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Anthelmintic efficacy and dose determination of *Albizia anthelmintica* against gastrointestinal nematodes in naturally infected Ugandan sheep

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Abstract

Weight loss, stunted growth, and death caused by gastrointestinal parasites are major constraints to livestock productivity, especially in tropical and developing countries where regular use, and misuse, of anthelmintics has led to nematode resistance. *Albizia anthelmintica* Brong. (Fabaceae) is traditionally employed throughout East Africa to treat helminth parasitosis in livestock. Reported efficacy has varied from 90% against mixed nematodes to just 19% against *Haemonchus contortus* alone. The objective of this study was to assess the anthelmintic effect of *A. anthelmintica* against naturally occurring infections of mixed gastrointestinal parasites, and to establish an effective treatment dose, in sheep under pastoral field conditions of northern Uganda. *A. anthelmintica* bark was collected and prepared according to local custom and packed into gel capsules. Fifty-five young female local mixed-breed lambs were randomly assigned to six groups, including a positive control group that received levamisole (synthetic anthelmintic) and a negative control group that received no treatment. Following the World Association for the Advancement of Veterinary Parasitology (WAAVP) dose determination guidelines, the other four groups were treated with varying doses of *A. anthelmintica*. Statistical analyses (using generalized linear models) were performed to assess treatment effect. There was a significant treatment (group) effect on parasite egg/oocyte counts per gram (EPG) for nematodes, but not for coccidia. The most effective dose against nematodes (0.8 g, 58.7 mg/kg) closely approximates what is usually given by traditional healers, 0.9 g/adult sheep. It provided major and significant reduction in EPG as compared to the negative control. Anthelmintic efficacy was estimated using percent faecal egg count reduction (FECR). Other than the positive control, animals in the standard dose group showed the greatest decline in shedding of nematode eggs, with an FECR of 78%. This study indicates that *A. anthelmintica* holds potential as part of an integrated management plan for the control of helminths in developing countries.

Keywords: Developing countries; *Albizia anthelmintica*; Dose determination; Integrated management plan; Gastrointestinal nematodes

1. Introduction

Weight loss, stunted growth, and death caused by gastrointestinal parasites are major constraints to livestock productivity, particularly for small ruminants in developing countries (Waller et al., 1996; Perry and

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Randolph, 1999). Currently, synthetic anthelmintics are the primary means of controlling parasitic infections. However, they have several disadvantages, including lack of availability in some areas, especially in developing countries; inconsistent quality in some countries; prohibitive cost; environmental contamination; and the potential for food residues (Hammond et al., 1997; Perry and Randolph, 1999; Krecek and Waller, 2006). Furthermore, regular use, and misuse, of anthelmintics has led to nematode resistance, a problem which is most serious in sheep and goats in the tropics and developing countries (Waller et al., 1996; Coles, 2002). Routine use of anthelmintics also reduces development of natural immunity against helminths (Ketzis et al., 2006). Targeted approaches such as improved grazing management may not be feasible for pastoralists who employ communal land ownership (Githiori et al., 2003).

These problems have led to the search for alternative methods of parasite control. Methods under investigation include parasite-based vaccines, nematophagous fungi, condensed tannins, and immunonutrition (Ketzis et al., 2006). These methods do not aim for total elimination of parasites; in fact, survival of some parasites *in refugia* can be of benefit (Vercruysse and Dorny, 1999; Waller, 1999; van Wyk, 2001). The aforementioned methods may act through direct parasiticidal activity, improving the immunity of the host, or decreasing exposure to the parasite, thus allowing reduced use of synthetic anthelmintics (Vercruysse and Dorny, 1999).

One promising area of investigation is the use of plant-based anthelmintics. Ethnoveterinary knowledge and plant-based anthelmintics were the mainstays of anthelmintic treatment prior to the advent of synthetic drugs, and are still widely used in many traditional societies (McCorkle et al., 1996; Gradé et al., 2007). Potential benefits of ethnoveterinary livestock anthelmintics are clear, as the latter societies often depend on livestock, and live in areas where synthetic anthelmintics are unavailable, unaffordable, and/or of poor quality.

The wider use and development of plant-based anthelmintics are often restricted, in part, by limited knowledge of the plants' actual efficacy against specific parasites, appropriate dosages, methods of preparation and administration for different livestock species, and possible toxicity (Githiori et al., 2005). Although many plant species have been listed as having anthelmintic activity, only a few have been subjected to rigorous scientific validation (Hammond et al., 1997).

Pastoralists throughout East Africa treat helminth parasitosis in livestock with *Albizia anthelmintica* Brong. (Fabaceae), a slow growing tree whose bark has previously been reported to contain triterpenoid saponins, histamine, tannins, and other phenolic compounds (Carpani et al., 1989; Khalid et al., 1996; Johns et al., 1999). In the past 15 years, anthelmintic properties of *A. anthelmintica* have been studied in Kenya, Sudan, and Ethiopia, using a variety of host animals, target parasites, and medicinal doses and preparations (Table 1). Efficacies of 100% have been reported against the liver fluke *Fasciola gigantica* (Koko et al., 2000) and *Moniezia* spp. (Gathuma et al., 2004) in sheep and against *Hymenolepsis diminuta* in rats (Galal et al., 1991). Reports of efficacy against nematodes have varied: although two studies found reductions in faecal egg counts of 77% (Grade and Longok, 2000) and 90% (Gathuma et al., 2004), a more standardized trial found only 19–34% efficacies against *Haemonchus contortus* (Githiori et al., 2003).

The objectives of this study were therefore to assess *A. anthelmintica*'s anthelmintic effect against natural infections of mixed gastrointestinal parasites, and to investigate an effective dose, in sheep under pastoral field conditions in northern Uganda.

2. Materials and methods

World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluation of anthelmintic resistance in ruminants were used to guide animal selection, treatment procedure, faecal egg counts, and interpretation of data (Wood et al., 1995). Field trial design was adopted from techniques used at University of Ghent, Belgium (J. Vercruysse, personal communication).

2.1. Plant material

A. anthelmintica was harvested and prepared according to local custom. In May 2006, at the start of the rainy season, a traditional Karamojong veterinary healer harvested stem bark from three naturally growing trees in Pian county of Uganda's northeastern Karamoja Region. Voucher herbarium specimens (JTG-251 and JTG-252) of these trees were confirmed to be *A. anthelmintica* at Makerere University Herbarium in Kampala (Angiosperm Phylogeny Group, 2003). These vouchers are kept at Makerere herbarium and at KACHEP field herbarium in Karamoja. Bark was shade-dried, and inner bark was removed and pounded into a fine powder. Powder was stored in a polythene

Table 1
Doses used and efficacies found in previous *Albizia anthelmintica* research

Reference	Parasite	Host	Part used[a]	Prep[b]	Dose[c]	*N*	Efficacy (%)
Gathuma et al. (2004)	Nematodes	Sheep 9–10 months	Rt crushed	CE	5 g	5	89.8
	Cestodes (*Moniezia* spp.)	Sheep 9–10 months	Rt powder	HE	26.5 g	6	100
Githiori et al. (2003)	Nematodes (*Haemonchus contortus*)	Sheep 6–8 months	Bk whole	CE	25 g	5	19
					50 g	5	19
					100 g	5	19
		Sheep 6–8 months	Bk whole	HE	25 g	5	28
					50 g	5	28
					100 g	5	28
		Sheep 9 months	Bk powder	CE	25 g	5	34
					50 g	5	34
					100 g	5	34
Koko et al. (2000)	Trematodes (*Fasciola gigantica*)	Goats 5 months	Bk powder	CE	9 g/kg[e]	3	95.5
Grade and Longok (2000)	GI count[e]	Rumnt[f]	Bk powder	CE	2 spoons	8	77
Desta (1995)	Cestodes	People	Bk powder	N	21.4 g	6	50[g]
Galal et al. (1991)	Cestodes (*Hymenolepsis diminuta*)	Rats	Bk	SE/BF	75 g/kg[d]	5	0
					150 g/kg[d]	5	0
					300 g/kg[d]	5	0
					450 g/k[d]	5	0
		Rats	Bk	SE/BFR	15 ml[c]	5	0
		Rats	Bk	SE/AF	30 g/kg[d]	5	68
					75 g/kg[c]	5	68
		Rats	Bk	SE/AF	150 g/kg[d]	5	100
					300 g/kg[d]	5	100
					450 g/kg[d]	5	100
		Rats	Bk	SE/PF	450 g/kg[d]	5	0

[a] Part used in the preparation, Bk: stem bark, Rt: root bark.
[b] Prep: method of preparation. CE: cold extraction, HE: heat extraction, SE: soxhlet extraction, BF: butanolic fraction, BFR: butanolic fraction residue, AF: aqueous fraction, PF: polar fraction, N: no extraction; ground bark powder was mixed with honey.
[c] Unless otherwise stated, dose was given once orally.
[d] One-third of this dose was given three consecutive days.
[e] Types of eggs were not differentiated.
[f] Rumnt: cattle, sheep, and goats.
[g] The median effective single dose, defined as the dose that expels the parasite, partially or totally, in 50% of infested subjects.

bag at room temperature for 2 weeks, then uniformly packed by hand into gelatine capsules.

2.2. Animals

Fifty-five female local mixed-breed lambs aged 3–6 months, predominantly variants of East African Black-headed Persian crosses or Karimojong sheep (T. Loquang, personal communication), and one nursing ewe were purchased from three different Karamojong counties (Pian, Bokora, and Matheniko). They were treated for external parasites topically with a synthetic pyrethroid (Protaid®), injected with 2 cm^3 of multi-vitamin solution (Coopers®) subcutaneously, weighed, given a complete physical exam, and treated for any conditions not related to parasitosis. They were weighed again at 35 days post-treatment. The flock was housed in a fenced area with free access to a commercial salt lick for ruminants. They were observed while grazing on local pasture for 8 h daily, commingling with one another and other animals throughout the study. Free-choice water for 1 h was provided daily. The milking ewe was restrained to supplement the diet of at least three younger lambs.

2.3. Treatment

The local standard *A. anthelmintica* dose used by several Bokora and Pian traditional healers for deworming sheep had previously been averaged and

Table 2
Treatments received and initial weight at day 0 by group

Group	Average live weight (kg)	Treatment received	Amount given (g)	Average dose (mg/kg)
A	9.31	0.5 standard dose of *A. anthelmintica*	0.4	32.5
B	10.13	1× standard dose of *A. anthelmintica*	0.8	58.7
C	9.35	2× standard dose of *A. anthelmintica*	1.8	135.5
D	9.00	5× standard dose of *A. anthelmintica*	4.7	358.5
E	8.97	Levamisole (Wormicid®) at labeled dose	0.185	14.11
F	8.72	None		

determined to be 0.9 g/sheep (Gradé et al., in press). Following WAAVP guidelines for dose determination studies (Wood et al., 1995), it was decided to give treatment groups approximately one-half, one, two and five times this standard dose (Table 2).

Baseline eggs/oocysts per gram (EPG) of faeces were determined using the modified McMaster technique, with a sensitivity of 50 EPG (Coles et al., 1992). Coccidia oocysts were not sporulated. However, coproculture samples were taken on day 0 for larval cross-section of nematodes. *Haemonchus* was the dominant larval species, although *Trichostrongylus*, *Nematodirus*, and *Ostertagia* were also seen. Lambs were stratified first by nematode EPG, then county of origin, coccidian EPG, and body weight. They were then randomly assigned to six groups ($n = 9$, except for group C, $n = 10$). Small, medium, and large gel capsules held 0.4 g, 0.7 g, and 1.1 g of *A. anthelmintica* bark powder, respectively. Therefore, lambs in group A received 0.4 g (one small capsule), those in group B received 0.8 g (two small capsules), those in group C received 1.8 g (one medium and one large capsule), and those in group D received 4.7 g (two medium and three large capsules). Animals in group E, the positive control, received the synthetic anthelmintic levamisole (Wormicid®) at the labelled dose (185 mg tablet for <20 kg). Group F was a negative control and animals received no treatment (Table 2).

Gelatine capsules and levamisole tablets were given orally in the morning at the base of the tongue. Pilling was followed with a small amount of water to facilitate swallowing.

2.4. Faecal egg counts

EPG determination was carried out twice before dosing, then every 7 days for 35 days. All sheep had total EPG over 2500 and nematode EPG over 800 prior to treatment. At least 3 g of faeces were collected directly from the rectum and put into clean, labelled containers for each sheep. Faeces were stored at 4 °C for up to 8 h while awaiting analysis. EPG was determined by the modified McMaster technique (Coles et al., 1992), differentiating between nematode eggs and coccidian oocysts. The first two authors independently determined EPG of each sample, for duplication. Faeces were maintained until results were compared and any discrepancies resolved.

2.5. Determination of anthelmintic efficacy

Anthelmintic efficacy was estimated calculating the percent faecal egg count reduction (FECR), according to the method described by Dash et al. (1988):

$$\mathrm{FECR} = \left(1 - \left(\frac{T_n}{T_0} \times \frac{C_0}{C_n}\right)\right) \times 100 \quad (1)$$

where T and C represent the arithmetic means of the EPG in treatment and negative control groups, and subscripts 0 and n denote counts before and after treatment, respectively.

2.6. Statistical analysis

Generalized linear models (GENMOD procedure in SAS version 9; SAS, 2003), which allow fitting various model types depending on the distribution of the response variable, were used to assess the treatment effect. In this procedure, fitting a normal distribution is equivalent to analysis of variance, while fitting a Poisson distribution corresponds to Poisson regression modelling. Response variables included EPG count on day 14 (hereafter ''EPG count'') as well as change in EPG between day 0 and day 14 (hereafter ''change in EPG'') for nematodes and coccidia. A logarithmic transformation for EPG count data of nematode ($\log_{10} + 3$) and coccidia ($\log_{10}$) fulfilled required assumptions for analysis of variance (i.e. GENMOD with a normal error distribution). The variable ''change in EPG'' data did not require transformation in order to meet the underlying assumptions of an ANOVA. The treatment or group was the only predictor variable and was entered in our models as a categorical variable with

six levels, as described above (see Treatment section, Table 2). When a significant group effect was detected, multiple comparisons were performed to determine which group(s) differed. Dunnett's test was used to compare means within each group to the negative control, as it is more powerful than other multiple comparison tests in this situation (Quinn and Keough, 2002). Means are however reported on an untransformed scale (±standard deviation). We used ANOVA to compare change in weight (weight gain) between groups, measured by the difference between weights on day 0 and day 35. All analyses were performed in SAS version 9 (SAS, 2003) and a significance level of 5% was adopted.

3. Results

3.1. Treatment effects

Using the generalized linear model, we found a significant treatment (group) effect for nematode EPG count (ANOVA model, $F_{5,49} = 27.43$, $P < 0.001$, $r^2 = 0.74$). Two groups, *A. anthelmintica* at a dose of 4.7 g (group B) (2313.89 ± 1859.15) and the levamisole group (group E) (38.89 ± 53.20), significantly differed from the negative control group (group F) (9930.56 ± 5385.00) (Dunnett's test, $P < 0.05$). We also found a group effect ($F_{5,49} = 3.40$, $P = 0.0102$; $r^2 = 0.26$) for change in EPG for nematodes. The major and only difference found using multiple comparisons was between animals receiving the standard plant dose, group B, and the negative control group, F (Dunnett's test, $P < 0.05$; Table 3). The difference between animals given levamisole (group E) and the negative control group also approached significance (Dunnett's test, $P = 0.055$; Table 3). There were no group effects on EPG count or on the change in EPG for coccidia (ANOVA, all $P > 0.05$).

Table 3
Group comparison for nematode change EPG count from day 0 to day 14

Group[a] comparison	Difference between means	Simultaneous 95% confidence limits	
A to F	1628	−5609	8,864
B to F	**9853**	**2616**	**17,089**
C to F	4138	−2916	11,191
D to F	6006	−1231	13,242
E to F*	7133	−103	14,370

Significant differences are shown in bold, differences based on Dunnett's test. *This group comparison is 'nearly significant' if $P < 0.06$.

[a] Group A received 0.4 g *A. anthelmintica*, group B received 0.8 g *A. anthelmintica*, group C received 1.8 g *A. anthelmintica*, group D received 4.7 g *A. anthelmintica*, group E (positive control) received levamisole at the labelled dose, and group F (negative control) received no treatment.

3.2. Anthelmintic efficacy

For nematodes, levamisole at the recommended dosage resulted in 99.2% FECR (see Eq. (1)), whereas for *A. anthelmintica* at the recommended dose of 0.8 g and the 4.7 g dose resulted in FECRs of 78.3% and 66.5%, respectively (Table 4). All groups had positive FECRs compared to the negative control after day 7. For coccidia, maximum efficacies observed were 90.5% for *A. anthelmintica* at 4.7 g, and 82.3% for group receiving *A. anthelmintica* at 0.4 g. All other groups had negative FECRs as compared to the negative control.

3.3. Change in live body weight

As seen in Table 5, on average lambs in the negative control group and the group receiving less than the healers' recommended dose of *A. anthlemintica* (group A) gained less than half as much weight as animals in the other groups. Weight gain was significant ($P = 0.0001$) within the sample as a whole, with a mean gain of 0.96 kg/animal. However, differences between groups were not statistically significant ($P > 0.05$).

4. Discussion

The results of our study indicate that *A. anthelmintica* has activity against gastrointestinal nematodes in

Table 4
Nematode EPG count (mean ± S.D.) and corresponding % FECR for each treatment group

Group	EPG pre-treatment	EPG post-treatment	FECR (%)
A	4547 ± 5754	7547 ± 5821	11.4
B	8794 ± 8844	3569 ± 2798	78.3
C	4770 ± 1977	5260 ± 4543	41.1
D	3689 ± 4881	2314 ± 1859	66.5
E	2544 ± 1685	38.9 ± 53.2	99.2
F	5303 ± 3302	9931 ± 5385	

Group A received 0.4 g *A. anthelmintica*, group B received 0.8 g *A. anthelmintica*, group C received 1.8 g *A. anthelmintica*, group D received 4.7 g *A. anthelmintica*, group E (positive control) received levamisole at the labelled dose, and group F (negative control) received no treatment. Percent reduction in fecal egg count, Eq. (1). A more positive FECR (up to a maximum of 100%) is associated with more effective treatment.

Table 5
Mean change in weight by treatment group

Treatment group	Mean change (kg) (S.D.)	Weight gain (kg) (min – max)		Inter-quartile range (kg) Q1–Q3
A	0.4 (1.5)	−2.5	+2.5	0.0–1.0
B	1.1 (1.3)	−5.0	+3.5	0.5–1.5
C	1.4 (2.1)	−2.5	+4.6	0.6–2.4
D	0.9 (2.0)	−1.5	+5.0	0.1–1.3
E	1.8 (1.4)	0.0	+4.0	0.9–2.4
F	0.4 (1.4)	−2.0	+2.0	−0.5–1.5

naturally infected sheep. At of 0.8 g/sheep (58.7 mg/kg), there was a significant reduction of EPG as compared to the negative control group, and as well as an efficacy level greater than 78%. This is somewhat lower than the 90% efficacy reported by Gathuma et al. (2004), but notably higher than the 19–34% found by Githiori et al. (2003). One possible explanation is the strength of medicine received by treated animals. Although Gathuma et al. (2004) and Githiori et al. (2003) both used much higher dosages than were used in this study (see Table 1), the parasiticidal element(s) of *A. anthelmintica* have not been isolated, and it is not possible to determine its concentration in any given formulation or if the active ingredient is present in the administered portion. There are many reasons why anthelmintic strength or activity may not be directly proportional to dose given, including region, season, plant part, preparation and extraction method. Githiori et al. (2003) found differences in efficacy between preparations of *A. anthelmintica* from different areas of Kenya. It has been proposed that a harsh and arid environment like that of Karamoja may be associated with more concentrated plant products, which may lead to more potent medicinal properties (Körner, 1999). Similarly, as *A. anthelmintica* is deciduous, seasonal variations in physiological processes may alter the composition or concentration of chemicals within the bark (Scogings et al., 2004). In this study, bark was collected at the beginning of the wet season, which may correspond with peak concentrations of certain secondary metabolites that are expressed more in during growth spurts. Çirak et al. (2007a,b, 2008) found variations in the concentrations of several bioactive substances in *Hypericum* species at different stages of plant growth and in different plant parts. Plant shoots were found to have the highest concentration of during growth phases, as opposed to the vegetative or dormant dry season. Similarly, woody species of semi-arid savannas in southern Africa had elevated phenols during the growth season (Scogings et al., 2004). Unfortunately, other *A. anthelmintica* studies have not noted the season or vegetative state the tree was in at bark collection, making comparisons difficult (Desta, 1995; Galal et al., 1991; Gathuma et al., 2004; Githiori, 2004; Githiori, 2004; Githiori et al., 2003; Grade and Longok, 2000; Koko et al., 2000).

Differences in methods of preparation may also have affected *A. anthelmintica* efficacy. Our study used shade-dried, powdered bark administered directly inside gel capsules, while Gathuma et al. (2004) used cold aqueous extractions and Githiori et al. (2003) used both cold and hot aqueous extractions. Additionally, Githiori et al., 2003 used whole, uncrushed bark for the extractions in two out of three trials (Table 1), rather than the powdered bark that healers commonly use in the above areas. Both Githiori et al. (2003) and Galal et al. (1991) found differences in efficacy dependent on method of preparation. Interestingly, neither study found a dose-related effect, indicating that method of preparation may be of paramount importance (see Table 1). It is possible the whole bark material administered in this study led to a stronger effect. Teichler, while at the Andalusia internment camp in South Africa, obtained better anthelmintic results from powdered bark than from a decoction (Teichler 1954, in Watt and Breyer-Brandwijk, 1962). However, no quantitative studies have been done to determine if administering aqueous extraction filtrate vs. whole plant material administration increases or reduces *A. anthelmintica*'s anthelmintic effect.

Susceptibility of parasites may also vary, according to strain, geography, or previous exposure to anthelmintics. Githiori et al. (2003) used an artificially induced infection with a single strain of *Haemonchus contortus*. The strain of *H. contortus* used in that study, however, may have been more resistant to the active element of *A. anthelmintica* than the strains present in our study's naturally occurring mixed nematode infection, even though it was primarily consisting of *Haemonchus* spp.

The FECR of 78.3% found in this study does not meet the proposed minimum standard of 90% for development of a new anthelmintic (Vercruysse et al., 2001) or even WAAVP's standard of 80% that indicates ''moderate'' efficacy (Wood et al., 1995). However, these standards were set for industrial development of pharmaceuticals and have been suggested to be too high for ethnoveterinary medicines and other novel approaches (Githiori et al., 2005; Ketzis et al., 2006). Githiori et al. (2005) proposed a standard of 70% for ethnoveterinary medications, as was used in some of their previous studies (Githiori et al., 2003; Githiori,

2004). This is in part because rather than seeking to eliminate parasitosis, novel control methods aim to keep infection levels below economic threshold, defined as the maximum level of infection that can be tolerated without causing production loss (Ketzis et al., 2006). Estimating a specific economic threshold is a complex task. This involves optimizing parameters such as decreased feed efficiency, time and financial costs of medication, and effects on local market prices, which is beyond the scope of this paper (for more discussion, see Perry and Randolph, 1999). Based on this 70% standard, *A. anthelmintica* is an effective treatment. No statistical difference between groups was observed for weight change, but the negative control tended to gain less weight, implying that the differences in worm-load had little short term affect on weight gain, the measure of production.

The second objective of this study was to evaluate the appropriate *A. anthelmintica* dose for sheep in our study area, as a follow-up to our preliminary findings (Gradé et al., 2007). The most effective dose against nematodes, 0.8 g/lamb or 58.7 mg/kg, closely approximates what Karamojong traditional healers give adult sheep (0.9 g/animal). This treatment group had the highest FECR, 78.3%, other than the positive control that received levamisole (group E). Moreover, it was the only group with a significantly greater change in EPG as compared to the negative control, group F (see Tables 3 and 4); however, we have to bear in mind that this treatment group did have an elevated initial worm load as estimated by EPG compared to other groups (Table 4) despite our effort to stratify the groups according to baseline EPG counts. So, although the healers' standard dose did not provide the highest efficacy, it did provide the highest absolute or raw reduction in infection load. This is most likely one reason why this dose is used by the local healers. Furthermore, as nematodes are by far the most clinically important gastrointestinal group of parasites in small ruminants in the tropics, it is not surprising that local healers have settled on using the dose most effective against them.

The dose we arrived at is markedly less than has been used in other studies. For example, Githiori et al. (2003) used 50 g/sheep, Gathuma et al. (2004) used 26.5 g/sheep, and Koko et al. (2000) used 9 g/kg for goats (3 g/kg for three consecutive days). All authors chose doses based on traditional healers' recommendations in their respective research settings. The vast discrepancies in doses may reflect geographic differences in *A. anthelmintica* potency, or may be cultural or traditional. In any case if, as this study suggests, some forms of *A. anthelmintica* are effective at this dose, there are clear benefits as to the amount of work and availability of bark required to treat a flock, and to sustainability and conservation in harvesting.

Given reports of anthelmintic activity in the plant's leaves and trunk bark (Desta, 1995), it is warranted to assess the anthelmintic activity of leaves, twigs, and other aerial parts that would allow for less destructive harvesting (Zschocke et al., 2000). Over-harvesting of bark could lead to the death of this slow growing tree and, if used routinely on a flock-wide basis, local supplies could be quickly depleted.

5. Conclusions

Although other studies have found *A. anthelmintica* to have less activity, this study indicates administration of *A. anthelmintica* holds potential as an effective treatment for nematode parasitosis in sheep. The best dose for mixed nematode infection was found to closely approximate what is usually given by traditional healers, 58.7 mg/kg. Plant-based treatments, such as *A. anthelmintica*, could be made of part of an integrated management plan for control of helminths in developing countries. Not only does our study provide evidence for the relevance of the traditional healing system, it also calls for a need to ensure long lasting availability for the species used to treat promote and maintain the health of livestock in the tropics.

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References

Angiosperm Phylogeny Group, 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141, 399–436.

Carpani, G., Orsini, F., Sisti, M., Verotta, L., 1989. Saponins from *Albizzia anthelmintica*. Phytochemistry 28, 863–866.

Çirak, C., Radušiené, J., Ivanauskas, L., Janulis, V., 2007a. Variation of bioactive secondary metabolites in *Hypericum origanifolium* during its phenological cycle. Acta Physiol. Plant. 29, 197–203.

Çirak, C., Radušiené, J., Janulis, V., Ivanauskas, L., 2007b. Secondary metabolites in *Hypericum perfoliatum*: variation among plant parts and phenological stages. Bot. Helv. 117, 29–36.

Çirak, C., Radušiené, J., Arslan, B., 2008. Variation of bioactive substances in *Hypericum montbretii* during plant growth. Nat. Prod. Res. 22, 246–252.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Coles, G.C., 2002. Sustainable use of anthelmintics in grazing animals. Vet. Rec. 151, 165–169.

Dash, K., Hall, K., Barger, I.A., 1988. The role of arithmetic and geometric worm egg counts in faecal egg count reduction test and in monitoring strategic drenching programs in sheep. Aust. Vet. J. 65, 66–68.

Desta, B., 1995. Ethiopian traditional herbal drugs. Part I. Studies on the toxicity and therapeutic activity of local taenicidal medications. J. Ethnopharmacol. 45, 27–33.

Galal, M., Bashir, A.K., Salih, A.M., Adam, S.E.I., 1991. Efficacy of aqueous and butanolic fractions of *Albizzia anthelmintica* against experimental *Hymenolepis diminuta* infestation in rats. Vet. Hum. Toxicol. 3 (6), 537–539.

Gathuma, J.M., Mbaria, J.M., Wanyama, J., Kaburia, H.F.A., Mpoke, L., Mwangi, J.N., Samburu and Turkana healers, 2004. Efficacy of *Myrsine africana, Albizia anthelmintica* and *Hilderbrantia sepalosa* herbal remedies against mixed natural sheep helminthosis in Samburu district, Kenya. J. Ethnopharmacol. 91, 7–12.

Githiori, J.B., 2004. Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and small holder farmers in Kenya. PhD. Department of Biomedical Sciences and Veterinary Public Health, Division of Parasitology and Virology, Swedish University of Agricultural Sciences; and International Livestock Research Institute (ILRI), 72 pp.

Githiori, J.B., Höglund, J., Waller, P.J., Baker, R.L., 2003. The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice. Vet. Parasitol. 116, 23–34.

Githiori, J.B., Höglund, J., Waller, P.J., 2005. Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. Anim. Health Res. Rev. 6 (1), 91–103.

Grade, J.T., Longok, A., 2000. Karamojong scientists: participatory field trial of a local dewormer. In: Proceedings of the Uganda Veterinary Association Scientific Conference, September 28–29.

Gradé, J.T., Tabuti, J.R.S., Van Damme P., in press. Four footed pharmacists: indications of self-medicating livestock in Karamoja, Uganda. Econ. Bot. DOI:10.1007/s12231-008-9032-9.

Gradé, J.T., Tabuti, J.R.S., Van Damme, P., Arble, B.L., 2007. Deworming efficacy of *Albizia anthelmintica* in Uganda: preliminary findings. Afr. J. Ecol. 45 (3), 18–20.

Hammond, J.A., Fielding, D., Bishop, S.C., 1997. Prospects for plant anthelmintics in tropical veterinary medicine. Vet. Res. Commun. 21, 213–228.

Johns, T., Mahunnah, R.L.A., Sanaya, P., Chapman, L., Ticktin, T., 1999. Saponins and phenolic content in plant dietary additives of a traditional subsistence community, the Batemi of Ngorongoro District, Tanzania. J. Ethnopharmacol. 66, 1–10.

Ketzis, J.K., Vercruysse, J., Stromberg, B.E., Larsen, M., Athanasiadou, S., Houdijk, G.M., 2006. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. Vet. Parasitol. 139, 321–335.

Khalid, H.S., Bashir, A.K., Mohmed, A.H., Alil, M.B., 1996. Histamine-like activity of *Albizia anthelmintica*. Pharm. Biol. 34 (3), 226–228 (3).

Koko, W.S., Galal, M., Khalid, H.S., 2000. Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazole. J. Ethnopharmacol. 71, 247–252.

Körner, C., 1999. Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems. Springer, Berlin, pp. 338.

Krecek, R.C., Waller, P.J., 2006. Towards the implementation of the "basket of options" approach to helminth parasite control of livestock: emphasis on the tropics/subtropics. Vet. Parasitol. 139, 270–282.

McCorkle, C.M., Mathias, E., Schillhorn van Veen, T.W. (Eds.), 1996. Ethnoveterinary Research and Development. Intermediate Technology Publications, London, pp. 339.

Perry, B.D., Randolph, T.F., 1999. Improving the assessment of the economic impact of parasitic disease and of their control in production animals. Vet. Parasitol. 84, 145–168.

Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, pp. 537.

SAS Institute Inc., 2003. The SAS System for Windows, Release 9.1. SAS Inst. Inc., Cary, USA.

Scogings, P.F., Dziba, L.E., Gordon, I.J., 2004. Leaf chemistry of woody plants in relation to season, canopy retention and goat browsing in a semiarid subtropical savanna. Aust. Ecol. 29, 278–286.

van Wyk, J.A., 2001. Refugia—overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort J. Vet. Res. 68, 55–67.

Vercruysse, J., Dorny, P., 1999. Integrated control of nematode infections in cattle: a reality? A need? A future. Int. J. Parasitol. 29, 165–175.

Vercruysse, J., Holdsworth, P., Letonja, T., Barth, D., Conder, G., Hamamoto, K., Okano, K., 2001. International harmonisation of Anthelmintic Efficacy Guidelines. Vet. Parasitol. 96, 171–193.

Waller, P.J., Echevarria, E., Eddi, C., Maciel, S., Nari, A., Hansen, J.W., 1996. The prevalence of anthelmintic resistance in nematode parasites of sheep in southern Latin America: general overview. Vet. Parasitol. 62, 181–187.

Waller, P.J., 1999. International approaches to the concept of integrated control of nematode parasites of livestock. Int. J. Parasitol. 29, 155–164.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone Ltd., Edinburgh, London, pp. 1457.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone Jr., J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.): second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet. Parasitol. 58, 181–213.

Zschocke, S., Rabe, T., Taylor, J.L.S., Jäger, A.K., van Staden, J., 2000. Substitution of plant parts—an alternative for sustainable use? J. Ethnopharmacol. 71, 281–292.