STATUS OF ANTHELMINTIC RESISTANCE IN SMALLHOLDER GOAT FARMS IN IPOH, PERAK BETWEEN 2013 AND 2014

PREMAALATHA B.[1]*, CHANDRAWATHANI P.[2], JAMNAH O.[1], RAMLAN M.[2], QUAZA NIZAMUDDIN H.N.[2] AND KHADIJAH S.[3]

1 Veterinary Research Institute, Jalan Sultan Azlan Shah, 31400, Ipoh, Perak.
2 Department of Veterinary Services, Putrajaya.
3 Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu.
* *Corresponding author:* princess_latha2280@yahoo.com

ABSTRACT. Helminth infection has been recognised as the most important cause of mortality and morbidity in small ruminants in Malaysia. In this study, nematode faecal egg count reduction tests after treatment with benzimidazole, imidazothiazole, macrocyclic lactone and salicylanilide, were conducted on eight free grazing smallholder goat farms in Ipoh, Perak from 2013 to 2014. Many of the farms were found resistant to three or four types of anthelmintic drugs. Major worm populations identified from these farms were *Haemonchus contortus* (55.5% to 76.4%), *Trichostrongylus colubriformis* (8.8% to 20.0%) and *Oesophagostomum* sp. (5.8% to 13.0%). Closantel and levamisole were found to be effective in the control of these worms in certain studied farms. The recommendation is to control helminths using alternative approaches such as cut-and-carry feeding, herbal medication using neem leaves (*Azadirachta indica*) and rotational grazing. Awareness on the correct use of anthelmintics is advocated.

Keywords: anthelmintic resistance, goats, smallholder, faecal egg count reduction test, Perak

INTRODUCTION

Anthelmintic resistance in sheep and goats in Malaysia was reported in early 1990s and helminthiasis has been recognised as a major problem causing diseases and production losses till now. There have been a number of studies on anthelmintic resistance conducted in Malaysia.

Dorny *et al*. (1994) reported benzimidazole resistance in 33 out of 96 goat farms using egg hatch assay method.

Pandey V.S. *et al*. (1994), found high benzimidazole resistance levels (albendazole, oxfendazole, fenbendazole) for *Haemonchus contortus* on the same farm. Closantel, levamisole and ivermectin in three other farms were found susceptible towards the worms.

Another study was conducted on 39 sheep farms and nine goat farms located in peninsular Malaysia and the results showed that 50% of sheep farms and 75% of goat farms had resistance to levamisole, closantel and ivermectin (Chandrawathani *et al*., 1999).

The eastern Malaysian state of Sabah had high-levels of stock losses due to infections with *H. contortus* for several years. Five government small ruminant breeding farms in Sabah had conducted anthelmintic efficacy using faecal egg count reduction test (FECRT) and the test showed total failure towards benzimidazole, imidothiazole, macrocyclic lactone and salicylanilide groups of anthelmintics (Chandrawathani *et al.*, 2004).

In 2006, Khadijah *et al.*, (2006a, 2006b) performed two different studies on anthelmintic resistance. In the first, tests were conducted in eighteen small ruminant private farms in Peninsular Malaysia and all the farms had resistance to salicylanilides closantel, thirteen farms had resistance to oxfendazole, eight farms had resistance to imidazothiazoles and four farms had resistance to moxidectin. In the second, five government small ruminant farms (Kedah, Perak, Selangor, Terengganu and Johor) had resistance to all anthelmintics tested. *Haemonchus contortus* (78%) was the most prevalent species, followed by *Trichostrongylus* spp. (22%). The animals from the farms may have been grazing on grass contaminated with helminth larvae.

In 2011, another study reported that all farms exhibited resistance towards benzimidazole and closantel while only two farms were still susceptible to levamisole and one farm suspected resistance to ivermectin. Four farms exhibited resistance to all anthelmintics tested (Nor-Azlina *et al.*, 2011).

In 2012, five farms had resistance to albendazole, ivermectin and closantel, only levamisole was found to be the most effective anthelmintic in Kelantan (Basripuzi *et al.*, 2012).

Two goat farms tested for anthelmintic resistance using four drugs and found resistance to all the drugs with high worm count in *H. contortus* and *T. colubriformis* (Chandrawathani *et al.*, 2013).

Anthelmintic resistance tested in six farms in Perak, found that three farms had resistance to all four groups of anthelmintic drugs used and the major worm population in these farms were *H. contortus* (49.75%), *Trichostrongylus colubriformis* (47.71%) and *Cooperia* sp. (2.53%). Two farms had resistance to three groups of drugs except levamisole, and the highest worm population was *Haemonchus contortus* followed by *Trichostrongylus* sp., *Oesophagostomum* sp. and *Cooperia* sp. Only one farm had resistance to benzimidazole and suspected resistance to three other groups of drugs (Premaalatha *et al.*, 2014).

In another study by Thongsahuan *et al.* (2014), FECRT was conducted on local goats in Ipoh, and tested with levamisole for resistance against strongyles. Ten animals were treated with levamisole and six animals were used as control in this study. Results showed that the strongyle worm population was resistant to levamisole with percentage reduction of 75%. The main worm population in this farm were *H. contortus* (71%), *Oesophagostomum* sp. (18%) and followed by *Trichostrongylus* sp. (11%).

Two goat farms from Terengganu were tested and both farms revealed resistance of nematode to albendazole, levamisole, and ivermectin. No information on nematode resistance to anthelmintic drugs in Terengganu was previously reported then. The farms were probably exposed to the parasite due to the climatic condition of the environment and pasture that favour their prevalence (Faisal Abubakar *et al.*, 2015).

From all these studies, anthelmintic resistance in small ruminants in Malaysia is the main problem causing diseases in ruminants and production losses in Malaysia. The main objective of this study was to deduce the efficacy of four drug groups for the effective control of helminthiasis based on analysis of faecal culture (third stage larvae, L3) in order to determine the status of anthelmintic resistance in local smallholder farms in Ipoh.

MATERIALS AND METHOD

Animals and Management

This study was carried out in eight local smallholder goat farms located around Ipoh, Perak, Malaysia. The goats used were Boer, Katjang, Angelo and mixed breeds. Six farms reared the goats with semi-intensive management on oil palm land and with grazing around their pen. Two other farms adopted intensive management where the goats were kept inside pens only. Goats of all the farms were fed with napier grass (*Pennisetum purpureum*) and guinea grass (*Panicum maximum*). Seven farms provided commercial concentrate pellets to the goats when put in shed. Two farms were using oil palm fronds, demerara sugar and soya as feed. Animals in the shed were given water *ad libitum*, and salt or mineral blocks. Mortality rate was between one to four animals a month due to free grazing in oil palm oil land and farm management. Deworming was carried out by two farms (Farm 1 and Farm 5) once or twice a year, five farms only when needed and one farm (Farm 2) had never. Most of the farmers recorded using ivermectins and some farms were unclear as no records were kept.

Laboratory tests

Animals were divided into five groups. Four groups of 10-15 animals each were treated according to the manufacturers' recommended dosage based on individual body weight. The fifth group, the control group, was not given any treatment. Rectal faecal samples were collected from each animal before treatment. 10 to 14 days after treatment, rectal faecal samples were collected again (H.S.M.O., 1986). Faecal culture method was conducted to identify the species of nematodes in all the farms by collecting the faecal samples for pre-treatment and post-treatment according to the drug groups. The samples were cultured for five to seven days and the larvae identified using taxonomy keys in the Manual of Veterinary Parasitological Laboratory Techniques (H.S.M.O., 1986).

Anthelmintic treatment

Four types of anthelmintics: benzimidazoles (albendazole, Vetpharm Laboratories (S) Pte. Ltd.), imidazothiazoles (levamisole, Bovet Pharmaceutical Ltd.), salicylanilides (closantel, Janssen Pharmaceutical Ltd.) and macrocyclic lactones (ivermectin, Kela N.V.) were given to treat 10 to 15 animals per group.

Data analysis

The data was analysed using calculations outlined by Coles *et al.* (1992), resistance was considered when the reduction in faecal egg count was less than 95% and the 95% confidence interval was less than 90%. The status was considered as 'suspected resistance' when only one of the two criteria was met. 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'.

RESULTS AND DISCUSSION

From Table 2, Farm 1 and 6 had resistance to three types of drug, i.e. benzimidazole (71%, 26%), imidazothiazole (95%, 91%) and macrocyclic lactone (40%, 64%) except salicylanilide (99%, 100%). Farm 2 had resistance to two types of drugs and susceptible to imidazothiazole (98%) and salicylanilide (96%). Three farms (Farm 3, 5 and 8) were found to have resistance to all four types of drugs used in this study. The result was resistance to benzimidazole (52%, -120%, 50%), imidazothiazole (levamisole) (90%, 25%, 89%), macrocyclic lactone (57%, -109%, 80%) and salicylanilide (67%, -224%, 75%). Only three types of drugs were used for Farm 4 because the number of animals were not enough to be divided into four groups. Farm 4 had resistance to benzimidazole (0%) and macrocyclic lactone (-1%) except imidazothiazole

Table 1. Summary of farm management in the 8 farms (Farms 1-8) involved in this study.

	Number of animals	Breed	Management type	Feed	Grazing area	Mortality rate	Deworning frequency	Drug
Farm 1	60	Mix	Semi-intensive	grass, pellet	Oil palm land	3-4/month	6 months once	Kelamectin
Farm 2	80	Boer Katjang Angelo	Semi-intensive	grass, pellet	Around the pen	4/month	None	Ivermectin
Farm 3	150	Mix	Semi-intensive	grass, pellet	Oil palm land	4/month	Needed	Ivermectin
Farm 4	34	Local Katjang	Semi-intensive	grass, napier	Oil palm land	None	Needed	Ivermectin
Farm 5	50	Local	Semi-intensive	grass, pellet, soya , gula hitam	Oil palm land	1/week	3 months once	Ivermectin
Farm 6	46	Local	Intensive	grass, pellet	None (in pen)	1/month	Needed	Ivermectin
Farm 7	50	Local	Semi-intensive	grass, pellet	Oil palm land	1/year	Needed	Kelamectin
Farm 8	100	Cross	Intensive	grass, pellet, napier, palm leaf	None (in pen)	10/month	Needed	Albendazole

Table 2. Anthelmintic resistance status on eight smallholder farms (Farms 1-8).

Farm	FECRT Percentage			
	Bz	Leva	ML	Clo
Farm 1	71%	95%	40%	**99%**
Farm 2	80%	**98%**	75%	**96%**
Farm 3	52%	90%	57%	67%
Farm 4	0%	**98%**	-1%	-
Farm 5	-120%	25%	-109%	-224%
Farm 6	26%	91%	64%	**100%**
Farm 7	**100%**	-417%	-83%	-681%
Farm 8	50%	89%	80%	75%

Note: FECR<50% = critical resistance. FECR 50% to 90% = severe resistance. FECR 91% to 95% = moderate resistance. FECR >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 from treatment and control groups for each farm (Farms 1-8).

		Mean faecal egg count (epg)				
		Control	Bz	Leva	ML	Clo
Farm1	Pre-treatment	1713	1488	1550	1138	1400
	Post-treatment	3450	1013	188	2075	57
Farm 2	Pre-treatment	8410	7060	10190	3290	2644
	Post-treatment	8137	2133	166	1963	386
Farm 3	Pre-treatment	2325	1163	1150	763	975
	Post-treatment	2337	1125	238	1014	763
Farm 4	Pre-treatment	2325	1163	400	433	-
	Post-treatment	2337	1125	50	2500	-
Farm 5	Pre-treatment	283	1475	1775	14625	1475
	Post-treatment	233	513	175	488	513
Farm 6	Pre-treatment	29	143	300	100	114
	Post-treatment	328	243	29	116	0
Farm 7	Pre-treatment	87.5	62.5	2025	1387.5	1187.5
	Post-treatment	75	0	387.5	137.5	585.7
Farm 8	Pre-treatment	62.5	362.5	1425	1275	1625
	Post-treatment	50	125	325	425	837.5

Bz = benzimidazole, Leva = levamisole, ML = macrocyclic lactone (ivermectin), Clo = closantel (flukiever).

Table 4. Prevalence of third stage larvae (%), pre-treatment and post-treatment for each farm (Farms 1-8) from treatment and control groups.

Farm	Pre-treatment		Post-treatment	Control	Bz	Imid	ML	Clo
Farm 1	*H. contortus*	98%	*H. contortus*	100%	95%	4%	100%	2%
	Trichostrongylus sp.	2%	*Trichostrongylus* sp.	-	5%	-	-	23%
Farm 2	*H. contortus*	73%	*H. contortus*	85%	92%	53%	88%	64%
	Trichostrongylus sp.	12%	*Trichostrongylus* sp.	8%	8%	41%	12%	20%
	Oesophagostomum sp.	15%	*Oesophagostomum* sp.	7%	-	6%	-	16%
Farm 3	*H. contortus*	84%	*H. contortus*	70%	74%	28%	62%	22%
	Trichostrongylus sp.	8%	*Trichostrongylus* sp.	30%	21%	33%	38%	40%
	Oesophagostomum sp.	8%	*Oesophagostomum* sp.	-	5%	39%	-	38%
Farm 4	*H. contortus*	70%	*H. contortus*	93%	91%	0	96%	-
	Trichostrongylus sp.	30%	*Trichostrongylus* sp.	7%	9%	0	4%	-
Farm 5	*H. contortus*	63%	*H. contortus*	52%	84%	52%	88%	2%
	Trichostrongylus sp.	15%	*Trichostrongylus* sp.	13%	16%	32%	12%	33%
	Oesophagostomum sp.	22%	*Oesophagostomum* sp.	35%	-	16%	-	65%
Farm 6	*H. contortus*	92%	*H. contortus*	100%	85%	2%	15%	0
	Trichostrongylus sp.	5%	*Trichostrongylus* sp.	-	7%	8%	7%	0
	Cooperia sp.	3%	*Cooperia* sp.	-	8%	-	-	0
Farm 7	*H. contortus*	44%	*H. contortus*	48%	0	52%	45%	79%
	Trichostrongylus sp.	23%	*Trichostrongylus* sp.	22%	0	14%	20%	5%
	Oesophagostomum sp.	33$	*Oesophagostomum* sp.	30%	0	34%	35%	16%
Farm 8	*H. contortus*	63%	*H. contortus*	95%	0	73%	90%	0
	Trichostrongylus sp.	21%	*Trichostrongylus* sp.	5%	1%	27%	10%	39%
	Oesophagostomum sp.	16%	*Oesophagostomum* sp.	-	0	-	-	61%

Bz = benzimidazole, Leva = levamisole, ML = macrocyclic lactones (ivermectin), Clo = closantel (flukiever).

(98%). From all these eight farms, only Farm 7 could use benzimidazole (100%) to control anthelmintics but had resistance to other drugs, i.e. imidazothiazole (-417%), macrocyclic lactone (-83%) and salicylanilide (-681%). Standard questionnaires were used to interview the management of the farms on deworming history. Six out of eight farmers were using macrocyclic lactone (ivermectin) to treat the animals and all the farms were found to have resistance to this drug (Table 1).

In Farm 1, pre-treatment L3 identification found *Haemonchus contortus* as the predominant species (98%), followed by *Trichostrongylus* sp. (2%). Post-treatment found resistance of *Haemonchus contortus*, *Trichostrongylus* sp. and *Cooperia* sp. to benzimidazoles, levamisole and macrocyclic lactones (ivermectin). Salicylanilide was the only effective drug.

In Farm 2, pre-treatment faecal culture found *Haemonchus contortus* as the predominant species (73%) followed by *Oesophagostomum* sp. (15%) and *Trichostrongylus* sp. (12%). Post-treatment found *Haemonchus contortus*, *Oesophagostomum* sp. and *Trichostrongylus* sp. resistant to benzimidazole and macrocyclic lactone. The other two drugs, imidazothiazole and salicylanilide, was very effective.

In Farm 3, *Haemonchus contortus* (84%), *Trichostrongylus* sp. (8%) and *Oesophagostomum* sp. (8%) was cultured in pre-treatment. Post-treatment faecal cultures showed resistance of *Haemonchus contortus*, *Trichostrongylus* sp. and *Oesophagostomum* sp. to all four types of drugs used.

In Farm 4, pre-treatment faecal culture found *Haemonchus contortus* as predominant species (70%) followed by *Trichostrongylus* sp. (30%). Post-treatment found *Haemonchus contortus* and *Trichostrongylus* sp. resistant to benzimidazole and macrocyclic lactone. Imidazothiazole was the only effective drug. Only three types of drugs were used because of insufficient number of animals.

In Farm 5, pre-treatment faecal culture found *Haemonchus contortus* as main nematode (63%), followed by *Oesophagostomum* sp. (22%) and *Trichostrongylus* sp. (15%). Post-treatment found *Haemonchus contortus*, *Oesophagostomum* sp. and *Trichostrongylus* sp. resistant to benzimidazole, imidazothiazole, macrocyclic lactone and salicylanilide.

In Farm 6, pre-treatment found *Haemonchus contortus* as the predominant species (92%), followed by *Trichostrongylus* sp. (5%) and *Cooperia* sp. (3%). Post-treatment found resistance of *Haemonchus contortus*, *Trichostrongylus* sp. and *Cooperia* sp. to benzimidazole, levamisole and macrocyclic lactone (ivermectin). Salicylanilide as a very effective drug for this farm.

In Farm 7, pre-treatment faecal culture found *Haemonchus contortus* (44%), *Trichostrongylus* sp. (23%) and *Oesophagostomum* sp. (33%). Post-treatment found *Haemonchus contortus*, *Oesophagostomum* sp. and *Trichostrongylus* sp. resistant to imidazothiazole salicylanilide and macrocyclic lactone. Benzimidazole was very effective.

In Farm 8, pre-treatment faecal culture found *Haemonchus contortus* (63%), *Trichostrongylus* sp. (21%) and *Oesophagostomum* sp. (16%). Post-treatment faecal cultures found resistance of *Haemonchus contortus*, *Trichostrongylus* sp. and *Oesophagostomum* sp. to all four types of drugs: benzimidazole, imidazothiazole, macrocyclic lactone and salicylanilides (Table 4).

The results show that anthelmintic resistance has been an escalating problem of small ruminants as the existing worm population were showing severe resistance to common anthelmintics. In order for the small ruminants industry to grow and perpetuate, alternative worm control methods are recommended to reduce morbidity and mortality in the sheep and goats.

As helminthiasis is the main cause of poor productivity, it is important to regularly monitor worm egg counts in small ruminants and employ novel, green management methods of worm control such as the use of herbal remedies. Studies on nematode control were in two ways, that is, as chemical and non-chemical methods.

In chemical methods, suppressive treatments have been used on small ruminants in the tropics and sub-tropics and its advantages has been very effective in the short term in reducing parasite populations and production losses.

Other than that, monitoring of *Haemonchus* infections using the FAMACHA system (Van Wyk and Van Schalkwyk, 1990) is cheap, easy to apply and easy for farmers to understand. It was reported to reduce the cost of drenching and will lower anthelmintic resistance.

There are many drugs in market to control anthelmintics, and some recommends to use the drugs in effective way, that is, to place the drench gun over the tongue. This will allow the drench to by-pass the rumen and will shorten the time for a sufficiently high concentration of drug to kill the worms. Before drenching, a reduction in feed intake will prolong drench uptake, extending the effective duration of the killing effect.

In non-chemical methods, pasture management have proved helpful in the control of helminths.

Rapid rotational grazing is an excellent tool, from the productivity point of view, as animals will consume a higher proportion of the available forage, thereby stimulating pasture re-growth (Barger, 1999). Use of this method will reduce use of chemicals, reduce risk of resistance development and improve pasture utilisation.

Another way in pasture management is alternate grazing. Alternate grazing is based on different age groups of the same species, or different species, grazing the pastures in sequence. This way will reduce the number of infective larvae on pasture because older animals, or different species, will act as a 'vacuum cleaner'.

The most important is supplement feeding. Improving nutrition will reduce production losses and mortality rates due to reduction of worm parasites.

CONCLUSION

As a conclusion, more studies need to be done in every district to create awareness among the farmers and overcome anthelmintic resistance.

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