LEVAMISOLE RESISTANCE TO A STRONGYLE POPULATION IN A SMALLHOLDER GOAT FARM IN MALAYSIA

THONGSAHUAN S.[1], PREMAALATHA B.[2], LILY ROZITA M.H.[2], ERWANAS A.I.[2], JAMNAH O.[2], CHANDRAWATHANI P.[2]*, RAMLAN M.[2] AND CHETHANOND U.[1]

1 Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla 90110, Thailand,

2 Veterinary Research Institute, No. 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia,

* Corresponding author: chandra1959@gmail.com

ABSTRACT. The faecal egg count reduction test (FECRT) was conducted on local goats in a smallholder farm on the outskirts of Ipoh. Levamisole drug tested for resistance against strongyles, was administered orally at the rate of 10 mg/kg body weight. Results showed that the strongyle worm population was resistant to levamisole. The percentage reduction was 75% and lower confidence limit less than 90%. The worm population was made up mainly of *Haemonchus contortus* (71%), followed by *Oesophagostomum* sp. (18%) and *Trichostrongylus* sp. (11%). This finding indicates that anthelmintic resistance is an existing problem in Malaysia, even on a small farm. Alternative approaches to chemical anthelmintics have been recommended to control nematodes in goats such as improved grazing management, herbal medication or biological control, to delay the occurrence of chemical resistance and prevent severe helminth infections in goat flocks.

INTRODUCTION

Gastrointestinal nematodes, namely strongyle worms have been recognised as a major cause of disease and production loss in small ruminants around the world, particularly in sheep and goats in Malaysia. The strongyle worm, *Haemonchus contortus*, is considered to be the most pathogenic parasite found commonly in the country (Cheah and Rajamanickam, 1997; Sani *et al*., 2004; Chandrawathani *et al*., 2009), while others such as *Trichostrongylus* sp., *Oesophagostomum* sp., *Bunostomum* sp. and *Cooperia curticei* also contribute to overall helminthic problems (Dony *et al*., 1995; Khadijah *et al*., 2006a; Rahman 1995). Infection is due to grazing activity, when the goats graze on pasture contaminated with the third stage larvae of nematodes.

Resistance to anthelmintic drugs, particularly in small ruminants, is of major concern in Malaysia, probably due to the intensive and indiscriminate use of both a broad and narrow spectrum of drugs for controlling nematodes. A nationwide

survey on anthelmintic resistance in Malaysia, as recorded by Dony *et al*. (1994), revealed the resistance status to benzimidazole in 33 out of 96 goat farms. Levamisole resistance also was detected in investigations on goat farms (Dony *et al*., 1994; Rahman, 1994). Later, resistance to benzimidazoles, levamisole, a combination of benzimidazoles/levamisole, macrocyclic lactones and closantel were detected in 48 small ruminant farms throughout the country (Chandrawathani *et al*. 1999). Anthelmintic resistance over the past decade also was recorded in both government and private goat farms (Chandrawathani *et al*., 2004b; Khadijah *et al*., 2006a; 2006b; Basripuzi *et al*., 2012). As government and private farms provide animals to smallholder farms throughout the country, there is a high probability that this severe problem has spread widely.

A reliable and common method for determining anthelmintic efficacy is the faecal egg count reduction test (FECRT) (Coles *et al*., 1992). While, *in vitro* assays such as the egg hatch assay (EHA), larval development assay (LDA) and larval migration inhibition assay (LMIA), although providing accurate results in an anthelmintic resistance survey, these methods are deemed expensive and required intensive procedures (Rahman 1993; Dony *et al.,* 1994; Demeler *et al*., 2012, 2013).

Recently, the anthelmintic resistance status of two smallholder goat farms in Malaysia documented that *H. contortus* and *Trichostrongyllus colubriformis* were resistant to four commercial anthelmintics i.e. albendazole, levamisole, ivermectin and closantel (Chandrawathani *et al*., 2013). Following this, an investigation by the Veterinary Research Institute was carried out in September 2013 at a smallholder goat farm on the outskirts of Ipoh with a complaint of ineffective anthelmintic therapy. The occurrence of anthelmintic resistance to Flukiver® (closanal 5% w/v), albenthic® (albendazole 2.5% w/v) and kelamectin® (ivermectin 10% w/v) was detected, while nematode populations were suspected resistant to levamisole (unpublished data). In order to prevent the spread of resistance and as a proactive initiative to institute suitable control measures, monitoring the efficacy of anthelmintic drugs should be done at least once a year. Hence, the aim of this study was to evaluate the current status of levamisole resistance in this local smallholder goat farm in Ipoh by using FECRT.

MATERIALS AND METHODS

Animals & Management

This study was carried out in a local smallholder goat farm located in Sungai Siput, Perak, Malaysia. The goats on this farm were Boer and mixed breeds. The animals were reared with semi-intensive management, whereby all of them were grazed on pasture, the roadside (private area) and around a shed during the day, and were housed in raised floor wooden

sheds during the night. Pasture grazing comprised Napier grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*) and Signal grass (*Brachiaria humidicola*). They were offered water *ad libitum*, salt or mineral licks, and the farmer also provided oil palm leaves and some commercial pelleted feeds. All goats in the treatment and control group had not been dewormed for at least 8 weeks prior to this study.

Laboratory tests

At least 3 grams of faeces were collected directly from the rectum, at pre-treatment (Day 0) and 7 days after treatment from both the control and treatment group. They were subjected to the McMaster technique for a faecal egg count (Coles *et al*., 1992). Consequently, a faecal culture was performed by pooling all pre-treatment samples in order to determine the species of helminths, as described by Chandrawathani *et al.* (2013). The morphology of infective stage larva (L3) was identified after 7 days by following van Wyk J.A. and Mayhew E. (2013).

Anthelmintic treatment

Ten goats in the treatment group were fed orally with levamisole. Dose rates were according to the manufacturer's recommendations and based on estimated individual body weight (10 mg/kg body weight). The goats in the control group did not receive any treatment.

Data analysis

The Faecal Egg Count Reduction Test (FECRT) was performed to evaluate anthelmintic resistance status. 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 if only 1 criterion of these 2 criteria was met. The status was considered as susceptible when the reduction in faecal egg count and the lower confidence limit were more than 95% and 90%, respectively (Coles *et al*., 1992).

RESULTS

All selected goats in both groups were suitable to be used in this study, as more than 150 eggs per gram faeces (e.p.g) were detected in each animals in the pre-treatment screening. Seven days post treatment, the average faecal egg count investigation revealed 633 and 160 e.p.g. in the control and treatment group, respectively. The drug showed a drop of 75% at the lower confidence limit to 27% (Table 1). This result indicated that the strongyle nematode population on this farm was resistant to levamisole. According to Coles *et al.*, 1992 the worm population in this farm can be considered severely resistant to levamisole with an FECR percentage of between 50-90%. Furthermore, *Strongyloides* sp. eggs and coccidian oocysts also were detected in the goats, as shown in Figure 1.

In addition, the infective larvae population recovered from pre-treatment faecal cultures revealed that *H. contortus* was the predominant strongyle nematode species (71%), followed by *Oesophagostomum* sp. (18%) and *Trichostrongylus* sp. (11%).

DISCUSSION AND CONCLUSION

Helminthiasis caused by strongyle worm is a major disease of concern in small ruminant flocks in Malaysia. The microenvironment in this country, such as high temperature, humidity and continuous rainfall throughout the year is responsible for the development of eggs to the infective stage on pasture. Generally, seasonal burdens of the nematodes in Malaysia were found correlate highly to rainfall (Rahman, 1995), with more than 2,000 mm per annum rainfall recorded (Malaysia Meteorological Department, 2013). Additionally, chemical anthelmintics are used regularly for long periods of time in small ruminant farms, which has led to development of resistant nematodes.

In this study, levamisole (Imidazothiazoles group) may not be used effectively for controlling strongyle nematodes in this smallholder farm, due to severe resistance against this drug, which developed within one year, with a faecal egg count reduction of lower than 95% and lower confidence limit level of less than 90%. This finding was in accordance with the study of Chandrawathani *et al.* (2013), where levamisole failed to control helminthiasis in two smallholder goat farms in Ipoh. The levamisole resistance of nematodes in goats was reported since 1994 (Dony *et al.*, 1994). However, nematode populations in sheep are generally susceptible to levamisole rather than goat

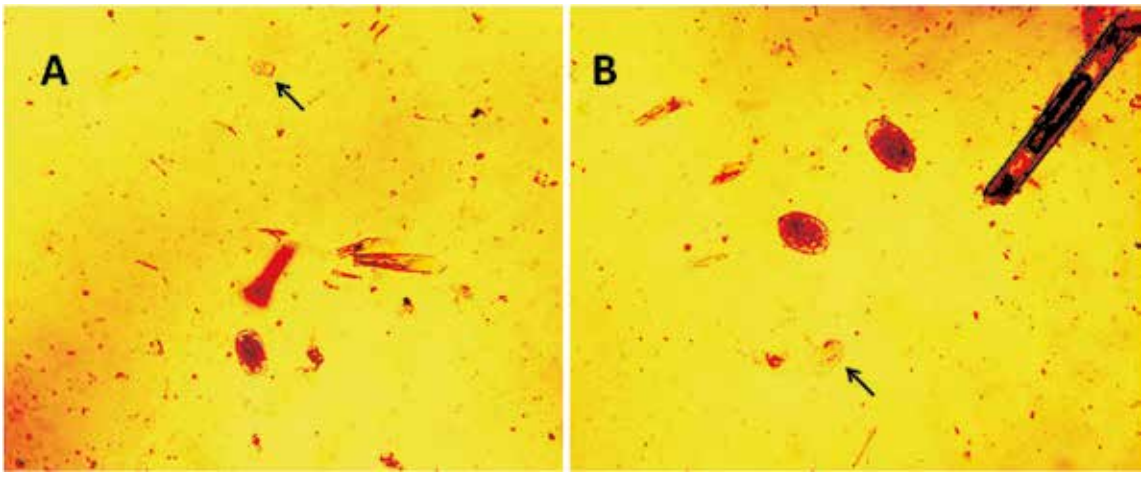

Figure 1. The low power-view of the McMaster floatation technique showing Strongyle egg and coccidian oocyst (marked in arrow) (A), two Strongyle eggs and Strongyloides sp. egg (marked in arrow arrow) (B)

Table 1. Faecal egg count from the treatment and control group and anthelmintic resistance status on a smallholder goat farm in Ipoh.

	Levamisole N=10	Control N=6
Mean epg Pre treatment	1,530	1,750
Mean epg Post treatment	160	633
Reduction	75%	
Upper confidence limit	91%	
Lower confidence limit	27%	

flocks in some areas (Chandrawathani 1999, Khadijah *et al*., 2006a). The result of this study was contrary, with high effectiveness of levamisole against strongyle nematodes in goats in Kelantan, as reported by Basripuzi *et al.* (2012). This may be due to all selected small ruminant farms had never or seldom used levamisole or other imidazothiazole drugs for controlling nematodes.

Furthermore, novel methods for controlling gastro-intestinal nematodes in small ruminants which are managed intensively is recommended to prevent infections rather than treat them. Alternative approaches to minimize the use of chemical drugs to delay the occurrence of resistance and increase immunity, such as cut and carry feeding, improved grazing management, use of herbal medication and biological control is nowadays more popular. According to the intensive management system, animals are not allowed to graze around sheds that may have been contaminated with nematodes larvae. Cut grass is given to the animals in sheds. However, this system was not successful in controlling nematode infection in sheep farms in Kedah (Khadijah *et al*., 2006b). This may be due to a permanent nematode burden on pasture all year round in selected areas, and the deworming program in animals on the farm. Based on the ability of larva development and survival on pasture, rotational grazing is suggested for controlling helminthiasis but this may depend on availability of pasture for rotation. Animals graze safely for 3-4 days on the pasture which was rested for 5-6 weeks (Chandrawathani *et al*., 2004b). Regarding herbal medication, goats can be fed with neem leaves (*Azadirachta indica*) instead of chemical drugs. This has been reported to give promising results in worm control (Chandrawathani *et al*., 2006). Another alternative method is biological control, with nematophagus fungi (*Duddingtonia flagrans*) being used successfully in controlling management against larvae on pasture (Chandrawathani *et al.,* 2004a). For water supply, farmers

should treat animal drinking water and clean the container as well. Additionally, smallholder farmers can monitor their animals regularly by using the FAMACHA technique, an anemia guide, in order to estimate *H. contortus* infections instead of faeces examination (Chandrawathani and Nurulaini, 2012). Anthelmintics are the last choice for controlling nematode populations, rotation and the appropriate use of drugs combined with alternative methods are recommended for reducing resistance problems.

In conclusion, the smallholder goat farm on the outskirts of Ipoh revealed the ineffectiveness of levamisole, seen by the strongyle population which was highly resistant, and *H. contortus* was the predominant nematode species on this farm.

REFERENCES

1. Basripuzi HB, Sani RA and Ariff OM (2012). Anthelmintic resistance in selected goat farms in Kelantan. *Mal J Anim Sci*. **15**; 47-56.
2. Chandrawathani P, Adnan M and Waller PJ (1999). Anthelmintic resistance in sheep and goat farms on Peninsular Malaysia. *Vet Parasitol*. **82**; 305-310.
3. Chandrawathani P, Chang KW, Nurulaini R, Waller PJ, Adnan M, Zaini CM, Jamnah O, Khadijah S and Vincent N (2006). Daily feeding of neem leaves (*Azadirachta indica*) for worm control in sheep. *Trop Biomed*. **23**; 23-30.
4. Chandrawathani P, Jamnah O, Adnan M, Waller PJ, Larsen M and Gillespie AT (2004a). Field studies on the biological control of nematode parasites of sheep in the tropics, using the microfungus *Duddingtonia flagrans*. *Vet Parasitol*. **120**:177-87.
5. Chandrawathani P and Nurulaini R (2012). Current trends in helminth parasite control in small ruminants in Malaysia and South East Asia. *Malaysian J Vet Res*. **3**; 23-28.
6. Chandrawathani P, Nurulaini R, Adnan M, Premalaatha B, Khadijah S, Jamnah O, Zaini CM, Khor SK and Zawida Z (2009). A survey of parasitic infection on small ruminant farms in Kinta and Hilir Perak districts, Perak, Malaysia. *Trop Biomed*. **26**; 11-15
7. Chandrawathani P, Premaalatha B, Nurulaini R, Erwanas AI, Zaini CM, Aizan M, Ramlan M and Khadijah S (2013). Severe Anthelmintic Resistance in Two Free Grazing Small holder Goat Farms in Malaysia. *J Veterinar Sci Technolo*. **4**: 4.
8. Chandrawathani P, Yusoff N, Wan LC, Ham A and Waller PJ (2004b). Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, East Malaysia. *Vet Res Comm*. **28**.
9. Cheah TS and Rajamanickam C (1997). Epidemiology of gastro-intestinal nematodes of sheep in wet tropical conditions in Malaysia. *Trop Anim Health Pro*. **29**, 165–173.
10. Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA and Waller PJ (1992). World Asosciation for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelminthic resistance in nematodes of veterinary importance. *Vet Parasitol*. **44**: 35-44
11. Demeler J, Gill JH, von Samson-Himmelstjerna G and Sangster NC (2013). The *in vitro* assay profile of macrocyclic lactone resistance in three species of sheep trichostrongyloids. *Int J Parasitol Drugs Drug Resist*. **3**: 109-118.
12. Demeler J, Kleinschmidt N, Küttler U, Koopmann R and von Samson-Himmelstjerna G (2012). Evaluation of the Egg Hatch Assay and the Larval Migration Inhibition Assay to detect anthelmintic resistance in cattle parasitic nematodes on farms. *Parasitology Int*. **61**; 614-618.
13. Dorny P, Claerebout E, Vercruysse J, Sani R and Jalila A (1994). Anthelmintic resistance in goats in peninsular Malaysia. *Vet Parasitol*. **55**; 327-342.
14. Dony P, Symoens C, Jalila A, Vercruysse J and Sani R (1995). Strongyle infections in sheep and goat under the tradition husbandry system in Peninsular Malaysia. *Vet Parasitol*. **56**; 121-136.
15. Khadijah S, Rahman WA, Chandrawathani P, Waller PJ, Vasuge M, Nurulaini R, Adnan M, Jamnah O, and Zaini CM (2006a). Small ruminants on private farms in peninsular Malaysia: nematode resistance to anthelmintics. *J Vet Malaysia*. **18**; 29-32.
16. Khadijah S, Rahman WA, Chandrawathani P, Waller PJ, Vasuge M, Nurulaini R, Adnan M, Jamnah O, Zaini CM and Vincent N (2006b). Nematode anthelmintic resistance in government small ruminant farms in peninsular Malaysia. *J Vet Malaysia*. **18**: 1-5.
17. Malaysia Meteorological Department. In General Climate Information. Available from http://www.met.gov.my/index.php?lang=english. cited 2014-03-28.

18. Rahman WA (1993). An assessment of thiabendazole-resistant nematodes in some smallholder goat farms of Malaysia using the egg hatch assay method. *Vet Parasitol.* **51**(1-2): 159-61.
19. Rahman WA (1994). Survey for drug-resistant trichostringyle nematodes in ten commercial goat farms in west Malaysia. *Trop Anim Health Prod.* **26**: 235-238.
20. Rahman WA (1995). Seasonal variation of trichostrongylid nematode populations in goats [Malaysia]. *Malays Appl Biol.* **24**: 5-10.
21. Sani, RA, Adnan M, Cheah TS and Chandrawathani P (2004). Worm control for small ruminants in goats in Serdang, West Malaysia. *Kajian Veterinar.* **17**: 127-131.
22. van Wyk JA and Mayhew E (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Onderstepoort J Vet Res.* **80**(1); 1-14.