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TABLE OF CONTENTS

ABBREVIATIONS

AH – anthelmintics, ALB – albendazole, AR – anthelmintic resistance, BC – back crosses i.e. 75 % D, BZ – benzimidazoles, EHT – egg hatch test, D – Dorper sheep, EPG – eggs per gram faeces, F1 – first generation of offspring of a RM and a D, F2 – the offspring of two F1s, FEC – faecal egg count, FECRT – faecal egg count reduction test, GIN – gastrointestinal nematodes, IVM – ivermectin, L3 – third stage larva, L4 – fourth stage larva, LEV – levamisole, ML – macrocyclic lactones, PCV – packed cell volume, RM – Red maasai sheep

SUMMARY

Haemonchus contortus is the most important bloodsucking gastrointestinal nematode in small ruminants worldwide. It is often controlled by anthelmintics. Even though other methods to control are available, the most efficient way to treat an animal with clinically manifested haemonchosis is to use one of the available broad spectrum anthelmintics: benzimidazoles (BZ), macrocyclic lactones (ML) or imidothiazoles-tetrahydropyromidines or the narrow spectrum drugs salisylanilides or nitrophenols. Anthelmintic resistance (AR) against all groups of anthelmintics in nematode parasites has been reported from many countries, and even multiple AR has been observed. In this study I have investigated the resistance levels to all major classes of broad spectrum anthelmintics in a naturally infected flock of sheep of Red Maasai sheep (RM), Dorper sheep (D) and their offsprings on a research farm in Kenya. Resistance to the BZ albendazole (ALB) and most likely also to the imidothiazole levamisole (LEV) had previously been seen in this flock. Faecal Egg Count Reduction Test (FECRT) was now performed. The efficacy of ALB, IVM and levamisole (LEV) were tested. Faecal egg counts (FEC) and reductions after deworming were analysed according to the AHs used. Accordingly, a total of 88 sheep were divided into three treatment groups per breed or crosses (n=5-7) i.e. the number of sheep subjected to each AH were 27-31 animals. One untreated control group (n=15) was also included in the study. Prior to AH treatment animals were weighed and they were then treated orally by a veterinarian with either of the three substances. In addition, blood and faeces were collected and examined for the packed cell volume (PCV) and the number of parasite eggs per gram of faeces (EPG) both at the day of treatment plus at day 10 and day 22-24 post treatment. Furthermore, nematode larvae were cultured from pooled faeces per group at day 0 and day 10 and larval differential counts were performed. Results showed that the majority of the nematodes were *H. contortus* both at days 0 and 10. The FECRTs showed efficacy of all three tested substances, between 98 % and 99 % reduction of egg counts per gram faeces, on days 22-24. Thus, there was no resistance against any of the tested substances, even though resistance had been seen only about two years earlier in the same flock.

SAMMANFATTNING

Den stora löpmagsmasken, *Haemonchus contortus*, är den viktigaste gastrointestinala nematoden hos små idisslare och är en blodsugande parasit som finns utbredd över hela världen. Parasiten kontrolleras ofta genom avmaskning. Även om det finns andra metoder att kontrollera *H. contortus*, är ofta den effektivaste metoden att behandla kliniskt sjuka djur med antihelmintika (AH). Tillgängliga AH på marknaden är benzimidazoler (BZ), makrocykliska laktoner (ML), imidothiazoler-tetrahydropyromidiner eller closantel. Läkemedelsresistens har rapporterats hos *H. contortus* från många länder, framför allt mot substanser inom gruppen BZ, men även resistens mot ML, levamisol och multipel resistens finns beskriven. Syftet med denna studie var att undersöka läkemedelsresistens hos nematoder i en fårflock med renrasiga Red Maasai- och Dorper-får samt olika korsningar av dessa på en fårfarm i Kenya. Tidigare hade man hos parasiter från denna besättning sett antihelmintikaresistens (AR) mot albendazol (ALB) och med stor sannorlikhet också mot levamisol (LEV). Vi utförde en så kallad ”faecal

egg count reduction test" (FECRT) där effekterna av ALB, IVM och LEV testades. Totalt 88 får ingick i studien. Djuren indelades i tre behandlingsgrupper per ras eller korsning (n=5-7). Antalet får som behandlades med respektive AH varierade mellan 27 och 31 djur. En kontrollgrupp (n=15) inkluderades också i studien. Innan avmaskningen vägdes alla får. Därefter behandlades de oralt av en veterinär med någon av de tre substanserna. I samband med detta togs även blod- och avföringsprover som analyserades avseende hematokrit (PCV) respektive antal parasitägg per gram avföring (EPG). Alla får provtogs på samma sätt även 10 och 22-24 dagar efter behandlingen. Äggutskiljningen (FEC) och reduktionen analyserades slutligen med avseende på behandlingen med respektive substans. Även nematodlarver odlades fram från poolade träckprover i samtliga behandlingsgrupper både från dag 0 och 10 varefter differentialräkningar utfördes, och som visade att en majoritet av larverna var *H. contortus* både dag 0 och dag 10. FECRT visade effekter mellan 98 % och 99 % hos alla de tre testade substanserna. Enligt FECRT fanns det således ingen resistens mot någon av de testade substanserna trots att man sett AR i samma flock endast cirka 2 år tidigare.

INTRODUCTION

Background

Weight loss and death caused by gastrointestinal parasites are frequently encoutered problems in grazing livestock production (Allonby & Urquhart, 1975) and especially in small ruminants in developing countries (Coles, 2002; Waller, 1997). *Haemonchus contortus* is the most important parasitic nematode of sheep and goats in the tropics and subtropics worldwide (Waller, 1997). *H. contortus* lives in the abomasums of ruminants and its eggs are shed with faeces of the host. It causes decrease in growth, anaemia and in serious cases even death of the ruminant host (Allonby & Urquhart, 1975).

H. contortus fourth stage larva (L4) and adults consume approximately 0.05 ml blood from the abomasal vessels, per day and worm (Allonby & Dargie, 1973). Thus, the severity of the infection varies with the number of adult worms in the abomasums but also with the nutritional state of the sheep. A female *H. contortus* can lay up to 10 000 eggs per day. A severely infected sheep can shed 10 000 eggs per gram faeces (EPG) (Radostits et al., 2007). The eggs hatch on pasture and develop within some days to some months into infective third stage larvae (L3). The prepatent period in sheep is 2-3 weeks (Urquhart et al., 1996). Hypobiosis is a way for *H. contortus* to survive dry or freezing periods. The larvae then stop their development and stay in the early L4 stage in the abomasum of the sheep (Gatongi et al., 1998)

Three different classes of broad-spectrum anthelmintics (AH) are frequently used for sheep today: benzimidazoles (BZ), macrocyclic lactones (ML) and imidothiazoles-tetrahydropyromidines (Table 1) (Kaplan, 2004; Kahn & Line, 2005). Two other classes of narrow-spectrum anthelmintics can be used to treat infection with only *H. contortus*: salisylanilides (closantel) and nitrophenols, as well as organophosphates (OP). The OPs are more toxic to the host than other anthelmintics. Thus the use of OPs is declining and they are now only available in some countries (Kahn & Line, 2005; Coles et al., 2006).

Table 1. Classes of anthelmintics and examples of anthelmintics from some of the classes. (Kaplan, 2004; Kahn & Line, 2005; Coles et al., 2006)

Broad spectrum anthelmintics	Subgroups	Example of anthelmintics
1. bezimidazoles		albendazole thiabendazole
2. imidothiazoles-tetrahydropyrimidines	imidothiazoles	levamisole
	tetrahydropyrimidines	pyrantel morantel
3. macrocyclic lactones	avermectins milbemycins	ivermectin
Narrow spectrum anthelmintics	Subgroups	Example of anthelmintics
1. salisylanilides-nitrophenols	salisylanilides	closantel
	nitrophenols	
2. organophosphates		

These three substance classes of AHs vary in their mechanisms of action. The MLs, i.e. milbemycin and the substances within the avermectin group exert their effect by opening glutamate-gated chloride channels on the nematode neuromuscular membrane. The effect is irreversible and the consequence is paralysis and death of the nematode (Prichard, 1994; Wolstenholme & Rogers, 2005). The imidothiazoles-tetrahydropyrimidines levamisole (LEV) and morantel stimulates the neuromuscular junctions. This group of AHs has a nicotine-like action (cholinergic agonistic action) and the worms become paralysed (Prichard, 1994; Rang et al., 2003). The BZs bind to helminth β-tubulin and prevent the polymerisation of the microtubules and excert its effects by interfering with cell division and the glucose uptake (Lacey, 1988; Prichard, 1994; Rang et al., 2003).

Resistance against different types of AHs has become a world-wide problem. The situation is serious especially in sheep and goats in the tropics and subtropical countries, including Kenya (Waruiru et al., 1997; Waruiru et al., 1998; Coles, 2002). Thiabendazole (TBZ) was the first BZ and also the first broad spectrum AH (Brown et al., 1961) that both had a high efficacy against nematode parasites and a low toxicity in mammals (Kaplan, 2004). This drug has therefore been widely used for the treatment of gastrointestinal nematodes (GIN) in sheep (Ghisi et al., 2007). Resistance to TBZ was reported already in 1964 in *H. contortus* in sheep (Kaplan, 2004). In the early 80's Prichard et al. (1980) reported that AR had also developed against both the imidothiazole LEV and the tetrahydropyrimidine morantel. The ML ivermectin (IVM) was released on the market in the early 1980's and has ever since then been the first drug of choice as resistance against many other AHs already existed at that time (Ghisi et al., 2007). However, in the late 1980's, resistance to IVM was also described (van Wyk & Malan, 1988). Today, AR is seen against all major classes of AHs (Kaminsky, 2003). Multiple AR was seen in *H. contortus* in the early 1980s (Kaplan, 2004; Prichard et al., 1980). One field strain of *H. contortus* in South Africa was resistant against all

major classes of AHs simultaneously, except for the narrow spectrum drug closantel (van Wyk et al., 1997). Some farms in South Africa have had to abandon sheep production as a consequence of failure of anthelmintic treatment. Resistance against all the three classes of broad spectrum AHs and closantel has been seen in Malaysia. (Waller, 1997)

The strategy of deworming all animals and then to move them to clean pastures, i.e. the dose-and-move strategy, has formerly been recommended in several countries, to reduce the negative impact of helminths. (Coles, 2002). It is still widely practised, but should according to Coles (2002) not be used any longer. It has been stated that the procedure may select for resistance. Only the resistant nematodes will survive in the sheep, and they will be spread onto the clean pasture (Kaminsky, 2003; van Wyk, 2001). In a situation like this there will be few larvae in "refugia" (the concept of refugia is explained below), and resistace will therefore develop rapidly (van Wyk, 2001). The larvae in refugia are those unselected larvae not being exposed to the AH, e.g. larvae in non-treated animals or larvae on pasture when the animals are dewormed (Kaminsky, 2003). There are two dry seasons per year in the Kenyan central highlands, separated by the long rains that lasts between March and May and the short rains in October to December (Nginyi et al., 2001). The only larvae in refugia during the dry periods are those in the non-treated sheep, since larvae will not survive on the pasture then. (Kaminsky, 2003). During the dry periods the EPG levels are also quite constant while they often increase during the rainy periods (Nginyi et al., 2001). The problem of resistance is in general more severe in larger commercial herds than in resource-poor farms with only a few animals, since AHs are costly, often comes in large packages and are not always available in the countryside in Africa (Vatta & Lindberg, 2006).

There is an ongoing discussion both about when the sheep should be treated and which individuals that should be included. Out of the perspective of increasing multiple AR, it would be preferable to only treat animals with clinical signs of helminth infection (Coles, 2002). One method is to only deworm individuals with high FECs. This is a somewhat insecure method since different species of worms have very different egg laying capacities (Coles, 2002). A FEC can be valuable in herd levels though, especially to see what types of parasites the sheep have. Another method to distinguish whether an animal should be treated against *H. contortus*, is the FAMACHA, which builds on the fact that small ruminants, that are heavily infected with *H. contortus* suffer from anemia. It scores the sheep or goats in different categories according to the colour of their mucus membranes. Only the animals with clinical infection and some level of anemia will be treated. This also ensures that there will be larvae in refugia in the non-treated sheep (Vatta & Lindberg, 2006).

It is important to dose the sheep according to how much they weigh, since underdosing may rapidly select for resistant worms (Kaminsky, 2003).

The use of AHs can also be decreased by the use of genetically resistant breeds of sheep that differ in their tendency to become heavily infected by *H. contortus*. It has been seen in a number of studies that RM sheep often has a lower FEC than for example D sheep. By using more resistant breeds, the need for regular

deworming of the entire herd will decrease and so will the rate of selection for AR against the chosen anthelmintics (Mugambi et al., 1996; Mugambi et al. 1997; Wanyangu et al., 1997). It has also been shown that there is a higher profit in raising RM sheep than Dorper in the Kenyan sub-humid regions (Waller, 1997).

Studies have been conducted on how different wildlife hosts, for example the Thomson gazelles, influence the amount of L3s on pasture, but their contribution is often small. Only if one wants to exterminate nematodes from a certain area, one has to take into account that gazelles or other wildlife hosts may function as reservoirs (Preston et al., 1979).

The most commonly used diagnostic method today in clinical praxis for the detection of AR, is the FECRT, which is an *in vivo* method that involves the parasites in the sheep as the experimental unit (Coles et al., 1992; Coles et al., 2006; Vatta & Lindberg, 2006). An advantage with the FECRT is that it can be used with all groups of anthelmintics that are available today. The disadvantage is that the EPG levels do not always correspond to the number of adult worms inside the animals. However, FECs in young sheep correlate fairly well to the burden of adult worms, at least compared to the situation in adult sheep (Coles et al., 2006; Coles, 2002). Furthermore, the FECRT will only detect AR if there are over 25% of resistant nematodes in a population (Coles et al., 2006). FECRT also requests a rather large number of sheep and is therefore difficult to use in small flocks (Vatta et Lindberg, 2006).

There are also several *in vitro* tests available that can be used to measure AR. One of them is the egg hatch test (EHT), which can only measure BZ resistance: fresh eggs are diluted either in increasing concentrations of thiabendazole (TBZ) or in a predetermined concentration of a TBZ solution (the discriminating dose) and incubated for 48 hours. The eggs hatched are then counted. Discriminating doses have been established in i.e. *H. contortus*. A discriminating dose is the dose required to prevent hatching of 99 % of susceptible eggs. The EHT can detect resistance if there are at least 2-3% resistant eggs (Coles et al., 2006).

I addition, there are several DNA-test under development, but none of them is currently used as screening tools under actual field conditions (Coles et al., 2006; von Samson-Himmelstjerna et al., 2009). The BZ resistance in *H. contortus* is partly caused by single nucleotide polymorphisms (SNPs) located to alleles in the codon positions 200, 167 and 198 of the beta-tubulin gene (Coles et al., 2006; Ghisi et al., 2007, von Samson-Himmelstjerna et al., 2009). Real-time specific PCR and pyrosequencing analyses for BZ resistance in *H. contortus* have been used in several research laboratories and it has for example been shown that pyrosequensing seems to be more sensitive than both the FECRT (Höglund et al., 2009) and the EHT (von Samson-Himmelstjerna et al., 2009). Another advantage with genetic tests are that they are are less time-consuming than the FECRT (von Samson-Himmelstjerna et al., 2009). However, no molecular tests are available for the other AHs, since their genetic basis of AR are not completely understood (Coles et al., 2006).

Aim of the study

The aim of this study was to investigate the current state of AR in a sheep flock on the ILRI research farm Kapiti in Kenya. According to Githiori AR has been seen both towards ALB and most likely also towards LEV in the sheep nematodes found on this farm about two years ago[1] [2]. We wanted to investigate if the parasitic nematodes of this sheep flock still display AR.

MATERIALS AND METHODS

The field study was conducted during April and May in 2009 and included 103 sheep from the ILRI research farm Kapiti in Kenya (about 200 km south-east of Nairobi). The sheep were between 1-2 years of age. The one year olds were selected after FECs have been performed from 120 sheep so that only the ones with a FEC of ≥1200 EPG were included. The two year olds were picked randomly without any prior testing. There were altogether 5 breeds or types of mixed breed sheep in the study: RM, Dorper, F1 (offspring of a Dorper and a RM), F2 (offspring of two F1:s) and their backcrosses (BC, 3/4 Dorper) (Fig. 1). A veterinarian treated the sheep orally with albendazole, ivermectin or levamisole at their recommended dosages. Each treatment group included sheep of all breeds and their crosses. One control group remained untreated. There were 5-7 sheep from each breed or cross in each treatment group while the untreated group had 3 animals of each breed and cross (Table 2). All control animals were male whereas the treated groups contained both sexes. The BC group contained only sheep born 2007 but the majority of the sheep in the other groups were born in 2008. Ear tags identified the sheep. Two sheep was excluded from the study since their breed could not be confirmed. Another sheep was excluded since it was not a pure breed and one animal was excluded since the father was unknown. Faeces and blood were collected from all animals at the same time as they were weighed (to closest half a kilo). The faecal samples were collected in plastic bags and the blood was taken from the ear in glass capillaries. The dosage of the treatments was conducted according to the weight of the sheep.

Table 2. Groups of sheep. Numbers of sheep in each group

	Levamisole	Albendazole	Ivermectin	Control	Sum
Red Maasai Sheep	5	6	6	3	20
Dorper	6	7	5	3	21
F1	6	7	6	3	22
F2	7	6	5	3	21
¾ Dorper	6	5	5	3	19
Sum:	30	31	27	15	103

The sheep were then sampled in the same way both 10 and 22-24 after the treatment.

[1] John Githiori, International Livestock Research Institute (ILRI), Nairobi, Kenya, e-mail 2009-12-10

[2] John Githiori, e-mail 2011-10-12

The sheep were kept in paddocks at night whereas they were grazing in the estate pastures under supervision of shepherds during the day (see Fig. 2). They could therefore not be regularly moved to new clean fields, but the estate was large. The sexes were seperated thoughout the experiment, but they all grazed the same pastures. They should therefore be considered as one and the same flock, though it is not known how often they went to the different areas of the estate.

Day 0 samples were taken in the beginning of the rain period when the first new green grass had started to grow. At this point some of the sheep were rather thin, since they were feeding mainly on the grass in the area. Some of the sheep had large ticks, which may have affected their PCV values.

Figure 1. Red Maasai Sheep (red)) in the foreground and a Dorper (white with black head) in the background. The sheep were identified by ear tags.

Figure 2. Shepherds grazing the sheep in the open fields on the estate.

Anthelmintics have previously been used on the farm. However, it could not be clarified to what extent the sheep included in this study had been treated with AHs earlier.

Faecal egg count reduction test

The faecal samples were tested individually with a modified McMaster method (Coles et al., 1992) based on 3 g of faeces in 42 ml of water and with a detection level of 50 EPG. Less than 10 percent of the samples taken on days 0 and 10, had too little faeces, so that we had to adjust the volume of the water added. Both on day 0 and 10, some faecal samples were soft due to diarrhoea and for six samples day 0 and three samples day 10, 5 g instead of 3 g was mixed with 42 ml of water.

Larval differentiation

Larvae cultures were grown from day 0 and day 10 samplings only. The faeces used for the cultures were first kept for several days in plastic bags, before the samples from each group were pooled in glass beakers with small air holes in the covering film. (Table 3). Sample material from some sheep was sometimes too scarce to allow larvae culture (Table 3). The four sheep that were excluded from the study were included in the pooled samples, since it became known after the larvae was cultured, that the breeds of those 4 sheep could not be confirmed or was not pure breed. The number of sheep from each group from which faeces were pooled is shown in Table 3. The pooled faecal samples were transferred to covered glass beakers with small air holes in the covering film. The environment in the beakers was kept moist by sprinkling water in them whenever they looked dry. The larvae were harvested approximately 10-11 days after sampling. The glass beakers were cleansed by soaking them for at least 10 minutes in water containing sodium hypochlorite, which would kill any remaining larvae. A differential count of 10-50 larvae per sample was then performed. In the samples from day 0 there were plenty of larvae, so 50 larvae could be counted, whereas on day 10 they were so scarce in some of the samples that less than 50 or no larvae was counted (Table 4 and 5).

Table 3. The number of sheep in each group is shown in bold type. The number of sheep from each group whose faeces were pooled for larvae culture on day 0 is shown in parenthesis. Numbers of sheep from each group whose faeces were pooled for larvae culture on day 10 is shown in square brackets. Sample material from some sheep was too scarce to allow larvae culture. Four sheep were later excluded from the study, since they were not pure breed or their breed could not be confirmed. This was seen after the larvae was cultured and they are included in the larvae differential counts. They were in the BC control group, IVM D group, IVM F2 group (only included day 0) and ALB F1 group respectively in the larval differentiation. One of the sheep was mistakenly placed in the D control group instead of the LEV D group.

	Levamisole	Albendazole	Ivermectin	Control	Sum
Red Maasai Sheep	**5** (5) [5]	**6** (5) [6]	**6** (6) [6]	**3** (1) [3]	**20**
Dorper	**6** (6) [5]	**7** (6) [7]	**5** (5) [6]	**3** (2) [4]	**21**
F1	**6** (5) [6]	**7** (7) [8]	**6** (6) [6]	**3** (2) [3]	**22**
F2	**7** (6) [7]	**6** (5) [6]	**5** (5) [5]	**3** (1) [3]	**21**
¾ Dorper	**6** (5) [6]	**5** (4) [4]	**5** (4) [5]	**3** (1) [4]	**19**
Sum:	**30**	**31**	**27**	**15**	**103**

Calculation

The statistical analyses were made in Excel spread sheets. The analyses of the reduction were done arithmetically in accordance to Waller (1989). When the reduction is less than 95 percent or the lower confidence interval is lower than 90 percent, there is a risk of AR in the nematode. If both of these criteria are fulfilled the nematodes are regarded as resistant to the treatment.

RESULTS

Larval differentiation and faecal egg count reduction test

Most of the larvae in this herd were *H. contortus*. The results of the larval differentiation counts, performed both at days 0 and 10, are shown in Fig. 3 and in Table 4 and 5. Very few larvae were present in the samples on day 10. In 7 of the samples there were less than 10 larvae, so in those groups differential count could not be performed.

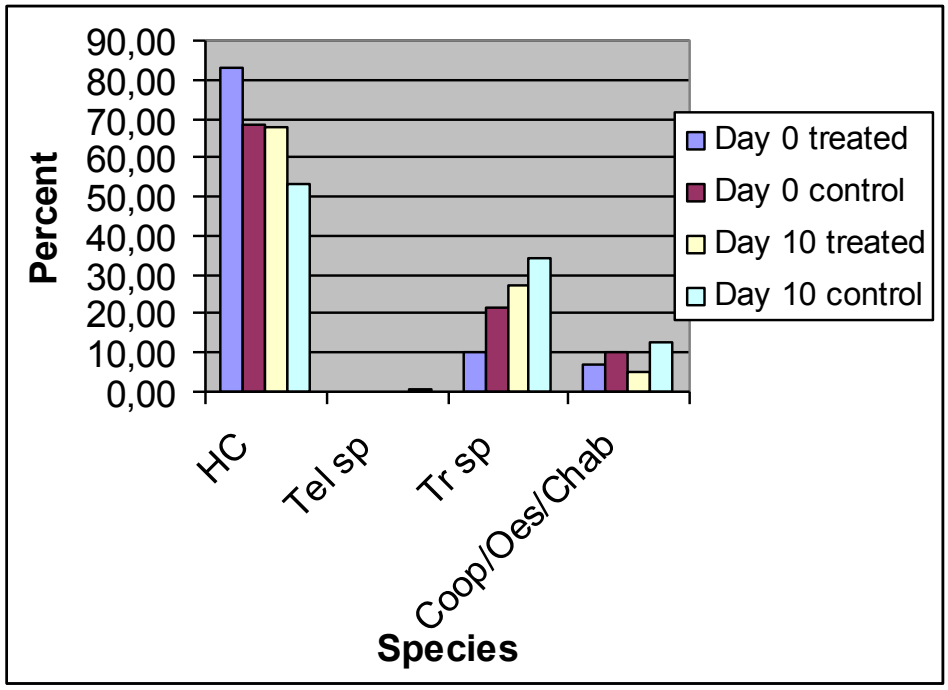

Figure 3. Larval differential counts. Percent Haemonchus contortus (HC), Teladorsagia species (Tel sp), Trichostrongylus species (Tr sp) and Cooperia sp./Oesophagostomum sp./Chabertina ovina (Coop/Oes/Chab) in the treated animals compared to the control group day 0 and 10. On day 10 there were very few larvae in the treated groups. In 7 of the 15 treated groups there were less than 10 larvae and reliable larvae differential counts could not be established in those groups.

Table 4. Larvae differential count day 0. Percent Haemonchus contortus (HC), Teladorsagia species (Tel sp), Trichostrongylus species (Tr sp) and Cooperia sp./Oesophagostomum sp./Chabertina ovina (Coop/Oes/Chab) in all of the 20 pooled groups. Number of counted larvae (No) is shown

Treatment, Breed	Hc in %	Tel sp in %	Tr sp in %	Coop/Oes/ Chab in %	No	Remarks
LEV RM	62	0	28	10	50	
LEV D	96	0	0	4	50	
LEV F1	88	0	2	10	50	
LEV F2	76	0	8	16	50	Larvae in very bad shape
LEV BC	94	0	6	0	50	
ALB RM	80	0	8	12	50	
ALB D	88	0	10	2	50	
ALB F1	72	0	28	0	50	
ALB F2	82	0	6	12	50	
ALB BC	82	0	18	0	50	Large amount of free-living larvae
IVM RM	84	0	12	4	50	
IVM D	92	0	4	4	50	Larvae in very bad shape
IVM F1	88	0	10	2	50	
IVM F2	78	0	10	12	50	
IVM BC	82	0	4	14	50	
CONT RM	66	0	32	2	50	
CONT D	90	0	6	4	50	
CONT F1	66	0	26	8	50	
CONT F2	50	0	32	18	50	
CONT BC	70	0	12	18	50	

Table 5. Larvae differential count day 10. Percent Haemonchus contortus (HC), Teladorsagia species (Tel sp), Trichostrongylus species (Tr sp) and Cooperia sp./Oesophagostomum sp./Chabertina ovina (Coop/Oes/Chab) in all of the 20 pooled groups. Number of counted larvae (No) is shown

Treat-ment, Breed	Hc in %	Tel sp in %	Tr sp in %	Coop/Oes/ Chab in %	No	Remarks
LEV RM	0	0	62	38	50	
LEV D						Only 4 Tr spp larvae found
LEV F1						Only 3 Tr spp larvae found
LEV F2						Only 1 Tr spp larva found
LEV BC	4	0	96	0	25	
ALB RM	96	0	4	0	25	
ALB D						Only 8 Hc larvae found
ALB F1						Only 3 Hc larvae found
ALB F2	100	0	0	0	25	
ALB BC						No larvae was found.
IVM RM	100	0	0	0	10	
IVM D	90	0	10	0	50	
IVM F1	72	0	24	4	50	A few free-living larvae
IVM F2	80	0	20	0	20	
IVM BC						No larvae found. Some more or less embryonised *Haemonchus*-like eggs are found.

No resistance was found with the FECRT (per treatment) (Figures 4 and 5). Compared to the control group, the efficacy on day 10 was 97 % in the IVM group, 96 % in the LEV group and 97 % in the ALB group respectively. Day 22-24 the efficacy had increased to 98% in the IVM group, 98% in the LEV group

and 99 % in the ALB group. All AHs had a good effect. LEV had lower activity, based on the confidence interval day 10, which was only 87%. On the other hand, at days 22-24 the lower confidence interval was 94 %, so there was no indication of resistance (Fig. 4). When the day 0 EPG values from each treatment group were used as the control instead of th values from the untreated group, the efficacy did not differ. All values were the same except for ALB day 10 where the efficacy was 98% (day 0 as control) instead of 97% (CONT as control). The upper and lower confidence intervals were also almost the same (Figures 4 and 5, Tables 6 and 7).

Table 6. Efficacy of the anthelmintics. Percent reduction in faecal egg count, compared to the control group, in the treatment groups ivermectin (IVM) (n=27), levamisole (LEV) (n=30) and albendazole (ALB) (n=31) day 10 and 22-24 post treatment. All 5 breeds and crosses are included in each treatment group. The upper (UC) and the lower (LC) 95 % confidence interval is shown

	Treatment	Efficacy	LC	UC
Day 10	IVM	97 %	93 %	99 %
	LEV	96 %	87 %	99 %
	ALB	97 %	95 %	99 %
Day 22-24	IVM	98 %	95 %	99 %
	LEV	98 %	94 %	99 %
	ALB	99 %	98 %	100 %

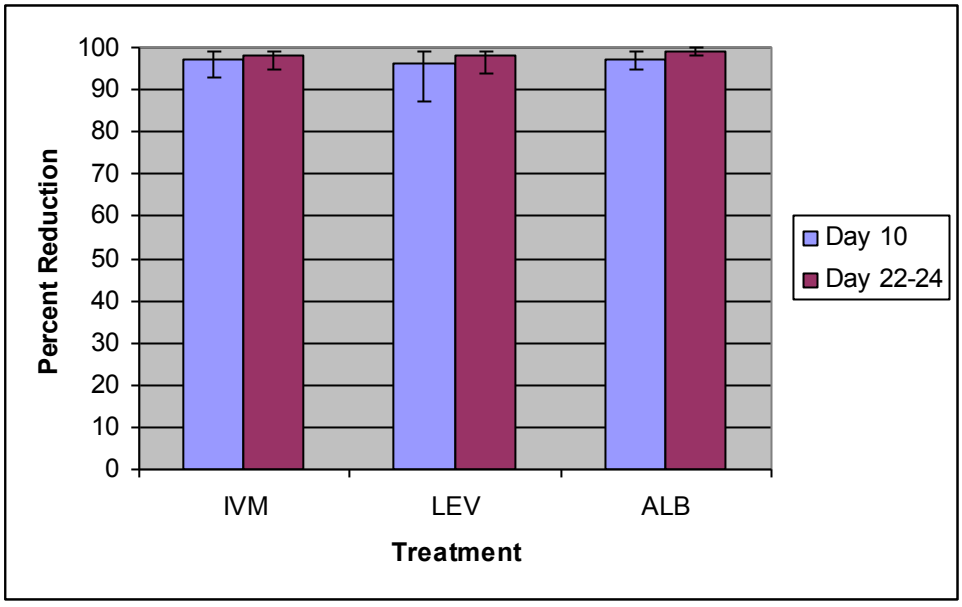

Figure 4. Efficacy of the anthelmintics. Percent reduction in faecal egg count, compared to the control group, in the treatment groups ivermectin (IVM) (n=27), levamisole (LEV) (n=30) and albendazole (ALB) (n=31) day 10 and 22-24 post treatment. All 5 breeds and crosses are included in each treatment group. Error bars show the 95% confidential intervals.

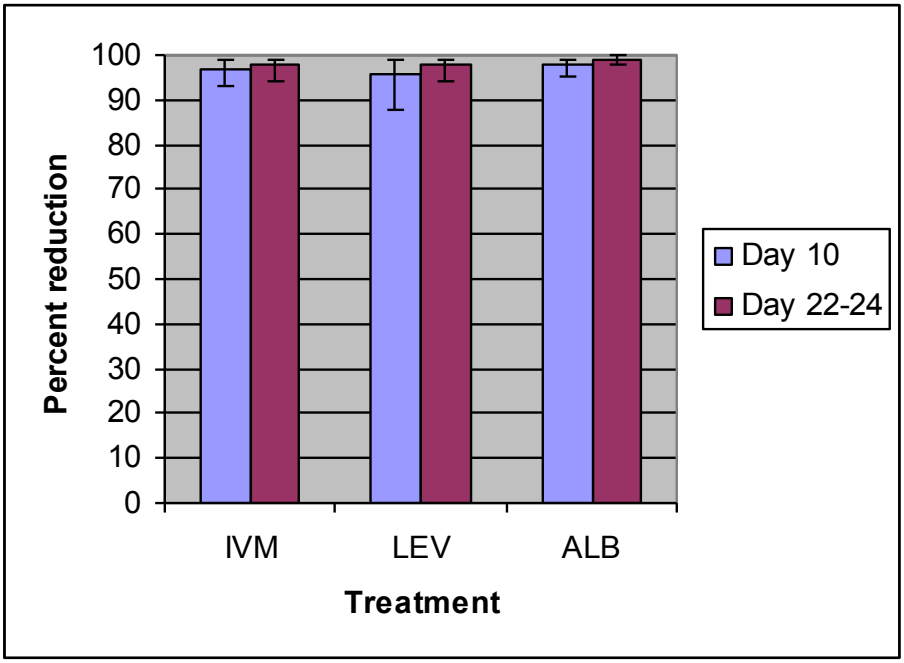

Figure 5. Efficacy of the anthelmintics. Percent reduction in faecal egg count, compared to the day 0 values, in the treatment groups ivermectin (IVM) (n=27), levamisole (LEV) (n=30) and albendazole (ALB) (n=31) day 10 and 22-24 post treatment. All 5 breeds and crosses are included in each treatment group. Error bars show the 95% confidential intervals.

Table 7. Efficacy of the anthelmintics. Percent reduction in faecal egg count, compared to the day 0 values, in the treatment groups ivermectin (IVM) (n=27), levamisole (LEV) (n=30) and albendazole (ALB) (n=31) day 10 and 22-24 post treatment. All 5 breeds and crosses are included in each treatment group. The upper (UC) and the lower (LC) 95 % confidence interval is shown

	Treatment	Efficacy	LC	UC
Day 10	IVM	97 %	93 %	99 %
	LEV	96 %	88 %	99 %
	ALB	98 %	95 %	99 %
Day 22-24	IVM	98 %	94 %	99 %
	LEV	98 %	94 %	99 %
	ALB	99 %	98 %	100 %

The EPGs per treatment are shown in Fig. 6 and in Table 8. Notable is the increasing numbers of eggs in the control sheep while there are very few eggs in the treated sheep at day 10 and days 22-24. This can also be seen in Fig.7, where the EPG per breed is shown and in Fig. 8, which shows the increasing EPG in the control group.

The RM sheep had as expected a lower EPG level than the other breeds on day 0. The D breed had more than double the number of EPG compared to the RM sheep, whereas the F1, F2 and BC had numbers in between (Fig. 7 and Table 9).

The all male control group had a markedly lower EPG value on day 0 than the treated groups, which consisted of both sexes (Fig. 6). The EPGs of the control

group increased during the time of the study indicating that all sheep increased during the course of the study (Fig. 8 and Table 10).

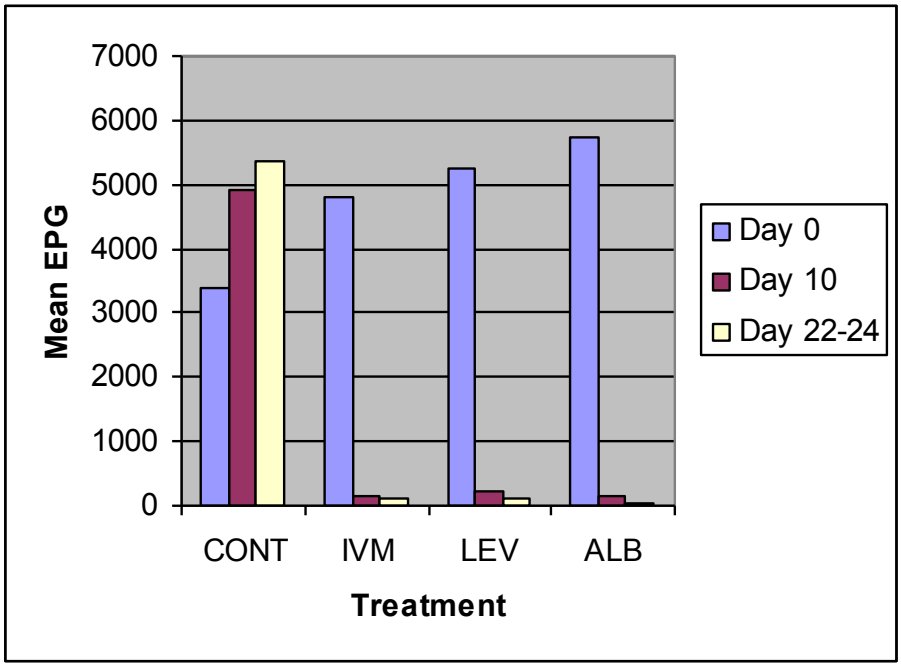

Figure 6. Arithmetic mean faecal egg count expressed as eggs per gram faeces of the sheep treated with levamisole (LEV) (n=30), albendazole (ALB) (n=31) and ivermectin (IVM) (n=27) assessed after 0, 10 and 22-24 days (called day 21 sampling). Control sheep (CONT) (n=15) were not treated.

Table 8. Arithmetic mean faecal egg count of all sheep expressed as eggs per gram faeces (Mean EPG) per treatment assessed after 0, 10 and 22-24 days and the standard deviations (SD). The treatments included in the study were ivermectin (IVM) (n=27), levamisole (LEV) (n=30) and albendazole (ALB) (n=31). There were also a non treated control group (n=15)

	Treatment	Mean EPG	SD
Day 0	CONT	3393	1912
	IVM	4785	3185
	LEV	5243	2934
	ALB	5733	4551
Day 10	CONT	4913	2660
	IVM	152	296
	LEV	213	599
	ALB	137	211
Day 22-24	CONT	5380	4486
	IVM	94	231
	LEV	110	287
	ALB	45	87

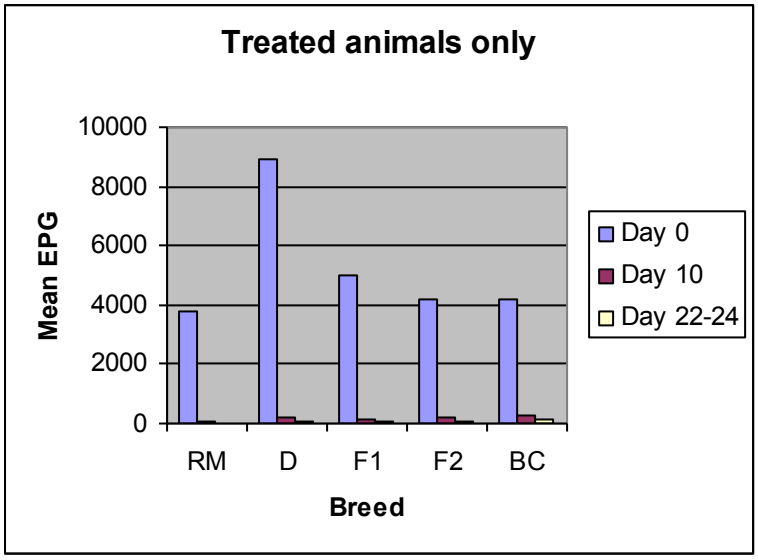

Figure 7. Arithmetic mean faecal egg count expressed as eggs per gram faeces per breed assessed after 0, 10 and 22-24 days. The diagram shows treated only. The breeds included in the study were Red Maasai Sheep (RM) (treated: n=17), Dorper (D) (treated: n=18) the first generation of their offspring (F1) (treated: n=19), the offspring of two F1s (F2) (treated: n=18) and Back crosses (BC) (treated: n=16) that were 75 % Dorper.

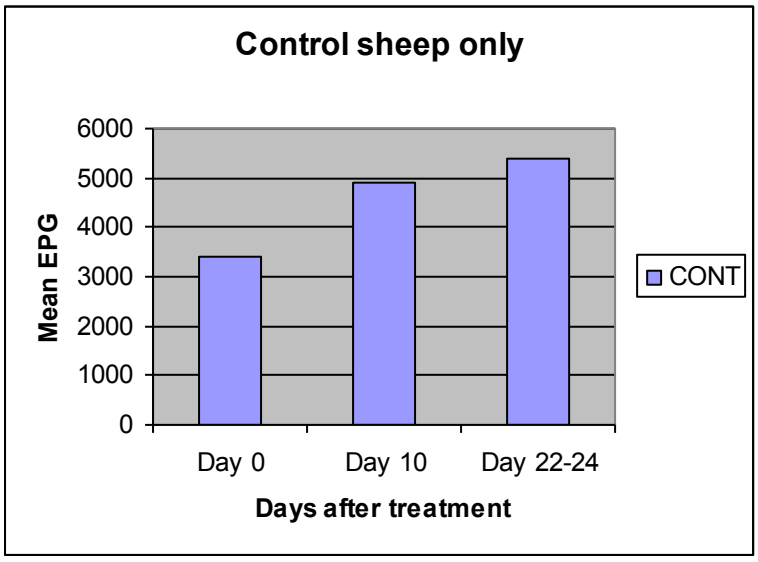

Figure 8. Arithmetic mean faecal egg count expressed as eggs per gram faeces per breed assessed after 0, 10 and 22-24 days. The diagram shows control animals only. The breeds included in the study were Red Maasai Sheep (RM), Dorper (D), the first generation of their offspring (F1), the offspring of two F1s (F2) and Back crosses (BC) that were 75 % Dorper. The control sheep were 3 sheep from each breed or cross (n=15).

Table 9. Arithmetic mean faecal egg count of the treated sheep expressed as eggs per gram faeces (Treat Mean EPG) per breed assessed after 0, 10 and 22-24 days and the standard deviations (Treat SD). The breeds included in the study were Red Maasai Sheep (RM) (treated: n=17), Dorper (D) (treated: n=18) the first generation of their offspring (F1) (treated: n=19), the offspring of two F1s (F2) (treated: n=18) and Back crosses (BC) (treated: n=16) that were 75 % Dorper)

	Breed	Treat Mean EPG	Treat SD
Day 0	RM	3753	2220
	D	8931	4735
	F1	5005	3064
	F2	4175	2246
	BC	4177	2500
Day 10	RM	79	132
	D	222	319
	F1	108	153
	F2	172	282
	BC	266	813
Day 22-24	RM	18	50
	D	81	173
	F1	68	195
	F2	92	152
	BC	167	403

Table 10. Arithmetic mean faecal egg count of the control sheep (Cont Mean EGP) expressed as eggs per gram faeces per breed assessed after 0, 10 and 22-24 days and the standard deviations (Cont SD). The breeds included in the study were Red Maasai Sheep (RM) Dorper (D), the first generation of their offspring (F1), the offspring of two F1s (F2) and Back crosses (BC) that were 75 % Dorper. The control sheep were 3 sheep from each breed or cross (n=15)

	Cont Mean EPG	Cont SD
Day 0	3393	35
Day 10	4913	4101
Day 22-24	5380	742

DISCUSSION

The faecal egg count reduction test (FECRT), is the clinically most commonly used diagnostic method today to analyse the level of anthelmintic resistance (AR) in nematode parasites of grazing livestock (Coles et al., 1992; Coles et al., 2006; Vatta & Lindberg, 2006). There are therefore a great number of studies to compare the received results to. Most of the FECRT studies in Africa have been carried out in Kenya and South Africa (Waller, 1997). The breeds in this study, the Red Maasai (RM) sheep and the Dorper (D) sheep, have also been investigated in a number of previous studies (Mugambi et al., 1997; Mugambi et al 1996; Wanyangu et al., 1997; Waruiru et al., 1998).

The fact that there was no resistance in this trial is a bit surprising since, according to Githiori[3] [4], resistance has been seen, about two years previously, in the same herd, both to albendazole (ALB) and most likely also to levamisole (LEV). That study had been conducted during the rainy period, so it was not likely that the larva were in hypobiosis.[5] The term "reversion" is used in the literature to describe when a parasite which is resistant against a specific dewormer, becomes more susceptible to that drug again. According to Leathwick et al. (2001) there are two processes that can lead to a reversion: a. when resistant genotypes are less fit to survive than the non resistant, or: b.when parasites are exposed to "counter-selection" by drugs from other classes of anthelmintics. i.e. the selection pressure is changed and strains that were resistant to the first anthelmintic are reduced in the population.

Most studies on the trend of reversion have been done on benzimidazole (BZ) resistant strains of *H. contortus, Teladorsagia (Ostertagia) cicumcinta* and *Trichostrongylus colubriformis.* In some cases there had been a level of reversion, but as soon as BZs had been used again, the level of resistance had increased and returned to previous status (Leathwick et al., 2001). Reversion seems to be rare also when it comes to LEV or the MLs and when it does occur it is only temporary, i.e. when the anthelmintic that previously caused resistance is reintroduced the resistance reappears rapidly. Full susceptibility hardly ever reoccurs (Leathwick et al., 2001; van Wyk et al., 1997). It is therefore likely, that the resistance against BZ and LEV will reoccur in the herd in this study when the sheep are treated with those substances again.

It is not known weather new sheep with susceptible nematodes have been introduced on the farm after the previous study in 2007, when AR was detected. The current anthelmintic (AH) treatment regimes on the farm are unknown, and it could not be confirmed wether the deworming regime had been dramatically changed since the previous study.

Further tests could be done on the same farm to detect to what extent the resistance reappears. FECRT is a suitable method for such studies. Pyrosequencing analyses can also be done to investigate if the beta-tubulin genes coding for BZ resistance are present in the herd. If such genes are present, it is only a question of time until there will be a failure of treatment because of resistance. A pyrosequensing analysis is a new method, up to now only used in research situations. The usefulness of the method in the Kenyan environment remains to be verified.

The RM had much lower eggs per gram faeces (EPG) values than the D. On day 0 the D had more than double level of EPG than the RM (Fig. 7). It has been shown in a number of previous studies, that the RM is less susceptible to *H. contortus* than other breeds, for example D, Blackheaded Somali and Romney Marsh (Mugambi et al., 1996; Mugambi et al. 1997; Wanyangu et al., 1997). RM sheep

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[3] John Githiori, International Livestock research institute (ILRI), Nairobi, Kenya, e-mail 2009-12-10

[4] John Githiori, e-mail 2011-10-12

[5] John Githiori, e-mail 2011-10-12

do not require as frequent treatment as the D sheep. The selection towards AR in the *H. contortus* can be retarded if the RM sheep are being bred instead of D sheep.

The first (F1) and second (F2) generations of offspring of RM sheep and D sheep had mean EPG values of 5005 and 4175 EPG respectively. That was only slightly above the value of the RM sheep (3753 EPG) on day 0. Also the BC that were 75 % D had an EGP level of 4177, which were not much higher than the level of the RM sheep. The D sheep had as high mean EPG level as 8931 on day 0. This means that there might be advantages to cross the RM sheep with the D sheep, which in the semi-arid areas of Kenya has a higher productivity (Okeyo & Baker, 2006; Cloete et al., 2000). In the humid and sub-humid parts of Kenya on the other hand, the RM sheep has a much higher productivity than the D sheep (Baker et al., 2003). Even though the sheep, in particular D sheep, had high EPG levels on day 0, their health condition was generally good.

All control sheep in this study were males and they had on an average a lower EPG value on the day of treatment than the treated animals, which were of both sexes, so the trustability of these values could therefore be questioned. On the other hand, when the resistance status of all treated animals also were checked with their own day 0 count as a control, those values did not differ much from the corresponding values received when compared to the separate control sheep. Our results can therefore be considered reliable.

The fact that the male control sheep had a lower EPG value than the treated animals of both sexes on day 0 was surprising. In some previous studies males have been shown to be more susceptible to infection with *H. contortus,* but in others the gender of the host did not affect the level of infection. Those divergences might depend on the various breeds used in the different studies (Gauly et al., 2006). Androgens reduces immunocompetence and the sex steriod hormones affects sexual behavior and disease resistance genes so that males of many species are more susceptible than females to different types of infections caused by bacteria, viruses, parasites and fungi (Klein, 2000).

The EPG of the control sheep increased during the study as expected, since it was in the beginning of the long rains, when there usually is a rise in the EPG levels (Nginyi et al., 2001). There were very few larvae from the treated groups on day 10 post treatment. In 7 of the 15 treated groups there were either no or so few (less than 10) larvae, so no proper differential count could be performed. In the larval differential count from day 0, the numbers of counted larvae were 50 in all of the groups. The day 0 count results are therefore much more reliable and they confirmed that the majority of the larvae in the herd were *H. contortus*.

The day 22-24 results were sampled and analyzed by other co-workers than the day 0 and day 10, and unfortunately the values from day 22-24 were not validated towards the originals. Still, larvae were not grown from day 22-24 samples, and therefore the percentage *H. contortus* in these samples remained unknown. No resistance was though seen in the day 10 results and the reduction was good, so there is no reason to question the results of the whole study.

CONCLUSION

The majority of larvae in this herd were *H. contortus*. There was no resistance to BZ, ML or LEV, which were the AHs tested in this study, even though resistance towards ALB and most likely towards LEV had been seen about two years previously. The reason for the reversion that has occurred is unclear but is most likely temporary and will reoccur if the sheep if further treated with BZ or LEV.

The all male control group had a markedly lower eggs EPG value than treated sheep of both sexes. In other studies males have had higher or the same EPG levels compared to the females.

The RM sheep, F1, F2 and BC has markedly lower EPG values than the D sheep. There might be advantages of crossing the helminth resistant RM sheep with the D sheep, which in the semi-arid areas of Kenya has a higher production level.

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