ANTHELMINTIC RESISTANCE IN SMALL RUMINANT FARMS: AN ONGOING CHALLENGE FOR PERAK FARMERS TO CONTROL HELMINTHS

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ABSTRACT. This study was done to evaluate the status of resistance to nematode populations on four major groups of anthelminthics. Nematode faecal egg count reduction tests (FECRT) were conducted on six free grazing small holder goat farms (namely Farms A-F) in Perak. The four drug groups tested in this study were Benzimidazoles, Imidazothiazoles, Macrocyclic Lactones and Salicylanilides. Faecal samples were subjected to the standard procedures of McMaster for worm egg estimation and also larval cultures for third stage larvae identification. Results of Farm A, Farm C and Farm D showed resistance to all 4 drugs groups of anthelminthic that were tested and the major worm population of this farm were *Haemonchus contortus* (49.75%), *Trichostrongylus colubriformis* (47.71%) and *Cooperia* sp. (2.53%). The results of Farm B and Farm F showed resistance to all the 3 groups of anthelminthics except Levamisole and the worm population of this farm was *Haemonchus contortus* (47.30%), *Trichostrongylus* sp. (38.44%), *Oesophagostomum columbianum* (13.50%) and *Cooperia* sp. (0.76%) Farm E was resistant to Benzimidazoles and suspected resistance to 3 drugs groups; Levamisole, Macrocyclic Lactones and Closantel. The worm population for Farm E was *Haemonchus contortus* (71.35%), *Trichostrongylus* sp. (27.6%) and *Oesophagostomum* sp. (2%). These farms have a history of using the same anthelminthics over the past few years, thus leading to the severe case of anthelminthic resistance. Recommendations have been made to control helminths using alternative approaches such as cut and carry feeding, herbal medication using Neem leaves (*Azadirachta indica*) and rotational grazing as well as improvement in management of animals to increase their immunity.

Keywords: FECRT, anthelminthic resistance, free grazing small holder

INTRODUCTION

Helminth infection has been recognised as the most important cause of mortality and morbidity in small ruminants in Malaysia (Fatimah *et al*., 1985; Sani and Rajamanickam, 1990; Sani *et al*, 2004). Anthelmintic resistance in sheep and goats in Malaysia was reported in the early 1990s, with demonstrations of resistance to

the benzimidazoles (Rahman, 1993;1994; Pandey and Sivaraj, 1994), levamisole (Dorny *et al*., 1994) and the first case of multiple anthelminthic resistance reported by Sivaraj and co-worker in 1994 (Sivaraj *et al*.,1994).

In 2006 FECRT was conducted in 18 small ruminants private farms located in Peninsular Malaysia and it was found that nematode populations on all farms showed resistance to salicylanilides closantel while 13 farms showed resistance to oxfendazole, 8 farms showed resistance to imidazothiazoles and 4 farms showed resistance to moxidectin (Khadijah *et al*., 2006).

In 2012, two goat farms experiencing severe mortality up to 30% in the flock were tested for anthelminthic resistance using the Faecal Egg Count Reduction Test (FECRT) with four anthelminthics from the group; Benzimidazoles, Imidazothiazoles, Macrocyclic Lactones and Salicylanilides. Results showed that *Haemonchus contortus* and *Trichostrongylus colubriformis* in these two farms were resistant to all the four anthelminthic groups tested (Chandrawathani *et al*., 2013).

In view of the escalating anthelmintic resistance problem in small ruminants in Malaysia, the Veterinary Research Institute has embarked on a programme to identify farms with severe helminthiasis whereby common anthelmintics seem ineffective and thereon establish a database of regions or areas which coincide with different anthelmintic resistance status. This is an ongoing study conducted on six free grazing small holder goat farms in several districts of Perak. The farms were investigated over a period of a year (January to December 2012). The objective of this study was to evaluate the current status of nematode anthelminthic resistance in these farms, as these farms experienced severe mortality and morbidity due to helminthiasis. From this study the farmers would be better able to control helminthiasis by acknowledging suitable and effective drugs and in the long term make their livestock venture profitable.

MATERIALS AND METHODS

The Faecal Egg Count Reduction Test (FECRT) was conducted on 6 goat farms near Ipoh, Perak. Four types of anthelminthics, Benzimidazoles (Albendazole Vetpharm Laboratories (S) Pte. Ltd.), Imidazothiazoles (Levamisole, Bovet Pharmaceutical Ltd.), Salicylanilides (Closantel, Janssen Pharmaceutical N.V.) and Macrocyclic Lactones (Ivermectin, Man./Fab: KELA N.V.)were used. Animals were divided to 5 equal groups of which, 4 are treated groups and 1 control group. Each group of 6-10 animals were treated according to the manufacturers recommended dose rates based on individual body weight and the control group were not given any treatment.

Pre-treatment rectal faecal samples were collected from each animal and treated respectively according to group, while the animals from control group were identified and no treatment was given. After

10 to 14 days post-treatment, rectal faecal samples were collected again for faecal egg count (Anon., 1986). Faecal culture method was conducted to identify the species of nematode in each farm. The faecal culture method was done by pooling all the pre-treatment samples and culturing it for 5 to 7 days. Identification of the species of larvae using the identification keys by Manual of Veterinary Parasitological Laboratory Techniques (1971).

The data were analysed based on the on the calculations outlined by Coles *et al*. (1992). Resistance to a particular anthelminthic was considered to be present if the reduction in faecal egg count was less than 95% and the 95% lower confidence limit was less than 90%. If only one of the two criteria was met, the status was considered as suspected resistance. If the reduction in faecal egg count was more than 95% and the lower confidence limit was more than 90%, the status was considered as susceptible .

Standard questionnaires were used to interview the management of the farms on deworming history, farm management, feed and water source and mortality records.

RESULTS

Animals and Management

Information on the management of the animals in the farms are summarised in Table 1.

All the farms involved in this study are commercial farms breeding goats for meat purposes. The animals were crossbred Boer and Katjang, as the farmers kept various breeds together in their farm since they had started farming. The farms had a population of between 50 and 150 heads per farm. Two farms practiced semi intensive management and other 4 farms practiced free grazing management. The animals grazed around 4 to 5 hours per day and kept in raised floor and wooden sheds during night. Four of farms provided commercial concentrate feed supplement while in the shed. Animals in the shed also were given water ad libitum, salt or mineral blocks. Their mortality rate was between 10 to 40% a year due to the animals were free grazing and the farm management. Five farms (Farm A, C, D, E and F) were dewormed once or twice a year with ivermectin,and benzimidazole. Only one farm (Farm B) was never dewormed. However, the length of time these drugs have been used in the farm was unclear as no records were kept. According to the district veterinary department which institutes treatment, the common drugs administered to all small ruminant farms are from the Benzimidazoles group as it was economical.

Anthelmintic resistance

Farm A, C and D shows severe resistance to all drug groups Benzimidazole (25%, -12%, 92%), Imidazothiazoles (33%, 93%, 97%), Salicylanilides (33%, -5%, -18%)

and Macrocyclic Lactones (94%, -63%, 62%). Farm B and F shows resistance to 3 type of drugs Benzimidazole (-46%, 64%), Salicylanilides (54%,4%) and Macrocyclic Lactones (-6%, 87% except Imidazothiazoles (97%, 96%). Farm E was resistant to one drug and susceptible to Imidazothiazoles, Macrocyclic Lactones and Closantel (Table 2). The mean egg counts for each treatment group are given in Table 3.

Pre-treatment faecal culture from Farm A indicated that *Haemonchus contortus* is the predominant species (96%) followed by *Trichostrongylus* sp (3%) and *Cooperia* sp (1%). Post-treatment faecal cultures from Farm A showed resistance of *Heamonchus contortus*, sp., *Trichostrongylus* sp. and *Cooperia* sp. to Benzimidazole, Levamisole, Macrocyclic Lactones (Ivermectin) and Closantel *Haemonchus contortus* is predominant species (50%) followed by *Oesophagostomum* sp. (38%) and *Trichostrongylus* sp. (12%) in Farm B. However, post treatment faecal cultures in Farm B showed resistance of *Haemonchus contortus*, *Oesophagostomum* sp., *Trichostrongylus* sp. and *Cooperia* sp. to Benzimidazole, Levamisole and Closantel. Macrocyclic Lactones is the effective drug for Farm B.

Pre-treatment faecal culture from Farm C showed *Trichostrongylus* sp. is the predominant species (74%) followed by *Haemonchus contortus* (26%). Farm C showed that *Haemonchus contortus* and *Trichostrongylus* sp. is resistant to all 4 drugs. The faecal cultures for Farm D indicated *Trichostrongylus* sp. (56%) was most common and followed by *Haemonchus contortus* (44%). For the post-treatment faecal cultures from Farm D, *Haemonchus contortus*, *Trichostrongylus* sp. were resistant to Benzimidazole, Imidazothiazoles, Salicylanilides and Macrocyclic Lactones.

Pre-treatment faecal culture from Farm E indicated that *Trichostrongylus* sp is the predominant species (67%) followed by *Haemonchus contortus* (22%) and *Oesophagostomum* sp. (11%). Post-treatment faecal cultures from Farm E showed resistance of *Haemonchus contortus*, *Trichostrongylus* sp. and *Oesophagostomum* sp. to Benzimidazole whereas Closantel, Levamisole and Macrocyclic Lactones is the effective drug for Farm E.

Pre-treatment faecal culture from Farm F showed *Haemonchus contortus* as the predominant species (48%) followed by *Trichostrongylus* sp. (28%) and *Oesophagostomum* sp. (24%). Post-treatment faecal cultures from Farm F showed resistance of *Haemonchus contortus,* sp., *Trichostrongylus* sp. and *Oesophagostomum* sp. to 3 drugs Benzimidazole, Macrocyclic Lactones and Closantel. The effective drug for Farm F is Levamisole (Table 4).

DISCUSSION

In Malaysia, anthelmintic resistance was suspected in the 1980's as small

Table 1: Summary of farm management in the 6 farms (Farms A-F) involved in this study

	Number of animals	Breed of animals	Management type	Feed	Grazing area	Mortality rate	Deworming frequency	Drug used
Farm A	60	Boer Mix	Semi-intensive	Grass when grazing and pellets while penned	Free roaming at oil palm plantation, always on the same area	33% a month	1/year	Ivermectin
Farm B	90	Boer Mix	Semi-intensive	Only grazing	Free roaming, always on the same area	13-40% a year	0	Animals were never dewormed
Farm C	140	Katjang crosses	Free grazing	Grass when grazing, pellet	Free roaming under oil palm plantation	33% a month	2x/year	Benzimidazole
Farm D	55	Boer	Free grazing	Only grazing	Free roaming under oil palm plantation	23% a month	2x/year	Benzimidazole
Farm E	65	Boer Mix	Free grazing	Grass when grazing and pellets while penned	Free roaming under oil palm plantation	10% a month	2x/year	Benzimidazole
Farm F	100	Boer Mix	Free grazing	Grass when grazing and pellets while penned	Free roaming under oil palm plantation	10% month	When Needed	Benzimidazole

Table 2: Anthelminthic resistance status on six smallholder farms (Farms A-F). .

	FECRT Percentage			
	Bz	**Leva**	**ML**	**Clo**
Farm A	25%	33%	94%	33%
Farm B	-46%	**97%**	-6%	54%
Farm C	-12%	93%	-63%	-5%
Farm D	92%	47%	62%	-18%
Farm E	95%	**99%**	**98%**	**96%**
Farm F	64%	**96%**	87%	4%

Note: FECR<50%-critical resistance; FECR 50% to 90%-severe resistance; FECR 91% to 95%- moderate resistance;>95% susceptible. Bold values indicate reduction greater than 95%. Bz = Benzimidazole, Leva = Levamisole, ML = Macrocyclic Lactones (Ivermectin), Clo = Closantel (flukiever)

Table 3: Pre-treatment and post-treatment of mean faecal egg count (±SE) from treatment and control groups for each farm (Farms A-F)

		Mean Faecal egg count (epg)				
		Control	**Bz**	**Leva**	**ML**	**Clo**
Farm A	Pre-treatment	625	2729	1775	2538	843
	Post-treatment	1550	2267	43	1643	713
Farm B	Pre-treatment	1010	937	1175	1362	3787
	Post-treatment	463	350	312	28	1842
Farm C	Pre-treatment	1010	890	333	1386	1970
	Post-treatment	1070	1200	80	1743	1122
Farm D	Pre-treatment	1200	1057	400	700	1271
	Post-treatment	750	57	400	288	883
Farm E	Pre-treatment	2617	800	600	1375	388
	Post-treatment	117	125	29	43	100
Farm F	Pre-treatment	2382	1929	1700	1738	2138
	Post-treatment	1227	857	86	320	2288

Table 4: Prevalence of third stage larvae (%), pre-treatment and post-treatment for each farm (Farms A-F) from treatment and control groups.

			Control	Bz	Imid	ML	Clo
Farm A	Pre-treatment	*H. contortus* (96%), *Trichostrongylus* sp. (3%), *Cooperia* sp. (1%)					
	Post-treatment	*H. contortus*	84%	86%	97%	76%	86%
		Trichostrongylus sp.	11%	1%	3%	19%	1%
		Cooperia sp.	5%	13%	–	5%	13%
Farm B	Pre-treatment	*H. contortus* (50%), *Trichostrongylus* sp. (12%), *Oesophagostomum* sp. (38%)					
	Post-treatment	*H. contortus*	47%	50%	6%	84%	33%
		Trichostrongylus sp.	22%	49%	28%	16%	43%
		Cooperia sp.	2%	1%	1%	–	3%
		Oesophagostomum sp.	–	–	65%	–	21%
Farm C	Pre-treatment	*H. contortus* (26%), *Trichostrongylus* sp. (74%)					
	Post-treatment	*H. contortus*	28%	30%	20%	39%	17%
		Trichostrongylus sp.	72%	72%	80%	61%	83%
Farm D	Pre-treatment	*H. contortus* (44%), *Trichostrongylus* sp. (56%)					
	Post-treatment	*H. contortus*	44%	50%	0%	22%	28%
		Trichostrongylus sp.	56%	37%	100%	10%	72%
Farm E	Pre-treatment	*H. contortus* (22%), *Trichostrongylus* sp. (67%), *Oesophagostomum* sp. (11%)					
	Post-treatment	*H. contortus*	86%	18%	13%	1%	19%
		Trichostrongylus sp.	14%	3%	31%	2%	3%
		Oesophagostomum sp.	–	–	–	–	2%
Farm F	Pre-treatment	*H. contortus* (48%), *Trichostrongylus* sp. (28%), *Oesophagostomum* sp. (24%)					
	Post-treatment	*H. contortus*	31%	35%	20%	63%	25%
		Trichostrongylus sp.	16%	65%	76%	16%	25%
		Oesophagostomum sp.	–	–	4%	–	6%

Bz = Benzimidazole, Leva = Levamisole, Imid = Imidazothiazoles, Clo = Closantel

ruminant farmers usually complained of ineffective drugs and has been rapidly escalating. With an increasing trend to import sheep and goats from Australia, South Africa and South America where anthelmintic resistance is common, it is not surprising that the resistant worm populations may have been imported too. Repeated treatment of local animals with anthelmintics selects parasites that have innate or acquired resistance to the drugs. Ultimately treatment becomes ineffective because a large proportion of the parasites in the population are resistant. It is now common for sheep to harbour at least one parasite species that is resistant to the major drench groups. It is becoming disturbingly common for worms to be resistant to anthelmintics in all of the major drench groups, including some combinations of unrelated drugs. Drench resistance is common and often highly developed in goats. Only a limited range of anthelmintics are registered for use in goats. Due to their altered drug metabolism goats may require higher dose rates than recommended for sheep (Sani & Gray, 2004).

Traditionally 4 drug groups, namely benzimidazole (BZ), imidazothiazoles (Leva) (LEV), Macrocyclic Lactones (IVM) were used control worms in small ruminants. However, with recent extensive spread of resistance in these chemical groups, other drenches including naphthalophos (NAP, Rametin TM), and its combinations with BZ and/or LEV, closantel (CLS), and other MLs such as abamectin, and moxidectin should be considered (Sheep101.info. 2014). Although some may recommend an increased drug dose to combat anthelmintic resistance, in practice, only double dose LEV or double dose LEV plus single BZ have been shown to be effective where the single dose is not effective. However, it is emphasized that the use of double dose rate or combinations of anthelmintics are not general recommendations. They are used to obtain a prompt answer to possibly a serious and immediate problem on specific farms, and now are infrequently used (Vatta & Lindberg, 2006).

In Malaysia, farmer awareness on anthelmintic resistance is encouraged and various other means of worm control are advocated such as rotational grazing, use of herbs or cut and carry method of feeding instead of grazing permanent pastures under rubber or oil palm plantations. It is vital that farmers are aware of the dangers of importing new animals into their farm. With the diagnosis of anthelmintic resistance, farmers must practice some measure of biosafety and security to prevent resistant worm populations from establishing in their farm. Conducting this study will increase farmer awareness on the pitfalls of indiscriminate purchases of animals from unknown sources.

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