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Comparing the Efficacy of Closantel (An Anthelmintic Drug) and *Azadirachta indica* (Neem Plant) Diet in Controlling Trichostrongylid Nematode Parasites in Goats in Malaysia

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A B S T R A C T

This study was conducted to compare the efficacy of closantel, an anthelmintic drug and neem plant diet (Azadirachta indica) against trichostrongylid nematode parasites of goats. Fecal egg counts (FEC) using modified McMaster and prevalence of nematode species in goats were investigated. Results indicated that the Fecal Egg Count Reduction Test (FECRT) for closantel was low, with approximately 29.22%, while FECRT for neem (A. indica) was lower than closantel at 4.25%. Both treated groups had no significant difference to the control group at P<0.05. Larval cultures of fecal samples revealed that Haemonchus spp. was the predominant species infecting the goats.

Introduction

Helminthiasis or infection with gastrointestinal nematodes such as *Haemonchus contortus* is a common problem in grazing animals which can lead to poor animal performance and economic loss to the livestock industry (Waller & Thamsborg, 2004). In Malaysia, the main methods of controlling gastrointestinal nematodes is using chemical anti-parasitic drug or anthelmintic and also by maintaining good pasture management (Rahman, 1994). As a result of frequent and unsupervised use of a wide variety of anthelminthic classes such as benzimendazoles, imidothiazoles and macrocyclic lactones, the helminth parasite has developed anthelminthic resistance which can lead to total failure of modern broad spectrum anthelminthic to control internal parasites of goats (Chandrawathani *et al.*, 2004).

Researches on finding alternatives to commercially available anthelminthic are rapidly increasing in order to combat resistance (Dorny *et al.*, 1994; Rahman 1994; Pandey and Sivaraj, 1994; Sivaraj *et al.*, 1994; Chandrawathani *et al.*, 1994). The increasing awareness in finding alternative anthelminthic from various plants has significantly reduced nematode parasite in small ruminants, with *Azadirachta indica* plant as a potential candidate for controlling worm problem (Chandrawathani *et al*., 2002).

The present study was initiated with the objective of determining the status of closantel efficacy and to compare with the anthelmintic activity of the Neem plant (*Azadirachta indica*) in controlling the helminthiasis problem in goats in Malaysia.

Materials and Methods

Study Area and experimental animals

A privately owned goat farm from Penang Island, Peninsular Malaysia (longitude 100°15’ E, latitude 5°17’ N) was chosen for this study. The goats were raised solely for their meat. Only adult, non-pregnant and non-lactating goats were selected. Also, the goats used showed mean nematode fecal egg counts exceeding 150 eggs per gram (EPG).

Goats were allocated into 3 groups, each group consisting of 10 goats; the first group was the control, the second treated with closantel, and the third given *A.indica* plant diet. All goats were weighed before (pre-treatment) and after treatment (post-treatment). Food and water were provided *ad libitum*.

Collection and preparation of plants

Fresh *A. indica* leaves were collected from trees from various sites of Penang Island, Peninsular Malaysia. The leaves were separated from the stalks and fed fresh to the treated group twice weekly before sample collection. Each goat was given 3 g of *A. indica* leaves per kg of body weight.

Anthelminthic administration

The goats for anthelminthic treatment were treated with 1 ml/20 kg (2.5 mg/kg) of closantel (Flukiver 5 Inject ® by Janssen Pharmateuticals) injected subcutaneously under the loose skin of the neck in a single dose.

Fecal egg counts

Fecal samples were collected *per rectum* twice weekly for a period of 6 weeks (duration of pre-treatment to post-treatment period) and the number of eggs per gram of feces was estimated using a modified McMaster technique (Whitlock, 1948) with flotation in saturated sodium chloride. The remaining feces were pooled and cultured with vermiculites for infective larvae. One hundred randomly collected larvae were obtained from the culture and identified according to the descriptions of Dikmans & Andrews (1933) and Gordon (1933).

Fecal Egg Count Reduction Test (FECRT)

Fecal samples were analyses by the Fecal Egg Count Reduction Test (FECRT) according to Coles *et al.* (1992). The FECRT provides estimation of the anthelminthic efficacy by comparing fecal egg counts (FECs) before and after treatment. Anthelminthic resistance was considered present if the FECR was less than 95% and the lower confident limit (CI) for the reduction was less than 90% (Coles *et al*., 1992).

Statistical Analysis

Descriptive statistics were produced using data analysis tools Microsoft Excel 2007 and SPSS 12.0 statistical software. Data for mean EPG and larval culture counts of the treated group were subjected to an analysis of variance (ANOVA). The differences in FEC and larval counts were determined using t-tests. The significant level for statistical tests was set to P<0.05.

Results and Discussion

Figure.1 shows the mean EPG counts for both pre- and post-treatment groups. At the beginning of the study, all goats showed high egg counts. In the closantel group, the mean EPG was 3625 while the *A. indica* group showed a mean EPG of 4775 compared to 2250 of the control group. For the mean of post-treatment EPG count, the control group recorded a mean of 9225. This value was slightly higher than that of post-treatment.

Treatment groups closantel and *A indica* recorded mean EPGs of 2565 and 4571 respectively. Both treatment groups showed reductions in EPGs. There was no significant difference in egg counts (P>0.05) between control and closantel groups and also between control and *A. indica* groups.

Table 1 shows the fecal egg count reduction (FECR) for the closantel and *A. indica* groups. FECR was 29.2% for closantel and 4.3% for *A. indica*.

Fecal larvae cultures indicated that *Haemonchus* spp. was the dominant gastro-intestinal parasite (Table 2). Other species recorded were *Trichostrongylus* spp., *Oesophagostomum* spp. and *Cooperia* spp. However, numbers of larvae for the different species varied greatly for all treatment groups.

Closantel treatment reduced all species of larvae except for *Trichostrongylus* spp (Table 3). A diet of *A. indica* succeeded in reducing the population of *Haemonchus* spp. (Table 4).

Haemonchus spp. seemed to be the most dominant species in the control group. Closantel treatment reduced all species except for *Trichostrongylus* spp. while treatment with *A. indica* group showed inconsistent generic larva prevalence.

Table.1 Fecal Egg Count Reduction (FECR) for Closantel and *A. indica* groups

Treatment	Pre-FEC	Post-FEC	FECR [%]
Closantel	3625	2566	29.2
A. indica	4775	4572	4.3

Table.2 Percentage mean of larvae for each treatment group.

Larvae species	Percentage mean of larvae		
	Control	Closantel	*A. indica*
Haemonchus spp.	52.7	29.4	41.8
Trichostrongylus spp.	19.6	44.7	6.9
Cooperia spp.	16.6	15.5	28.9
Oesophagostomum spp.	11.1	10.4	22.4

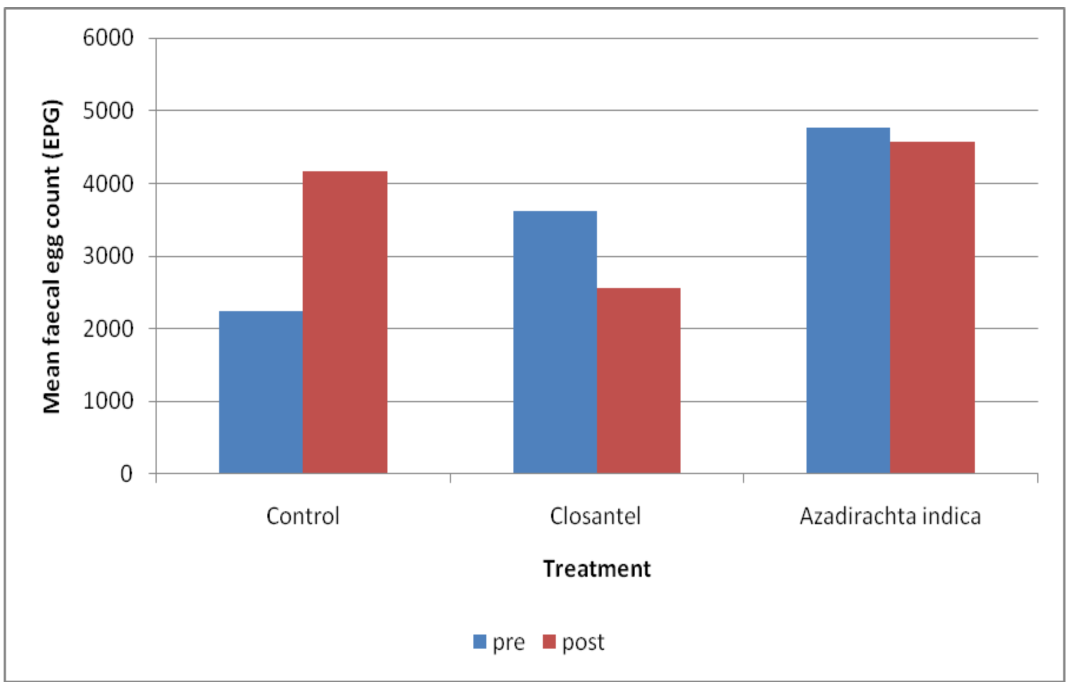
Table.3 Percentage mean of larvae for pre- and post-treatment for closantel treatment

Larvae species	Percentage (%) mean of larvae species		
	Pre-treatment	Post-treatment	% of reduction
Haemonchus spp.	44.25	22.00	50.28
Trichostrongylus spp.	14.75	59.63	-304.24
Cooperia sp.	25.25	10.63	52.25
Oesophagostomum sp.	15.75	7.75	50.79

Table.4 Percentage mean of larvae for pre- and post-treatment for *A. indica* treatment

Larvae species	Percentage (%) mean of larvae species		
	Pre-treatment	Post-treatment	% of reduction
Haemonchus sp.	51.25	37.25	27.32
Trichostrongylus sp.	5.25	7.75	-47.62
Cooperia sp.	22.00	32.38	-47.16
Oesophagostomum sp.	21.50	22.88	-6.40

Fig.1 Pre- and post-treatment mean fecal egg count (FEC)



In this study, the trichostrongylid nematodes that were recovered in all groups were *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., and *Oesophagostomum* spp., with *Haemonchus* spp. being dominant over the others. Based on FECRT, closantel has a low efficacy of 29.2% and did not reduce the total number of nematode eggs, as previously reported in the country by Pandey & Sivaraj (1994) and Chandrawathani *et al.*, (1999) where this drug had failed to control trichostrongylid nematodes both in sheep and goats, possibly attributed to the development of resistance to the closantel.

There were several factors that have caused this resistance problem. Lack of anthelminthic history from sheep and goat importers into the country had possibly worsened the problem. The introduction of goats carrying resistant worms into farms with non-resistant worm populations may have promoted the spreading of resistance in the country. Also, imported animals were sometimes not quarantined adequately before being integrated into the farms. The other factor that had resulted in the resistance problem was the long-term use of the same anthelminthic without verifying its efficacy, thus leading to an increase in the proportion of resistant nematodes in the farms. Also, the goats used in the present study were imported from neighboring Thailand and resistance records of these imported animals were unclear.

It is clear that anthelminthic resistance in Malaysia is escalating and the dependence on anthelminthic to combat worms should be minimized; potentials of plants which possess anthelminthic activity should be explored (Githiori *et al*., 2003; Chandrawathani *et al.*, 2006). However, based on this study, *A. indica* plant had a low efficacy against gastrointestinal parasites as indicated by the FECR, concurring with the findings of Khadijah *et al.*, (2005) who had used pellet *A indica* as well as fresh leaves of the plant. Thus, in conclusion, other methods of biological control should be explored such as the use of nematophagous fungi as reported by Chandrawathani *et al.*, (2002, 2003, 2004).

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