	82.6	450	1200	5100
G	250	250.6	79.23	175	800	2500
H	225	190.4	60.21	175	550	2250
I	85	66.87	21.15	50	200	850
J	110	128.7	40.69	50	400	1100

Arithmetic mean FEC with standard deviation (SD) at first sampling and second sampling are combined in fig 1. Notable, already at this stage, is the lack of efficacy on the nematode egg reduction at the second sampling of flock G.

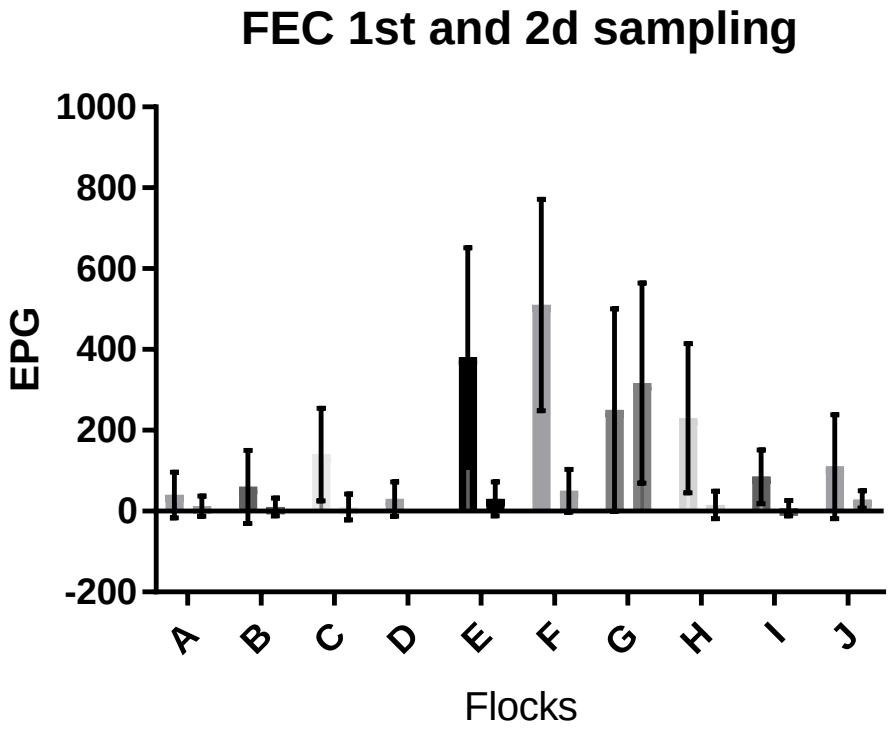

Figure 1. Combined arithmetic mean EPG of first and second sampling, with the first sampling to the left and the second sampling 10-14 days post treatment shown to the right in each group. Note that most of the SD are indicating that the variables do not have a Gaussian distribution. As mentioned, flock G does not seem to respond to the anthelmintic treatment, as indicated by actual higher egg counts at second sampling.

The FECRT is presented below in fig 2 as well as in table 3. Flock G had a higher mean and total sum of EPG after deworming than at sampling 1 (2850).

Figure 2. Results of the FECRT with the error bars representing the CI at 95%. Note that flock G, which had no reduction in egg counts, are not within the percentage axis. Flock D had a total reduction, and therefore no error bars.

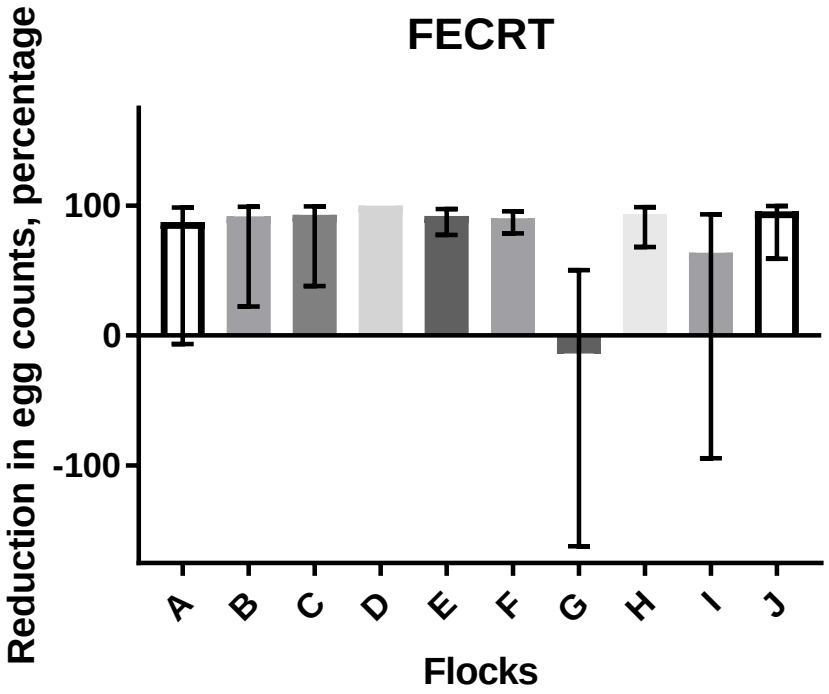

Table 3. Results of FECRT, showing the numbers that did not fit the model of percentage reduction in the figure above.

Area	FECRT (%)	UC (95%)	LC (95%)
A	87,5	98,5	6,6
B	91,7	99,1	22,2
C	92,9	99,2	38,2
D	100		
E	92,1	97,2	77,5
F	90,2	95,5	78,7
G	-14	50,4	-162
H	93,5	98,7	68,1

I	63,6	93,2	-94,3
J	95,5	99,5	59,1

According to Kruskal-Wallis test EPG levels was significantly different between the investigated flocks. Post hoc testing with Mann-Whitney showed that there were significant differences between several flocks (see Appendix B), but notably flock F in Kopong had EPG levels significantly higher than all the other flocks except from flock E in the same area. The three different areas were all significantly different from each other when tested with Mann-Whitney.

PCR of larval cultures showed that *H. contortus* was present at least in flock D, F and I from the first set of sampling, and in flock F and G from the second set of sampling.

The correlation between FAMACHA® scores and PCV/HCT was evaluated with a Spearman test, showing a significant negative correlation with a coefficient of -0,513.

DISCUSSION

Trichostrongylid eggs were present in all of the 10 flocks, but varied significantly between some of the flocks. The management on all 10 farms were close to the same, and all of the flocks were grazing/browsing freely in areas around their homestead. All animals were owned by small holder farmers and practiced communal grazing. This kind of semi-extensive management seemed to be a suitable system to keep worm burdens at a reasonably low level on most farms.

Some flocks did have their homestead close to water and green grazing grounds. This was particularly an observation in Kopong (flocks E and G), but also in the flock from Gakuto (I). All of these three flocks had their homestead within a kilometer from river beds, with lush green vegetation such as bushes and greener pasture grounds. This was especially evident in the flocks from Kopong, and flock G had a green field close by. It is most likely that these flocks do not graze or browse as extensively as those flocks who have longer distances to water and where there is no access to green grazing grounds.

While the animals in close range to water and greener pastures mentioned above (E, G, I) would have access to more green bushes and would be able to obtain most feed from browsing it is likely that they would also graze from time to time, on a green pasture with required moist for keeping a population of infective larvae. Animals in drier areas, with long distances to water, and no green grazing grounds, probably browse in greater degree. Thus these animals are thereby probably not exposed to infective larvae to the same degree as those that are grazing. The highest EPG, both mean and individual, were also noted in the flocks from Kopong (E, F, G) where at least two of the herds had close access to green grazing grounds. Flock I, on the other hand, had close access to water and greener grounds but had the fourth lowest mean EPG.

Compared to earlier studies with goats in Botswana, the FEC in the present study were on a similar or somewhat lower level. In the present study the mean FEC were in the range of 30 to 510 EPG, whereas it was 257 EPG during winter and 741 EPG in spring in a study by Nsoso (2001). In contrast, Ramabu (2015) reported a mean of 959 EPG. Possible reasons for these differences may be the time of sampling, but also animal density and management of the goats, including anthelmintic treatments.

The anthelmintic dose used herein for the goats was the same as is recommended for sheep, which in theory is too low (Hennessy et al., 1993). The dose recommendation for sheep according to the manufacturer is 0.5 ml/25 kg (0,2 mg/kg). However, in this study there was a quite large interval of bodyweight, and some of the goats were probably closer to 25 kg than 50 kg. Thus several animals had low bodyweights and therefore received the accurate dose. This probably explains why the treatment had a good effect on worm burdens in the majority of the investigated flocks.

However, one exception was observed in flock G, where the FEC increased 11 days post treatment. The most reasonable explanation for drug failure is presence of anthelmintic resistance. This flock was larger and more commercialized than the rest of the flocks and had probably received more frequent treatments against gastrointestinal parasites than the other flocks. Although a journal was kept for notes on treatments and other events, such as animals sold, it is unclear which anthelmintic that had been used. It was however known that the latest deworming was performed in June the same year. This coupled with the management of the flock, with frequent deworming, facilitates a strong suspicion of anthelmintic resistance towards ivermectin in flock G, which actually involved the most pathogeneic species *H. contortus*.

Other reasons for the poor efficacy in flock G could be that there was something wrong with the anthelmintic drug or that it was something wrong with the way of drug administration. Both of these reasons are unlikely; because the anthelmintic used in this flock was also used in all other flock, and it was also administered in the same way. It has already been stated that the dose used herein was not ideal for goats but nevertheless, the majority of the investigated flocks responded on the treatment, with this one exception.

Although the statistical FECRT, has been widely used for decades, it have shortcomings (Waller, 1997), especially in this case when inclusion criteria was not fulfilled. First, in half of the cases less than 10 animals with a ≥150 EPG was identified. Secondly, some animals most likely received a sub-optimal dosage of anthelmintics, which could lead to false signs of resistance. Because of this, anthelmintic resistance can not be assessed with accuracy in all flocks.

H. contortus was shown to be present in 4 out of 10 flocks; D, F, G and I, with PCR and gel electrophoresis. With this, all three areas are represented, and it could be that some of the other herds are falsely negative. This could be because of a number of reasons; low density of DNA from the larvae cultures (even though larvae were confirmed present before DNA extraction was done), too low concentration of extracted DNA, or sup-optimal handling of the FTA-cards,

which warrants further investigation. Furthermore, for the internal control of the PCR results, the manufacturer of the FTA-cards recommends a negative control, a positive control, a negative control with a washed, no-sample punch (so that the punch does not cause a positive result) and a positive control standard added to a washed, no-sample punch (so that the punch does not inhibit the reaction). Since this was not done, the PCR results can not be considered entirely reliable.

It is also possible that there was a low egg output from *H. contortus* females at the time. Especially *H. contortus* is known to undergo hypobiosis to survive unfavorable climatic conditions, and at the time of sampling the weather was still dry with no rains. Nematodes have only been studied earlier in one of the areas also included herein; Modipane. However, it is unclear during which period FECs and enumerations of larvae were done, but they showed a higher presence of *Trichostrongylus spp.* at 86 %, with *H.contortus* occurring at less than 5 % (Ramabu et al. 2015). These results could be because of a generally low prevalence of *H. contortus*, or due to the seasonal variations that commonly occur when dealing with this particular nematode.

The correlation between the FAMACHA® scores and HCT was a slightly negative correlation at -0,513, indicating that when the scores go up, i.e. the membranes become paler, the hematocrit drops. The results could be less strongly correlated than other study results due the operators inexperience with the chart (Besier, 2012). Flock G, which were positive for *H. contortus* and strongly suspected to carry resistant worms, had the highest mean FAMACHA® score and the lowest mean HCT, indicating that the worms could indeed be a factor in causing anemia and supporting the results of the flock being positive for the blood sucking *H.contortus*.

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APPENDIX A

Questionnaire

1. Number of animals in the flock? Adults and kids?
2. Any other animals of different species, specifically any ruminants?
3. Where do the animals graze/browse?
4. Do the animals get into contact with goats or other ruminants from other flocks or villages?
5. What species of wildlife are observed in this region? Do wildlife ever come into contact with the flock?
6. How do you consider the health of the animals? Have you ever observed abortions, stillbirths, diarrhea or respiratory symtoms?
7. Do you use medicines such as antibiotics or anthelmintics? What kinds?
8. Do you vaccinate your animals?
9. Do you use any anti-tick treatment?
10. How do you acquire new stock?
11. How important are your goats to you?

APPENDIX B

Flocks compared		P-value	Significantly different? (P < 0.05)
A	B	0.7449	No
A	C	0.0220	Yes
A	D	0.8800	No
A	E	<0.0001	Yes
A	F	<0.0001	Yes
A	G	0.0072	Yes
A	H	0.0127	Yes
A	I	0.0950	No
A	J	0.1560	No
B	C	0.0611	No
B	D	0.6449	No
B	E	0.0002	Yes
B	F	<0.0001	Yes
B	G	0.0124	Yes
B	H	0.0154	Yes
B	I	0.1681	No
B	J	0.3706	No
C	D	0.0094	Yes
C	E	0.0119	Yes
C	F	<0.0001	Yes
C	G	0.4198	No
C	H	0.3762	No
C	I	0.3348	No
C	J	0.4650	No
D	E	<0.0001	Yes
D	F	<0.0001	Yes
D	G	0.0041	Yes
D	H	0.0062	Yes
D	I	0.0358	Yes
D	J	0.1197	No
E	F	0.0984	No
E	G	0.2036	No
E	H	0.2353	No
E	I	0.0001	Yes
E	J	0.0018	Yes
F	G	0.0189	Yes
F	H	0.0072	Yes
F	I	<0.0001	Yes
F	J	<0.0001	Yes
G	H	0.9518	No
G	I	0.0549	No
G	J	0.1149	No
H	I	0.1020	No
H	J	0.1447	No
I	J	0.9079	No