RECENT STATUS OF LEVAMISOLE RESISTANCE IN SELECTED GOAT FARMS IN KOTA BHARU AND BACHOK, KELANTAN.

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CERTIFICATION

This is to certify that we have read this research paper entitled 'Recent status of Levamisole resistance in selected goat farms in Kota Bharu and Bachok, Kelantan.' By Nur Alawiyah Binti Mohd Awalluddin, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 55204 – Final year project.

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Thank you

DEDICATIONS

I WOULD LIKE TO DEDICATE ALL MY JOURNEY AND OUTCOMES OF MY PROJECT AND THESIS TO MY SUPERVISOR, FAMILY, FRIENDS AND TO ALL THOSE WHO HAS HELPING AND BEEN PART OF THE PROCESS.

HONESTLY,

ALAWIYAH AWALLUDDIN

Table of content

List of Tables

Page

List of Figures **Page**

List of Appendices **Page**

List of symbols and abbreviation

FEC	Faecal Egg Count
FECRT	Faecal Egg Count Reduction Test
FECR%	Faecal Egg Count Reduction Percentage
epg	Egg per gram
NaCl	Sodium chloride
PGE	Parasitic Gastroenteritis

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine in partial requirement on the course course DVT 55204 – Final year project.

RECENT STATUS OF LEVAMISOLE RESISTANCE IN SELECTED GOAT FARMS IN KOTA BHARU AND BACHOK, KELANTAN

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Anthelmintic resistances is a widespread problem in small ruminant industry. Levamisole was the only anthelmintic that was found to be effective against gastrointestinal nematode in goat farms in Kelantan about 10 years ago. However, the current status of levamisole in Kelantan is still unknown. Hence, this study aims to investigate the latest status of levamisole resistances in selected goat farms located in Kelantan. The study was conducted on goat farms located in Bachok (n=1) and Kota Bharu (n=1). The goats in these farms were screened for the criteria of Faecal Egg Count Reduction Test (FECRT) and divided into control and treatment groups with approximately similar means of faecal egg count (FEC). Faecal samples that were collected during pre- and post-treatments were subjected to McMaster and faecal culture. The obtained data was recorded and analysed by an automated FECRT spreadsheet. Reduction of post-treatment mean FEC were observed in both farms. Faecal Egg Count Reduction percentage (FECR%) of 88% and 100% were observed in Farm 1 and Farm 2 respectively. Infective stage larvae (L3) identification revealed three genera of gastrointestinal nematode namely *Oesophagostomum sp.*, *Trichostrongylus sp.* and *Haemonchus contortus* which predominant the infection. This study shows levamisole resistance has been developed in Farm1. Farm 2 status was observed with low count thus levamisole resistance was not suspected in this farm.

Keyword: Anthelmintic resistance, levamisole, FECRT, FECR%, infective stage larvae

ABSTRAK

Status terkini terhadap ketidakberkesanan ubat cacing levamisole di dalam ladang kambing tertentu di Bachok, Kelantan

Oleh

Nur Alawiyah Binti Mohd Awalluddin

Kerintangan ubat cacing merupakan satu cabaran besar pada industri ruminan kecil. Levamisole merupakan satu-satunya ubat cacing yang masih berkesan melawan kecacingan di ladang kambing Kelantan pada 10 tahun yang lalu. Namun, status terkini levamisole di Kelantan masih belum diketahui. Maka, kajian ini bertujuan untuk mengkaji status terkini kerintangan ubat cacing levamisole di ladang-ladang kambing terpilih di Kelantan. Kajian ini dilakukan di ladang kambing di Bachok (n=1) dan Kota Bharu (n-1). Kambing-kambing di ladang disaring untuk menepati kriteria Ujian Penurunan Kiraan Telur Dalam Tinja (FECRT) dan dibahagi kepada kumpulan rawatan dan kawalan dengan jumlah kiraan telur cacing (FEC) yang sama nilai setiap kumpulan. Sampel tinja kambing yang telah dikutip sebelum dan selepas rawatan dihantar untuk McMaster dan kultur tinja. Data yang diperoleh direkod dan dianalisis menggunakan hamparan automatik FECRT. Penurunan pada purata FEC selepas rawatan dapat dilihat pada kedua-dua ladang. Peratus penurunan kiraan telur dalam tinja (FECR%) 88% dan 100% telah dilihat di Ladang 1 dan Ladang 2. Identifikasi larva (L3) menemui tiga genera cacing gastrousus iaitu *Oesophagostomum sp. Trichostrongylus sp*, dan *Haemonchus contortus* yang mendominasi kecacingan ini. Kajian menunjukkan kerintangan ubat cacing sudah berlaku pada Ladang 1. Manakala, Ladang 2 dilihat mempunyai kiraan rendah maka kerintangan ubat cacing levamisole tidak disuspek di ladang ini.

Keyword: Kerintangan ubat cacing, levamisole, FECRT, FECR%, larva infektif,

1.0 INTRODUCTION

Levamisole is a chemical anthelmintic that belongs to a group called Imidazothiazole. It serves the purpose of eliminating gastrointestinal worms, specifically nematodes by acting on the nematode nervous system. However, there was no effect on the nematode eggs as it does not have ovicidal properties (Boothe, 2014). Furthermore, levamisole is commonly used in the livestock industry to combat helminthiasis infestation. According to Basripuzi et al. (2012), levamisole was the most effective anthelmintic used in goat farms in Kelantan in comparison to the other anthelmintics such as albendazole, ivermectin and closantel.

Helminthiasis was a common infection in animals, especially in ruminant livestock. According to Basripuzi et al. (2012), small ruminants especially goats were more susceptible compared to sheep to be infested by gastrointestinal helminth, resulting in a disease condition called as parasitic gastroenteritis (PGE). PGE can be defined as an infection of more than one gastrointestinal helminth species in ruminants. The examples of helminth that commonly caused PGE in Malaysia were *Haemonchus contortus*, *Trichostrongylus sp*. and *Oesophagostomum sp*. (Basripuzi et al.,2012). On the other hand, the second most significant disease among small ruminants in Malaysia is PGE (Sani and Chandrawathani, 1996)

1.1 **Research problem**

In Malaysia, anthelmintic resistance has been a rising issue as it has a long-term effect on the livestock industry. It happens as the consequences of some farmers practising unsystematic deworming strategies and incorrect dosage of anthelmintic given to the goat on the farm. Furthermore, according to Chandrawathani et al. (1999), the repeated and improper use of anthelmintics are regularly being practised by farmers. Anthelmintic resistance can lead to a serious problem to the livestock industry if it is not being tackled properly. The ineffectiveness of anthelmintics could lead to PGE hence clinical sign of poor body condition score, retarded growth, and delayed obtain of the market body weight.

Referring to Basripuzi et al. (2012), the level of concern regarding the status of anthelmintic resistance in Kelantan has reached a critical state where the anthelmintics used during that time not being effective except levamisole. Thus, there is a need to re-evaluate the current status of levamisole whether resistant has been developed or it still remains effective.

1.2 Research question

1.2.1 What is the mean FEC before and after levamisole treatment in each farm?

1.2.2 What is the current resistance status of levamisole in goat farms in Kelantan?

1.2.3 Which genus of endoparasite that are affected and/or survive levamisole treatment?

1.3 Research hypothesis

1.3.1 Null Hypothesis: Levamisole remains effective in combating endoparasites in the selected goat farms of this study.

1.3.2 Alternate Hypothesis: Levamisole resistant has been developed in the selected farms of this study.

1.4 Research objective

1.4.1 To calculate the mean faecal egg counts before and after levamisole treatment in each farm.

1.4.2 To determine the status of levamisole resistance in selected farms in Kelantan.

1.4.3 To identify the genus of endoparasites before and after levamisole treatment.

2.0 LITERATURE REVIEW

2.1 Helminth control approaches

Over the past decades, goat farmers of smallholder or large companies have implemented many approaches to control and minimise the helminth infestation from infecting their livestock. However, study conducted by Chandrawathani & Raimy (2012), stated that helminth infestation in small ruminants in Malaysia were highly controlled by anthelmintics which have been given frequently, usually once every month. This action led to consequences of having serious anthelmintic resistance in the helminth population. Hence, there is a need to seriously look for alternative methods of helminth control.

Chandrawathani & Raimy (2012) had introduced several practices to farmers that have encountered cases of anthelmintic resistance in the farm. Examples of such practise were rapid rotational grazing, cut and carry system or zero grazing method, improving the flock nutrition, usage of herbal remedies, tethering and biological control method. Furthermore, along with each practice, the author also included a brief explanation, the advantages and disadvantages of each method. From this study, there are various combination methods of helminth control to obtain the best outcome in raising their flock, but with financial and available facilities to keep in mind

Kumar *et al.* (2012) concluded that pasture, housing system and nutritional management play important roles in combatting helminthiasis. Additional nutrition such as mineral and vitamin have a direct relation with animal susceptibility to helminth. For example, adequate vitamin A will enhance the intestinal epithelial integrity. Meanwhile, zinc helps in improve body immunity by ensuring the function of cell mediated cytotoxicity and function of T helper cell (Kumar *et al.*, 2012). The condition and hygiene of the housing influences the animal health as animal kept in good condition has better tolerant against helminth infection compared to animal in poor housing condition. Management of pasture such as rotation grazing in which the livestock are let to graze on clean pasture fields which has not been grazed six to 12 months, and also practise regular burning of old pasture field to gained a free parasite pasture land (Kumar *et al.*, 2012).

Nisbet et al. (2016) and Claerebout & Geldhof, (2020) proved the establishment of vaccines and its development can promote immunity against *H. contortus* in small ruminant. The vaccinated young animals were found to be protected greatly by the vaccine and proved by 80% reduction of FEC (Claerebout & Geldhof, 2020). On the other hand, this vaccine practise was use in sheep and cattle, but only available in Australia and South Africa. However, this could be one of the methods of future helminth control in Malaysia.

2.2 Faecal egg count reduction test (FECRT)

There is an approach to evaluate the present of anthelmintic resistance in the farm. Based on a study conducted by Basripuzi et al. (2012), the author states that anthelmintic resistance is an ability of helminths to survive anthelmintic treatment. Hence, this would explain the reason behind anthelminthic ineffectiveness that was faced by the small ruminant farmers. Evaluation of anthelmintic resistance status can be obtained through FECRT, in which it was based on the method described by the World Association for Advancement of Veterinary Parasitology (Coles, 1992). Prior to the FECRT test, the goats must not be dewormed for eight to 12 weeks. Screening of the samples must be performed by the modified McMaster technique to determine if the goats has at least 150 eggs per gram (epg) of faeces thus eligible for FECRT

FECRT percentage was calculated as in FECR% formula listed below, where Xt represent the mean epg of post-treatment in the treated group and Xc represent the mean epg of post-treatment of control group. Coles, (1992) has pointed out that resistance is present when two criteria are fulfilled: i) FECR% is less than 95%, and ii) 95% lower confidence limit is less than 90%. In addition, if only one of the two elements achieved, anthelmintic resistance was suspected.

FECRT formula:

Faecal Egg Count Reduction Percentage (FECR%) = $(1 – Xt / Xc) \times 100$

2.3 Anthelmintic resistance in Malaysia

A number of authors have investigated anthelmintic resistance among small ruminants in our country, including Chandrawathani et al. (1999), Chandrawathani et al. (2004), Basripuzi et al. (2012), Chandrawathani et al. (2013), Abubakar et al (2015) and Premaalatha et al. (2019).

Chandrawathani et al. (1999) has investigated anthelmintic resistance in ovine and caprine farm in Peninsular Malaysia and concluded that in studied farms, resistance toward benzimidazole (6 farms), levamisole (4 farms) and closantel (2 farms) have been developed. The study also found that benzimidazole (1 farm), levamisole (2 farms) and combination of anthelmintics (1 farm) were suspected resistance while it is susceptible for benzimidazole, levamisole and ivermectin each in one farm and two farm for moxidectin.

Chandrawathani et al. (2004) discovered total anthelmintic failure in small government ruminant breeding farms in Sabah, East Malaysia and has found that benzimidazole, ivermectin, levamisole and closantel resistance have been developed in the studied farms from September 2002 to February 2003.

Basripuzi et al. (2012) has conducted a study of anthelmintic resistance in chosen goat farms in Kelantan. The result from the studied shows various degree of resistance (critical, severe and moderate) and suspected resistance for albendazole, ivermectin levamisole and closantel in eight studied farms. In the study, levamisole was found to have moderate resistance (2 farms), susceptible (2 farms) and suspected resistance (1 farm).

Chandrawathani et al. (2013) detected about severe anthelmintic resistance in two free grazing smallholder goat farms in Malaysia. The study had found that both farms were suffering from anthelmintic resistance for benzimidazoles, levamisole and closantel. However, one farm was susceptible to ivermectin while the others had developed ivermectin resistant.

Moreover, Abubakar et al (2015) has conducted a preliminary study of helminth resistance to anthelmintic drugs in two goat farms in Terengganu. In the study, two farms in Terengganu were studied and tested with albendazole, levamisole and ivermectin. One farm had critical resistance to all three anthelmintics. Meanwhile other farms were found to had critical resistance to ivermectin and severe resistance toward albendazole and levamisole.

Premaalatha et al. (2019) identified anthelmintic resistance in small scale goat farms in Perak. Four types of anthelmintics were tested in this study. The study was conducted in 2013 to 2014 has found that two farm had resistance to benzimidazole, levamisole and ivermectin. Closantel and levamisole resistance were found in one farm while three farms were found to have resistance for all anthelmintics tested.

3.0 MATERIAL AND METHODS

3.1 Ethical approval and consent

This study was approved by the Institutional Animal Care and Use Committee (AICUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The approval code is UMK/FPV/ACUE/FYP/025/2022).

3.2 Faecal sample collection

Faecal samples were collected from two goat farms. Goat faeces was collected per rectum from at least 20 goats that was randomly chosen in each farm. Each goat was properly restrained prior to faecal sampling. Minimum 8 pellets of goat faeces were collected from each goat in this study using a plastic glove and lubricant. The collected faecal samples were then labelled based on ID number of individual goat or based on the particular pen where the goat was located. Once labelled, the faecal samples were kept in a cold container to be transported to the Parasitology Laboratory. In this study, collection of faecal samples was conducted twice in each farm for pre-treatment and post-treatment data.

3.3 Faecal sample preparation for McMaster and faecal culture

Sample preparation was conducted prior to McMaster and faecal culture methods. One grams of goat faeces was separated from each collected faecal sample and kept in a plastic glove at 4°C for McMaster method. The remaining faecal samples were pooled in plastic containers and stored in a dark place at room temperature for faecal culture.

3.4 McMaster Technique

One gram of faeces was weighted from the prepared samples and placed in a numbered plastic container. Saturated sodium chloride (NaCl) solution of 15ml (SG 1.2) was added into the plastic container. Faecal pellets and saturated sodium chloride was mixed and filtrated. The filtrate was filled into two McMaster chambers using a dropper.

The helminth eggs were identified with criteria of having thin-shelled, broad ellipse and barrel-shaped side wall and contains blastomere. The eggs that were within ruled area of McMaster chamber was identified and counted under the microscope with ten times (10x) magnification.

$$\text{McMaster calculation (epg)} = \frac{\text{Total no of eggs counted within 2 chambers}}{\text{Weight of faeces (g)}} \times \frac{\text{Volume of NaCl solution}}{2(0.15)}$$

3.5 Faecal culture

The pooled faecal samples were broken using mortar and pestle and packed in a container. Distilled water was added to provide moisture to the faeces. The lid of container was covered using clean gauze and tightened with rubber band. The faecal cultures were kept in a dark area for 7 days at room temperature. Distilled water was added on a daily basis to maintain moisturized condition of the faecal culture. The infective stage larvae (L3) were harvested, and stored in falcon tubes based on pre and post-treatment sampling for treatment and control group. The L3 were kept at 4°C for identification.

3.6 Method of L3 identification

The L3 were collected from the sedimentation in the falcon tube using a dropper and placed on a petri dish. Presence and movement of the L3 were observed under a dissecting microscope. A drop of Lugol's iodine was added into the L3 droplet thus killed the L3. The killed L3 were transferred onto a glass slide and covered with a coverslip to be observed under 40x magnification of a compound microscope for genus identification based on the guidelines provided by the Ministry of Agriculture, Fisheries and Food of Great Britain (1986). The percentage of each identified L3 genus were calculated from the total number of observed L3.

3.7 Faecal Egg Count Reduction Test (FECRT)

The method of FECRT was conducted following Coles *et al.* (1992) and Coles *et al.* (2006). Thirty out of 49 goats were detected with more than 150 epg of faeces and was subjected to FECRT. The goats were divided into control and treatment group levamisole in each farm with five to 10 animals in each group. The control and treatment levamisole group was divided with approximately similar mean pre-treatment FEC ranged from 160 epg to 180 epg in one farm while another farm ranged from 1933.5 epg to 1945 epg

The second faecal sampling was conducted 7 days after treatment to obtain post-treatment FEC. Percentage of FECRT was calculated as in FECR% formula listed below, where Xt is the mean epg of post-treatment in the treated group and Xc is the mean epg of post-treatment of control group. The helminth population will be classified as resistance if the FECR% value is less than 95% and the confidence level of 95% is lesser than 90% (Coles *et al.*, 1992). The obtain data of both FECRT and FECR% was later recorded in FERCT calculation spreadsheet.

4.0 RESULT

4.1 Identification of L3 from faecal culture

H. contortus was observed to have a pointed head, slender body and sharp tail (Figure 1). *Trichostrongylus sp.* was identified having a tapered head, short and stout body with a shorter tail than *H. contortus* that have blunt internal end, sharp and pointed external end (Figure 2). *Oesophagostomum sp.* was identified for having a rounded head, long and stout body, and a long, filamentous tail (Figure 3).

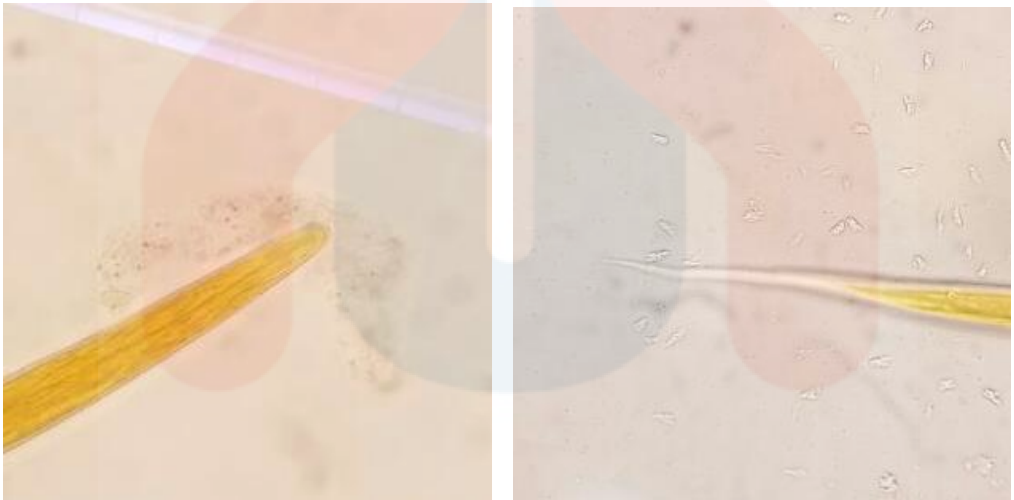


Figure 1: pointed head and sharp tail *H. contortus*

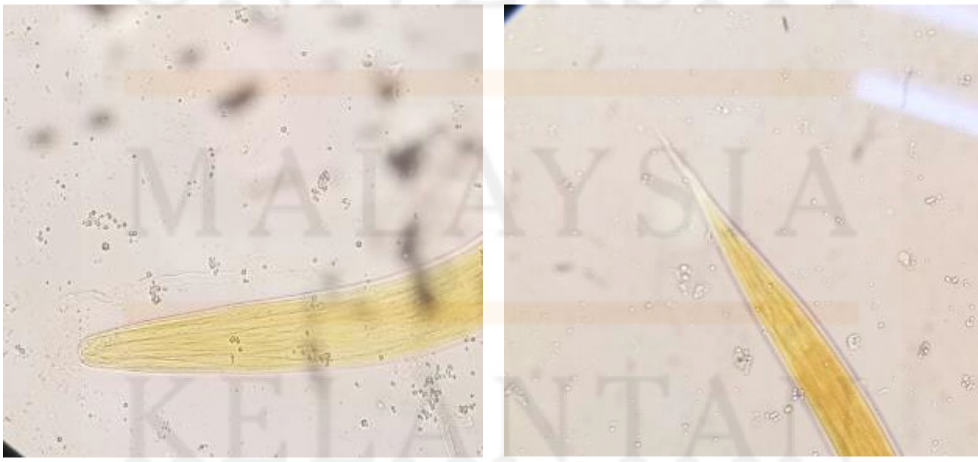


Figure 2: *Trichostrongylus sp.* characteristic of having tapered head and shorter tail

Figure 3: round head and long, filamentous tail was observed from *Oesophagostomum sp.*

4.2 Determination of mean faecal egg count before and after levamisole treatment in each farm.

Table 1 represents the pre-treatment and post treatment FEC of each animal in Farm 1 as well as the mean FEC during pr-treatment and post-treatment sampling of 3063.3 epg and 1061.1 epg respectively. All goats with zero epg in the post-treatment FEC have been treated with levamisole. However, although goats with ID 013, 0921 and P28HTP have been treated with levamisole, the goat still have high post-treatment FEC ranging from 500 – 2400 epg.

Table 2 shows the individual pre-treatment and post-treatment FEC in Farm 2. Pre-treatment FEC consist of 20 animals, while post-treatment FEC was taken from 10 animals. The mean FEC of pre-treatment and post-treatment samples were 115 epg and 35 epg respectively.

Table 1: Pre-treatment and post-treatment faecal egg count in Farm 1

Animal ID	Pre-[1](epg)	Group	Post-[2](epg)
0921	4450	Levamisole	1000
P28HTP	3700	Levamisole	550
4186(F)	3150	Levamisole	0
2683	10850	Control	Removed
P14(M)	15050	Levamisole	Died
P15	3950	Control	NA[3]
P27(M1)	5950	Control	NA
TH4614	950	Control	NA
P27(M2)	2000		Sold
P12(M)	6850	Levamisole	50
TL	800	Levamisole	0
P30(M)	1850	Levamisole	0
P19	1100	Levamisole	0
013	2300	Levamisole	2400
G1973	2250	Levamisole	0
P28(BW[3])	2400	Levamisole	0
P33(W[4])	600	Levamisole	0
4189	550	Levamisole	0
023	750	Levamisole	0
4155	3050	Control	4155
P34	3550	Control	5100
P28M4400N	1700	Control	850
P23(BW[3])	2700	Control	2450
P29	1150	Control	2400
4190	1350	Control	1150
P23M(B[5])	3050	Control	950
TM4614	135	Control	1350
(F)008	1500	Control	1450
P15(F)	1150	Control	550
Average	3063.3		1061.1

[1]pre-treatment1; [2]post-treatment; [3]Not available

Table 2: Pre-treatment and post-treatment faecal egg count in Farm 2

Animal ID	Pre-[1](epg)	Group	Post-[2](epg)
1	150	Levamisole	0
2	0	NIL[3]	NT[4]
3	150	Control	50
4	200	Levamisole	0
5	0	NIL	NT
6	200	Control	50
7	150	Control	100
8	150	Control	0
9	50	NIL	NT
10	150	Treatment	0
11	0	NIL	NT
12	150	Control	150
13	100	NIL	NT
14	50	NIL	NT
15	100	NIL	NT
16	100	NIL	NT
17	100	NIL	NT
18	250	Levamisole	0
19	150	Levamisole	0
20	100	NIL	NT
Average	115		35

[1]pre-treatment; [2]post-treatment; [3]Not taken; [4]Not in list

4. 3 Determination of levamisole resistance status

Table 3 shows the status and the level of levamisole resistance in each farm. Calculation was conducted on FECRT calculation spreadsheet based on the mean of both FEC post-treatment in levamisole and control groups. It shown 100% reduction in the faecal egg count from levamisole treatment in Farm 2, but only 88% in Farm 1. Anthelmintic resistance status in Farm 1 was resistant while Farm 2 was low count, which based on the 100% FECR% and the lower confidence limit of less than 90%. Farm 1 has moderate level of anthelmintic resistance as FECR% was 88%. Meanwhile, no anthelmintic resistance was observed on Farm 2 as FECR% was 100%.

Table 3: Status and level of levamisole resistance in each goat farm

Farm	N[1]	Status	Level	Post-treatment mean FEC[2]		FECR[3](%)	95% CI[4]	
				Levamisole	Control		Lower	Upper
Farm 1	20	Resistant	Moderate	245	2041	88	1	99
Farm 2	10	Low count	-	0	70	100	70	100

[1]No. of animals; [2]Faecal egg counts; [3]Faecal egg count reduction; [4]Cl = confidence interval

4.4 Identification of endoparasite genus before and after levamisole treatment

Figure 4 and 5 shows the genus of helminth that were identified from the faecal culture of pre-treatment and post-treatment of levamisole treated group in Farm 1 and Farm 2. The identified helminth were *H. contortus*, *Trichostrongylus* sp. and *Oesophagostomum* sp. *H. contortus* was the predominant helminth detected in both farms before and after levamisole treatment. *Oesophagostomum* sp. and *Trichostrongylus* sp. were identified in Farm 1 but not detected in Farm 2. Meanwhile, reduction in *Oesophagostomum sp.* (4% to 1%) and increased in *Trichostrongylus sp*. was observed in Farm 1 after levamisole treatment (5% to 9%). In contrast, increased in *H. contortus* was observed after treatment with levamisole in Farm 2 (2% to 6%) while reduction was seen in Farm 1 (91% to 90%).

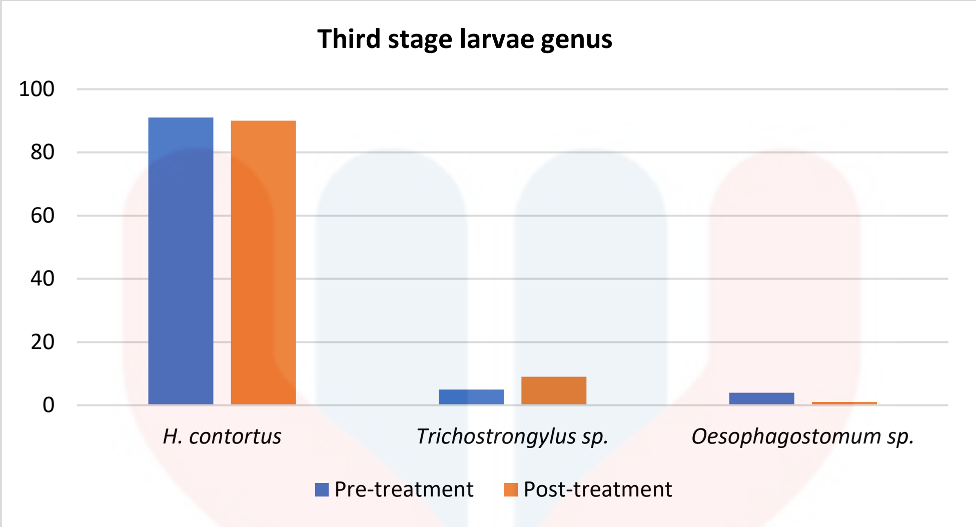


Figure 4: Percentage of identified L3 genus during pre-treatment and post-treatment of levamisole treated group in Farm 1

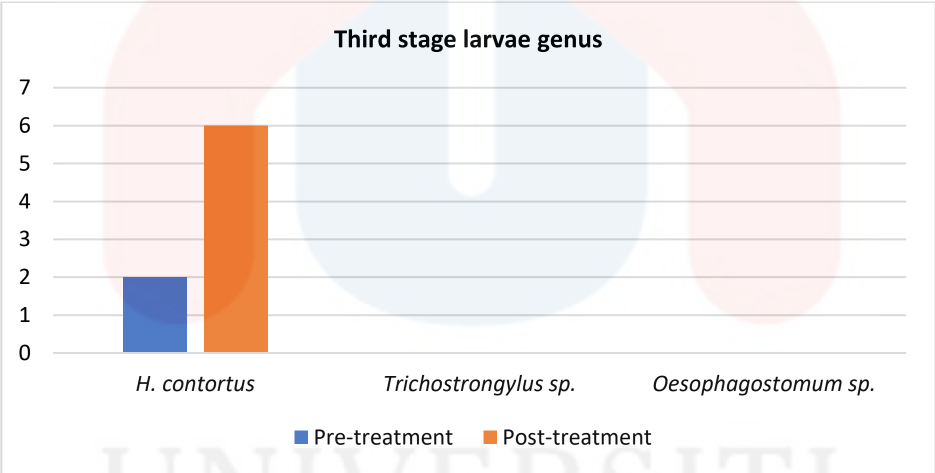


Figure 5: Percentage of identified L3 genus during pre-treatment and post-treatment levamisole treated group in Farm 2

5.0 DISCUSSION

Helminthiasis caused major negative effect to ruminant health especially small ruminants. It results in decreased meat production, anaemia, diarrhoea, dehydration, and also mortality. Helminthiasis in small ruminant in Malaysia mostly caused by *Oesophagostomum spp., Strongyloides spp., Haemonchus spp., and Trichostrongylus spp.,*(Sani and Gray, 2004). Among these helminths, *H. contortus* is known to be the most pathogenic nematode in small ruminants (Chandrawathani et al., 2006). In this study, *H. contortus* were found to have the highest prevalence among helminth population in both farms. Li et al., (2016) revealed that infestation of *H. contortus* has increased the risk of secondary bacterial infection in the abomasum of parasitized goat. Apart from that, *H. contortus* was known as a major caused for the reduction of blood in infected ruminant due the helminth feed directly on blood and can caused intestinal haemorrhage (Dorny *et al.*, 1995). Thus, causing infected goat to become severely anaemic. Hence, helminthiasis is a serious threat to small ruminant industry in Malaysia and a major effect on the profitability.

Anthelmintic resistance mainly happened due to improper usage of anthelmintic drugs. It developed due to the increase in resistance helminths that survive a normal dose of anthelmintic in animal (Beleckė et al., 2021). Reproduction of survived worm gradually increased resistance worm population in the infected animals. Thus, it could be the reason FECR% from Farm 1 was not achieving 100%. However, only one goat had a heavy worm burden thus influencing the FECRT findings. Furthermore, all other goats in the treatment group from Farm 1 have 0 to 50 epg after the treatment. This suggests that the one particular goat had contributing to high mean FEC compared to other members of the herd due to the ineffectiveness of levamisole against infecting helminth.

From the result, levamisole resistance has been developed in Farm 1 (Table 3). The farmer may need to consider other options for helminth control. This included the usage of non-chemical approaches such as the use of herbal medicine like Neem leaves. Studies conducted by Chandrawathani *et.al* (2006) stated that treatment with Neem leave (*Azadirachta indica*) was able to reduce the worm burden in small ruminants, as it has a significant low number of helminth and low FEC compared to the control group in the study. Farmers can also be educated to use FAMACHA tool in identifying clinical sign of anaemia in small ruminants. According to Chandrawathani *et al.* (2006), FAMACHA score is used in small ruminant to observed the anaemic status of animal especially animals that were affected with *H. contortus.* Hence, both of it aided farmers, especially the small holder farmers in early detection of helminthiasis severity status in their farm.

Result of anthelmintic resistance in Table 3 shows low FEC in Farm 2. This could happen due to small number of sampled animals (n=5) that meet the criteria of FECRT for each of control and treatment groups as well as the 100% effectiveness of levamisole against helminths. This indicated that identified helminths that caused Parasitic Gastroenteritis (PGE) in Farm 2 were susceptible to levamisole treatment.

In this study, the initial study population of goats in Farm 1 were 29. However, nine out of 29 goats were removed from the study population due to several reasons such as dead, being sold, unable to be located and to balance the mean FEC between the control and treatment groups. Hence, only 20 goats were involved in this study from Farm 1. The number of animals included in this study was accepted as it still follows the minimum number of animal approved by the Animal Ethic Committee (UMK/FPV/ACUE/FYP/025/2022).

In this study, the technical errors occurred during laboratory works may influenced the result of L3 genus identification such as insufficient heat of lukewarm water may contribute to lesser amount of L3 found and harvested. Apart from that, confusion and misidentification of individual L3 may happened, especially while differentiating between *H. contortus* and *Trichostrongylus sp.* larvae.

6.0 CONCLUSION AND RECOMMENDATION

In conclusion, research questions were answered and anthelmintic resistance toward levamisole has been develop in one of the goat farms in this study. However, more study sites were needed to investigate the status of levamisole resistance in goat farm in Kelantan. It is recommended for farmers to implement the alternative methods of helminth control such as utilize herbal anthelmintic in feed such as neem and cassava leaves, practise proper drenching of chemical anthelmintics and rotational grazing, also to switch to complete intensive management with zero grazing or cut and carry system.

REFERENCES

Abubakar, F., Kari, A., Ismail, Z., Haruna Usman, T., & Baba, A. R. (2015). PRELIMINARY STUDY OF NEMATODE RESISTANCE TO ANTHELMINTIC DRUGS IN TWO GOAT FARMS IN TERENGGANU. Jurnal Teknologi, 77(24). https://doi.org/10.11113/jt.v77.6690

Basripuzi, H.B., Sani, R.A. and Ariff, O.M. (2012). Anthelmintic resistance in selected goat farms in Kelantan. Malaysian Journal of Animal Science. 9: 1-9.

Beleckė, A., Kupčinskas, T., Stadalienė, I., Höglund, J., Thamsborg, S. M., Stuen, S., & Petkevičius, S. (2021). Anthelmintic resistance in small ruminants in the Nordic Baltic region. *Acta Veterinaria Scandinavica*, *63*(1). https://doi.org/10.1186/s13028-021-00583-1

Boothe, D. M. (2014) Imidazothiazoles, Pharmacology, MSD veterinary manual.

Chandrawathani, P., Adnan, M., and Waller, P. (1999). Anthelmintic resistance in sheep and goat farms on peninsular Malaysia. Veterinary Parasitology, 82(4), 305-310.

Chandrawathani P. AND Nurul Aini R. (2012). Current Trends in helminth parasite control in small ruminant in Malaysia and South East Asia. Malaysia Journal of Veterinary research. 23-28.

Chandrawathani, Chang, K., Nurulaini, R., Waller, P., Zaini, C., Jamnah, O., & Khadijah, S. (2006). Daily feeding of fresh Neem leaves (Azadirachta indica) for worm control in sheep. *Tropical Biomedicine*, *23*(1), 23–30. http://www.msptm.org/files/23_-_30_Chandrawathani_P.pdf

Chandrawathani P., Premaalatha B. , Nurulaini R. , Erwanas A.l. , Zaini C.M. , Aizan M. , Ramlan M. and Khadijah S. (2013). Severe anthelmintic resistance in two free grazing smallholder goat farms in Malaysia. J. Veterinar Sci technol. 4:137

Claerebout, E., & Geldhof, P. (2020). Helminth Vaccines in Ruminants. *Veterinary Clinics of North America: Food Animal Practice*, *36*(1), 159–171. https://doi.org/10.1016/j.cvfa.2019.10.001

Coles, G.C., Bauer, C., Borgsteede, F.H.M, Geerts, S., Klei, T.R., Taylor, M.A., and Waller, P.J. (1992). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology. 44: 35-44.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary parasitology. 19; 1-19

Dorny, P., Symoens, C., Jalila, A., Vercruysse, J., & Sani, R. (1995). Strongyle infections in sheep and goats under the traditional husbandry system in peninsular Malaysia. *Veterinary Parasitology*, *56*(1-3), 121–136. https://doi.org/10.1016/0304-4017(94)00657-x

Kumar, N., Rao, T. K. S., Varghese, A., & Rathor, V. S. (2012). Internal parasite management in grazing livestock. *Journal of Parasitic Diseases*, *37*(2), 151–157. https://doi.org/10.1007/s12639-012-0215-z

Li, R., Li, W., Sun, J. et al. The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome. Sci Rep 6, 20606 (2016). https://doi.org/10.1038/srep20606

Ministry of Agriculture, Fisheries and Food of Great Britain. (1986). Manual of veterinary parasitological laboratory techniques 418. HM Stationery Office

Nisbet, A. J., Meeusen, E. N., González, J. F., & Piedrafita, D. M. (2016). Immunity to Haemonchus contortus and Vaccine Development. *Haemonchus Contortus and*

Haemonchosis – Past, Present and Future Trends, 353–396. https://doi.org/10.1016/bs.apar.2016.02.011

Paul, B. T., Jesse, F. F. A., Chung, E. L. T., Che'Amat, A., & Mohd Lila, M. A. (2020). Risk Factors and Severity of Gastrointestinal Parasites in Selected Small Ruminants from Malaysia. *Veterinary Sciences*, *7*(4), 208. https://doi.org/10.3390/vetsci7040208

Premaalatha B., Chandrawathani P., Jamnah O., Ramlan M., Quaza Nizamuddin H.N. and Khadijah S. (2019, January) Status of anthelmintic resistance in smallholder goat farms in Ipoh, Perak between 2013 and 2014. Malaysian Journal of Veterinary Research. 9: 1-9

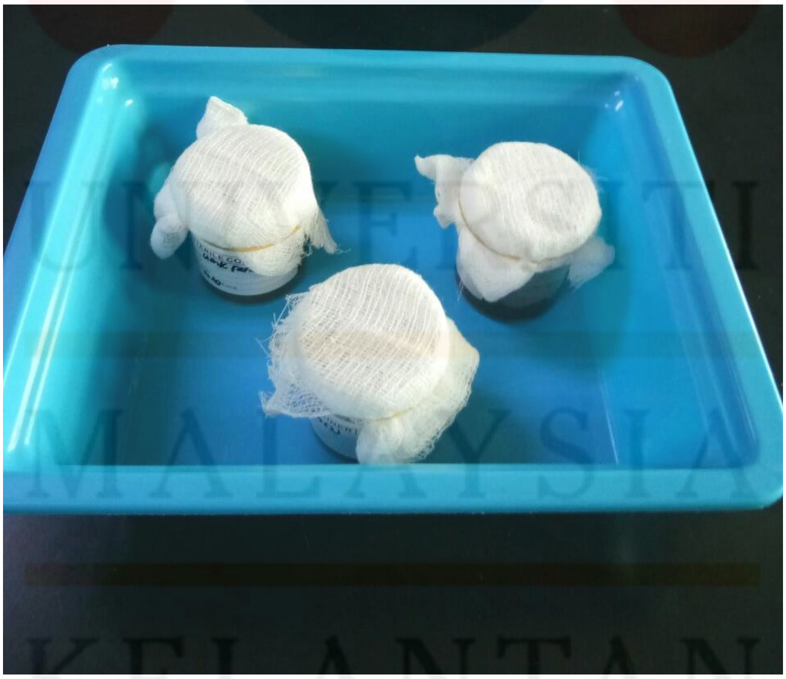
Appendix A

Picture 1: Restraining goat for faecal sample

Picture 2: Faecal sampling

Appendix B

Picture 3: McMaster procedure



Picture 4: Faecal culture