Anthelmintic resistance in selected goat farms in Kelantan

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Abstract

The widespread use of chemical anthelmintics to control goat gastrointestinal nematodes has resulted in the occurrence of resistant nematode populations that has become a global problem including Malaysia. Faecal samples were collected from 202 goats from eight farms in Kelantan for the confirmation of gastrointestinal nematode infection. A total of 161 goats were chosen for the Faecal Egg Count Reduction Test (FECRT) to detect resistance to four anthelmintics: albendazole, ivermectin, levamisole and closantel. Varying levels of resistance were detected for each anthelmintic where resistant nematode populations to albendazole, ivermectin, levamisole and closantel were observed in goats in six, five, two and two farms, respectively. Resistance was suspected against albendazole, ivermectin and levamisole in one farm. Nematode populations in two farms were found to be susceptible to levamisole while those in only one farm was susceptible to albendazole. In the present study, levamisole was found to be the most effective anthelmintic. Pre-treatment faecal cultures revealed *Haemonchus contortus* (73%) as the predominant infective larvae followed by *Trichostrongylus* spp. (26%) and *Oesophagostomum* spp. (1%). Based on the present study, the anthelmintic resistance status in Kelantan is of critical concern as the current anthelmintic based nematode control in goat farms may not be effective on a long term basis. There is an urgent need to evaluate non-chemical methods to control parasitism in local goats.

Keywords : Anthelmintic resistance, gastrointestinal nematodes, goats

Introduction

Parasitic gastroenteritis (PGE) caused by strongyle worms is considered the second most important disease of small ruminants in Malaysia causing high morbidity and mortality (Sani and Chandrawathani, 1996) especially in goats which are known to be more susceptible than sheep (Sani *et al.*, 2004). The significant nematodes in Southeast Asia include *Haemonchus contortus, Trichostrongylus, Strongyloides* and *Oesophagostomum* (Sani and Gray, 2004). *H.contortus* was reported to be the most prevalent species in small ruminant farms in Peninsular Malaysia (Khadijah *et al*., 2006a and 2006b). Annual production losses in small ruminants in Indonesia attributable to roundworm parasites was reported to be $US13 million in 1999 (McLeod, 2004).

In many goat farms PGE is controlled almost exclusively using chemical anthelmintics but their effectiveness is restricted by the development of anthelmintic resistance (Waller, 2002) and the inherited ability of parasites to survive anthelmintic treatments (Sangster, 2001). As a result of indiscriminate use of chemical drugs for the control of PGE in goats and sheep, anthelmintic resistance has now been identified in many countries all over the world, affecting the profitability of the small ruminant industry (Kaplan and Vidyashankar, 2012).

The emerging threat of anthelmintic resistance in the small ruminant industry in Malaysia has been reported since early 1990s. Thiabendazole resistance was investigated using the egg hatch assay method in some smallholder goat farms in Malaysia (Rahman, 1993). Further research revealed the presence of anthelmintic resistance against gastrointestinal nematodes of sheep and goats to benzimidazoles, macrocyclic lactones, imidazothiazoles and salicylanilides (Pandey and Sivaraj, 1994; Rahman, 1994; Sivaraj *et al., 1994;* Dorny *et al*., 1994; Chandrawathani *et al*., 1999, 2003, 2004; Khadijah *et al*., 2006a, 2006b, 2007, 2008; Nor-Azlina, 2010). Studies to evaluate the status of anthelmintic resistance in small ruminant in Peninsular Malaysia including goat farms in Kelantan (Dorny *et al*.,1994; Chandrawathani *et al*.,1999; Khadijah *et al*., 2006b) revealed that anthelmintic resistance was on the rise. Therefore the objective of the present study was to evaluate the current anthelmintic resistance status in selected goat farms in the state of Kelantan to four locally available anthelmintics, namely albendazole, ivermectin, levamisole and closantel.

Materials and Methods

Study sites

A total of eight goat farms in Kelantan were selected for the study. Four goat farms were located in the district of Kota Bharu while the other four farms were in the district of Pasir Mas. The farms were selected based on faecal egg count (FEC) screening and Faecal Egg Count Reduction Test (FECRT) criteria as described by Coles et al. (1992). The study was conducted between June to October 2011.

Management of goats

Details on feeding system, types and frequency of anthelmintic usage of each farm are given in Table 1. Feed in the form of cut native grasses, improved grasses or a mixture of both were provided daily to the goats in these farms. The improved grasses included Napier grass (*Pennisetum purpureum*), native signal grass (*Brachiaria humidicola*) and Kazungula grass (*Setaria sphacelata cv Kazungula*). The animals on all of the farms were housed on raised wooden sheds with slatted floor and provided with water *ad libitum* and fed limited amount of commercial pelleted feed with supplemental mineral licks, molasses and vitamins.

A total of 202 goats of both sexes from the eight farms that were not dewormed for at least the last eight weeks before the study was conducted, were screened for infection with gastrointestinal nematodes by modified McMaster technique for FEC (Lyndall-Murphy, 1993). Goats having more than 150 eggs per gram (epg) of faeces were selected for FECRT which is the most effective test for estimating anthelmintic resistance in ruminants, horses and pigs (Coles *et al.*, 1992). A total of 161 goats were selected for the FECRT ranging from less than 1 to 5 years in age. Details of the worm control programmes conducted in these farms were obtained through a questionnaire.

Treatment

The goats were randomly assigned to the treatment groups of anthelmintics tested on each farm with at least 5 animals per group, based on pre-treatment faecal egg count. The number of groups available determined the number of anthelmintics which could be tested including a control group. The animals were treated with albendazole, ivermectin, levamisole and closantel, according to the manufacturers' recommended dose rates and based on individual body weight estimation as shown in Table 2. No treatment was given to the animals in the control group.

Parasitological techniques

Faecal sample of each selected goat was collected per rectum in the morning on two occasions, firstly on Day 0 pre-treatment and secondly from Days 10 to 14 post-treatment. Faecal samples collected were subjected to the modified McMaster technique and faecal culture method. Pre-treatment faecal samples from each farm and post-treatment faecal samples from each group were pooled and cultured for 7 days. The infective larvae (L3) from the cultures were identified to the genus level.

Anthelmintic resistance status was evaluated by FECRT based on methods described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992).

Faecal Egg Count Reduction Percentage (FECR%) = $(1 – X_t /X_c) \times 100$
where X_t is the arithmetic mean of post-treatment epg of the treated group and X_c is the arithmetic mean of post-treatment epg of the control group.

Resistance is present when two criteria are met (1) FECR% is less than 95% and (2) 95% lower confidence limit is less than 90%. Resistance is suspected if only one of these two criteria is met (Coles *et al.*, 1992). A susceptible status is established if the FECR% is more than 95% and the lower 95% confidence limit is more than 90% (Chandrawathani *et al*., 1999, Khadijah *et al*., 2006b, Nor-Azlina, 2010). Following the classification of Khadijah *et al*. (2006a) anthelmintic resistance is considered as critical, severe and moderate when FECR% is less than 50%, 50-90% and 91-95%, respectively. Critical and severe resistance indicate serious ineffectiveness of a particular anthelmintic, but moderate resistance is an acceptable level of resistance as reduction of FEC is still more than 90%.

Results and Discussion

Presence of anthelmintic resistance in the goat farms selected for the present study is shown in Table 3. There were varying degrees of resistance of each anthelmintic detected in the farms. Strongyle populations in Farm 1 were severely resistant to albendazole, ivermectin and closantel but susceptible to levamisole which was 100% effective, as it was never used in the farm.

Critical resistance to closantel also existed on Farm 2. In this farm resistance was suspected against albendazole, ivermectin and levamisole. Even though Farms 1 and 2 had no history of using closantel, resistance may have developed from the source of origin. Usage of closantel against nematode and liver fluke infection was encouraged by the Department of Veterinary Services (DVS) in 1980s in government farms (Chandrawathani *et al*., 1996). The practice of using closantel continued until the early 2000s in farms where animals were distributed to smallholder farmers. (Khadijah *et al*., 2006b). Therefore the use of closantel has been practiced for over two decades, leading to the critical nematode resistance against closantel in the farms under the goat distribution programme.

In other farms where albendazole and ivermectin were tested, severe and critical resistance cases were detected for both anthelmintics except in Farm 5 which was still susceptible to albendazole. As shown in Table 1, animals in this farm had no history of being drenched with any anthelmintic which resulted in 100% effectiveness of albendazole. In the other farms, resistance to albendazole was expected due to the availability of oxfendazole for many years, a benzimidazole drug that can be purchased cheaply from DVS Kelantan. In addition, the most critical resistance against albendazole in this study was detected on Farm 7 with FECR percentage of 0%. Prolonged regular treatment of oxfendazole contributed to the development of oxfendazole resistance which subsequently resulted in cross resistance to albendazole (Sangster, 1999; Leathwick, 2012).

Small ruminant farmers in Kelantan conceived that macrocyclic lactones such as doramectin, biomectin and ivermectin are solely for ectoparasite infections. Thus these groups of anthelmintics are used extensively when goats and sheep have skin infection. The farmers also administered oral anthelmintics such as albendazole to the animals as prophylaxis or therapeutic agents to combat gastrointestinal parasitism. Consequently, varying degrees of resistance to albendazole and ivermectin in the farms were detected in this study.

The study showed that resistance to levamisole in the goat farms in Kelantan was negligible compared to other anthelmintics. Two out of five farms tested with levamisole were found to have moderate resistance, two farms were susceptible and one had suspected resistance. Moderate resistance was tolerable as FECR% of all levamisole groups was above 90% (Nor-Azlina, 2010). This outcome was predicted from the questionnaire survey as none of the farms selected for FECRT used levamisole or other imidazothiazole drugs for worm control. Hence levamisole was found to be the most effective anthelmintic currently in the goat farms in Kelantan.

Third stage infective larvae (L3) yield from pre-treatment faecal cultures from eight goat farms in Kelantan revealed that *H. contortus* was the predominant gastrointestinal nematode (73%), followed by *Trichostrongylus* spp. (26%) and *Oesophagostomum* spp. (1%) as shown in Figure 1. Similar trends were also shown in Figure 2 for post-treatment percentage of infective strongyle larvae in control, albendazole and ivermectin groups where the survival of L3 was more than 70% for *H.contortus*, followed by 15-25% *Trichostrongylus* larvae and 1-6% *Oesophagostomum* larvae (Figure 2). This suggests that albendazole and ivermectin were not effective in eliminating *H.contortus*.

On the contrary, less than 30% *H.contortus* infective larvae survived levamisole and closantel treatments (Figure 2). As a narrow-spectrum salicylanilide anthelmintic that specifically targets *H.contortus*, this result is expected even though severe and critical resistance were detected on two farms tested with closantel. Nevertheless, percentage of surviving *H. contortus* infective larvae in closantel group (26%) was higher than that of levamisole group (19%) since levamisole was found to be the most effective anthelmintic in this study. Furthermore, *Oesophagostomum* spp. was found to be the most susceptible gastrointestinal nematode in both pre-treatment and post-treatment faecal cultures. Although *H.contortus* and *Oesophagostomum* spp. populations succumbed to treatment with levamisole and closantel , *Trichostrongylus* spp. populations prevailed.

Conclusion

Varying degrees of resistance to albendazole, ivermectin, levamisole and closantel were detected in eight selected goat farms in Kelantan. In this study levamisole was found to be the most effective anthelmintic against strongyle worms. Hence levamisole should be introduced to the goat farmers in Kelantan with advice to rotate anthelmintics as well as to adopt non-chemical control approaches to delay occurrence of resistance. Thus there is an urgent need for research on non-chemical sustainable approaches in controlling PGE.

Table 1 : Management and anthelmintic usage in selected goat farms in Kelantan

Farm	Breed	Grazing	Grass type	Anthelmintics used currently	Drenching frequency per year
1	Boer	6 to 7-month-old only 24 hours around the barn	Improved	Doramectin Fenbendazole Albendazole	2
2	Boer Katjang Jamnapari Saanen Toggenburg	Afternoon, around the barn	Mixed	Fenbendazole	3
3	Boer	Zero		Biomectin Fenbendazole	3
4	Boer Katjang	Afternoon, at oil palm plantation	Native	Ivermectin Oxfendazole	3
5	Boer Katjang	Zero	Mixed	No	Never
6	Boer	Zero	Mixed	Albendazole	2
7	Boer	Zero	Mixed	Ivermectin	3
8	Boer Katjang	Afternoon, round the barn and roadside	Mixed	Ivermectin Oxfendazole	3

Table 2 : Anthelmintics used in Faecal Egg Count Reduction Test

Anthelmintic group	Registered trade name	Generic name	Manufacturer	Dosage	Mode of administration
Benzimidazoles	Alben 10%	Albendazole	Nova Laboratories Sdn. Bhd	0.1ml/kg	Oral
Macrocyclic lactones	Baymec	Ivermectin	Bayer Korea Ltd	1ml/50kg	Injection (*s.c)
Imidazothiazoles	Coopers® Nilverm	Levamisole	Coopers Animal Health	2.5ml/10kg	Oral
Salicylanilides	Flukiver 5 Inject	Closantel	Janssen Animal Health	1ml/10kg	Injection (*s.c.)

Note : *s.c = subcutaneously

Table 3 : Faecal Egg Count Reduction Percentage with 95% confidence limit (in parenthesis) against four anthelmintics on selected goat farms in Kelantan

Farm	Albendazole	Ivermectin	Levamisole	Closantel
No. of farms tested	8	6	5	2
Farm 1	68% (-20, 90%)	67% (-3, 90%)	100%	63% (-131, 94%)
Farm 2	95% (78, 99%)	97% (77, 100%)	95% (54, 99%)	39% (-215, 88)
Farm 3	71% (3, 97%)	NT*	NT	NT
Farm 4	53% (-30, 83%)	11% (-86, 58%)	98% (92, 100%)	NT
Farm 5	100%	NT	NT	NT
Farm 6	57% (-181, 93%)	87% (83, 91%)	91% (75, 97%)	NT
Farm 7	0% (-185, 65%)	78% (24, 94%)	92% (86, 95%)	NT
Farm 8	70% (-12, 92%)	41% (-145, 86%)	NT	NT

Note : *NT = not tested

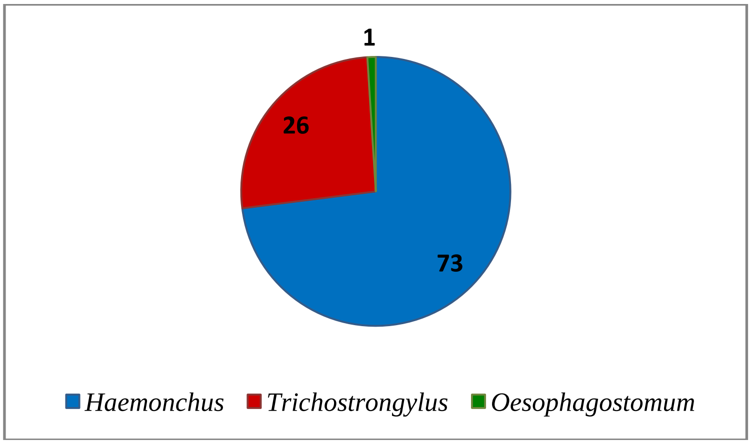

Figure 1 : Pre-treatment infective larval population percentage by nematode genera in 8 goat farms

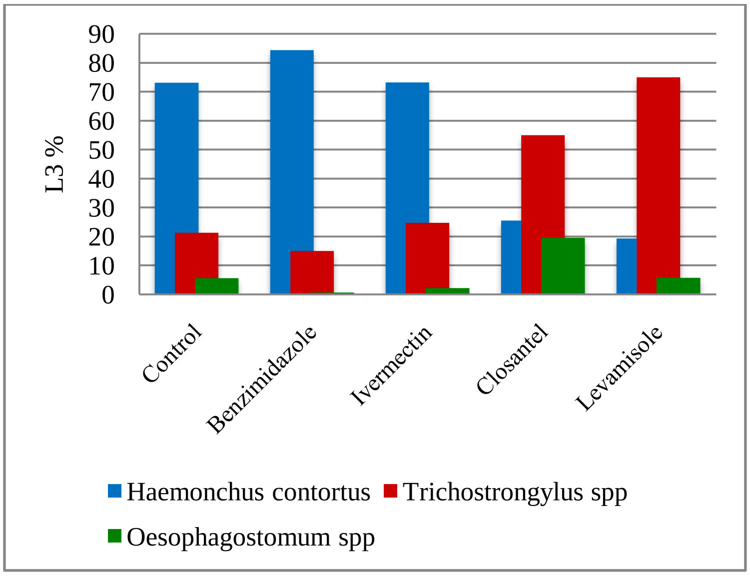

Figure 2 : Post-treatment infective larval population percentage of control and treatment groups in 8 farms by nematode genera (control, n = 8; benzimidazole, n = 8; ivermectin, n = 6; levamisole, n = 5; closantel, n = 2)

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