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IDENTIFICATION OF PARASITIC INFECTION IN LABORATORY RODENTS THROUGH CONTRAST MANAGEMENT METHODS

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By

NURUL AIN FATIN BINTI RASLAN

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IDENTIFICATION OF PARASITIC INFECTION IN LABORATORY RODENTS THROGUH CONTRAST MANAGEMENT METHODS

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Parasites may be found in the skin and intestine of the laboratory mice (*Mus musculus*) and rat (*Rattus norvegicus*). High parasitic burden are known to influence experimental outcomes and results. Therefore, it is important to determine the impact of parasitic infestations on rodent study, especially in Malaysia where some rodent colonies are still being kept in conventional systems. This study allows for the identification of common parasitic infection of laboratory rodents, assess the parasitic infection based on management methods of stocking density and environmental condition, and compare the parasitic infection between two conventional animal facilities. Firstly, seventy-two (72) laboratory rodents of BALB/c, ICR and Sprague Dawley (SD) were chosen from an animal facility located in Klang Valley and subjected to identification of common parasitic infections. Secondly, one hundred and eight (108) male BALB/c mice were randomly chosen and placed in three groups to reflect different stocking densities of 3, 6 and 9 mice per group, under different environmental settings of regulated and non-regulated environment for 5 weeks. Thirdly, sixty (60) BALB/c mice and sixty (60) SD rats were chosen to compare the parasitic infection between two conventional animal facilities. Helminths, ectoparasites and blood parasites were examined using conventional techniques. Parasites were identified based on observation and classification of their distinct characteristics under a compound microscope. Results showed that mice were commonly infected with pinworms; *Syphacia obvelata* (*S. obvelata*) and *Aspiculuris tetraptera* (*A. tetraptera*) whereas, rats were infected with *Syphacia muris* (*S. muris*) and *A. tetraptera.* The prevalence of the pinworms; *S. obvelata* in the mice range from 20.83% to 41.67%, *S. muris* in the rats at 83.33%, and *A. tetraptera* range from 8.33% to 45.83% in both species. Although the second findings revealed BALB/c mice placed in two different management settings had no association between parasitic infections with various stocking density and between different environmental condition using repeated-measures

ANOVA, but association was observed using gastrointestinal examination and tape impression test when using one-way ANOVA. Ectoparasites suspected to be immature mites detected in non-regulated environment at a prevalence of 20.4%, with an association found using the tape impression test under one-way ANOVA. Finally, the comparison between two different conventional animal facilities demonstrated that ICR mice were infected with a common fur mite; *Myocoptes musculinus* (*M. musculinus*) and lice; *Polyplax serrata* (*P. serrata*) while the SD rats were infected by uncommon parasites; *Heterakis spumosa* (*H. spumosa*) that is normally found in wild rats, and *Chirodiscoides caviae* (*C. caviae*), a common fur mite in guinea pigs. The results also revealed an association between parasitic infections and different management of animal facilities for laboratory mice and rats using the Chi-square test. Overall, management plays an important factor in parasitic infestation of laboratory rodents. The findings highlight the parasites identified in laboratory rodents varied according to the parasitological methods used following the contrast management method of stocking density, environmental condition and facility.

Keywords: ectoparasites, endoparasites, laboratory rodents, management factors, pinworms

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PENGENALPASTIAN JANGKITAN PARASIT DALAM RODEN MAKMAL MELALUI KAEDAH PENGURUSAN KONTRAS

Oleh

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Parasit boleh didapati dalam kulit dan pada usus mencit (*Mus musculus*) dan tikus makmal (*Rattus norvegicus*). Beban parasit yang tinggi dikenali untuk mempengaruhi hasil eksperimen. Oleh itu, penting untuk menentukan kesan jangkitan parasit terhadap kajian menggunakan roden makmal, terutama di Malaysia di mana beberapa koloni masih disimpan dalam sistem konvensional. Kajian ini membenarkan pengenalpastian jangkitan parasit yang lazim dalam roden makmal, menilai tahap jangkitan parasit berdasarkan faktor pengurusan iaitu kepadatan stok dan keadaan alam sekitar yang boleh dijadikan panduan sebagai cara pengurusan yang tepat untuk dilaksanakan, dan membandingkan jangkitan parasit antara dua fasiliti haiwan makmal konvensional yang berlainan. Pertama, tujuh puluh dua (72) mencit makmal BALB/c, ICR dan Sprague Dawley (SD) dipilih dari sebuah fasiliti haiwan makmal yang terletak di Lembah Klang bagi mengenalpasti jangkitan parasit yang lazim dalam roden makmal. Kedua, seratus lapan (108) mencit BALB/c jantan dipilih secara rawak dan ditempatkan dalam tiga kumpulan untuk mencerminkan kepadatan stok yang berbeza iaitu 3, 6 dan 9 mencit dalam satu kumpulan dan juga ditempatkan dalam tiga kumpulan persekitaran yang berbeza untuk tempoh 5 minggu. Ketiga, enam puluh (60) mencit BALB/c dan enam puluh (60) tikus SD dipilih untuk membandingkan tahap jangkitan parasit di antara dua fasiliti haiwan makmal yang konvensional. Helmint, ektoparasit dan parasit darah diperiksa menggunakan teknik-teknik konvensional. Parasit dikenal pasti berdasarkan pemerhatian dan klasifikasi ciri khasnya di bawah mikroskop kompaun. Hasil menunjukkan bahawa mencit biasanya dijangkiti cacing; *Syphacia obvelat*a (*S. obvelata*) dan *Aspiculuris tetraptera* (*A. tetraptera*) manakala, tikus makmal dijangkiti *Syphacia muris* (*S. muris*) dan *A. tetraptera*. Prevalens cacing; *S. obvelata* dalam mencit dalam lingkungan antara 20.83% hingga 41.67%, *S. muris* dalam tikus makmal pada 83.33% dan *A. tetraptera* antara 8.33% hingga 45.83% dalam kedua-dua spesies. Walaupun penemuan objektif kedua menunjukkan mencit BALB/c yang ditempatkan dalam dua faktor pengurusan yang berbeza tidak mempunyai

kaitan antara tahap jangkitan parasit dengan kepadatan stok dan antara pelarasan keadaan alam sekitar yang berbeza dengan menggunakan ANOVA berulang, tetapi hubungan diperhatikan dengan menggunakan pemeriksaan usus dan ujian kesan pita ketika menggunakan ujian ANOVA satu arah. Ektoparasit yang disyaki tungau hanya ditemui dalam keadaan alam sekitar yang tidak dilaras dengan prevalens 20.4%. Bagi perbandingan antara dua fasiliti haiwan makmal konvensional yang berbeza, hasil menunjukkan bahawa mencit ICR dijangkiti oleh hama bulu yang lazim iaitu; *Myocoptes musculinus* (*M. musculinus*) dan kutu; *Polyplax serrata* (*P. serrata*) manakala tikus SD dijangkiti oleh parasit yang tidak lazim seperti *Heterakis spumosa* (*H. spumosa*) yang biasanya hanya dijumpai dalam tikus liar dan *Chirodiscoides caviae* (*C. caviae*), hama yang kebiasaannya dijumpai dalam tikus belanda. Keputusan juga menunjukkan hubungan antara parasit dan pengurusan fasiliti haiwan makmal yang berbeza untuk mencit dan tikus makmal menggunakan ujian 'Chi-square'. Secara keseluruhan, pengurusan memainkan peranan penting dalam jangkitan parasit pada roden makmal. Hasil kajian menekankan parasit yang dijumpai dalam roden makmal berbagai mengikut kaedah yang digunakan berikut kaedah pengurusan yang bebeza iaitu kepadatan stok, keadaan alam sekitar dan fasiliti.

Kata kunci: cacing pin, ektoparasit, faktor pengurusan, helmint, roden makmal

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiv
LIST OF FIGURES		xvi
LIST OF ABBREVIATIONS		xix
CHAPTER		
1	**INTRODUCTION**	1
	1.1 Justification	3
	1.2 Objectives	3
	1.3 Hypothesis	3
2	**LITERATURE REVIEW**	4
	2.1 Laboratory mice	4
	2.1.1 Conventional laboratory animals	7
	2.1.2 Husbandry of the laboratory mice	7
	2.1.3 Effects of management methods on laboratory mice	9
	2.2 Laboratory rat	12
	2.2.1 Husbandry of the laboratory rat	13
	2.2.2 Effects of management methods on laboratory rat	14
	2.3 Parasitic diseases of laboratory rodents	17
	2.3.1 Endoparasites	18
	2.3.2 Ectoparasites	20
	2.3.3 Blood parasites	21
	2.3.4 Effect of parasites on laboratory rodents	21
	2.4 Diagnosis of parasites on laboratory rodents	23
	2.4.1 Endoparasites	23
	2.4.2 Ectoparasites	24
	2.4.3 Blood parasites	24
3	**MATERIALS AND METHODS / METHODOLOGY**	25
	3.1 Ethical approval	25
	3.2 Animals and experimental deisgn	25
	3.2.1 Identification of opportunistic parasites in laboratory rodents	27

	3.2.2	Comparison of effects of management methods associated with parasitic infection in laboratory rodents	27
	3.2.3	Comparison of parasitic infection in laboratory rodents at different animal facilities	28
3.3	Sample collection		28
3.4	Endoparasite examination		29
3.5	Ectoparasite examination		31
3.6	Blood parasite examination		32
3.7	Statistical analysis		33
4	**IDENTIFICATION OF OPPORTUNISTIC PARASITES IN LABORATORY RODENTS**		34
4.1	Introduction		34
4.2	Material and methodology		36
	4.2.1	Animals and experimental design	36
	4.2.2	Environmental setting of study location	37
	4.2.3	Parasite detection	38
	4.2.4	Statistical analysis	38
4.3	Results		39
	4.3.1	Detection of endoparasites in laboratory mice	39
	4.3.2	Detection of ectoparasites and blood parasites in laboratory mice	42
	4.3.3	Detection of endoparasites in laboratory rats	43
	4.3.4	Detection of ectoparasites and blood parasites in laboratory rats	43
	4.3.5	Summary of parasitic infection for laboratory rodents	44
4.3	Discussion		46
4.4	Conclusion		48
5	**MANAGEMENT METHODS ASSOCIATED WITH PARASITIC INFECTION IN LABORATORY RODENTS**		49
5.1	Introduction		49
5.2	Materials and Methodology		51
	5.2.1	Experimental design	51
	5.2.2	Experimental animals	52
	5.2.3	Location of study	56
	5.2.4	Identification of endoparasits and ectoparasites	56
	5.2.5	Detection of blood parasites	56
	5.2.6	Statistical analysis	56
5.3	Results		57
	5.3.1	Effects of stocking density	57

5.3.1.1 Detection of endoparasites 57
5.3.1.2 Detection of ectoparasites 63
5.3.1.3 Detection of blood parasites 63
5.3.2 Factor of environmental condition 63
5.3.2.1 Detection of endoparasites 63
5.3.2.2 Detection of ectoparasites 69
5.3.2.3 Detection of blood parasites 71
5.4 Discussion 72
5.5 Conclusion 75

6 COMPARISON OF PARASITIC INFECTION IN LABORATORY RODENTS AT DIFFERENT ANIMAL FACILITIES 76
6.1 Introduction 76
6.2 Materials and methodology 77
6.2.1 Animals study 77
6.2.2 Location of study and setting 77
6.2.3 Sample collection 79
6.2.4 Detection of endoparasites and ectoparasites 79
6.2.5 Detection of blood parasites 79
6.2.6 Statistical analysis 79
6.3 Results 80
6.3.1 Detection of endoparasites in laboratory mice 80
6.3.2 Detection of endoparasites in laboratory rats 81
6.3.3 Detection of ectoparasites in laboratory mice 82
6.3.4 Detection of ectoparasites in laboratory rats 84
6.3.5 Prevalence of parasitic infection in laboratory rodents 85
6.3.6 Statistical analysis 87
6.4 Discussion 88
6.5 Conclusion 91

7 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 92
7.1 General discussion 92
7.2 Conclusion 95
7.3 Recommendations 95

REFERENCES 96
APPENDICES 108
BIODATA OF STUDENT 123
LIST OF PUBLICATIONS 124

LIST OF TABLES

Table		Page
1	Environmental requirements for an adult mouse	8
2	Cage space recommendations according to weight and number of mice for non-breeding population	10
3	Environmental requirements for an adult rat	13
4	Cage space recommendations according to weight and number of rats for non-breeding population	15
5	Presence of helminths and ova; *Syphacia spp.* and *Aspiculuris tetraptera*, ectoparasites and blood parasite in laboratory rodents (N=48)	44
6	Detection of endoparasite by pooled direct faecal smear from Week 0 to Week 4 in groups of different stocking densities within three replicates.	58
7	Detection of endoparasite by faecal floatation from Week 0 to Week 4 in groups of different stocking densities within three replicates.	60
8	Relationship between stocking density and parasitic infections in BALB/c mice using gastrointestinal examination by one-way ANOVA method.	62
9	Detection of endoparasite by pooled direct faecal smear from Week 0 to Week 4 in groups of regulated and non-regulated environment within three replicates.	64
10	Detection of endoparasite by faecal floatation from Week 0 to Week 4 in groups of regulated and non-regulated environment within three replicates.	67
11	Relationship between environmental condition and parasitic infections using gastrointestinal examination by one-way ANOVA method.	68
12	Relationship between environmental condition and ectoparasite infections using fur pluck method by one-way ANOVA method.	69
13	Relationship between environmental condition and ectoparasite infection using tape impression test by one-way ANOVA method.	70

14	Relationship between parasites infection between Premise 1 and Premise 2 using Chi-square test	87
A	Relationship between stocking density and parasitic infection in BALB/c mice using pooled direct faecal smear by repeated-measures ANOVA.	117
B	Relationship between stocking density and parasitic infection in BALB/c mice using perianal tape test by repeated-measures ANOVA.	118
C	Relationship between stocking density and parasitic infection in BALB/c mice using faecal floatation by repeated-measures ANOVA.	119
D	Relationship between environmental condition and parasitic infection in BALB/c mice using pooled direct faecal smear by repeated-measures ANOVA.	120
E	Relationship between environmental condition and parasitic infection in BALB/c mice using perianal tape test by repeated-measures ANOVA.	121
F	Relationship between environmental condition and parasitic infection in BALB/c mice using faecal floatation by repeated-measures ANOVA.	122

LIST OF FIGURES

Figure		Page
1	BALB/c mice	5
2	ICR mice	6
3	Open-top cages	8
4	Sprague Dawley rat	12
5	Common endoparasite ova of laboratory rodents	17
6	Layout of research design	26
7	Design of experiment to identify the presence of opportunistic parasites in laboratory mice and rats using different parasitological methods at ARU, UPM.	36
8	Layout of animal facility studied.	37
9	Presence of pinworms. A - Direct faecal smear technique showed presence of larva (yellow arrow) and an adult worm of *Syphacia obvelata* (red arrow); B - Evidence of an adult worm of *Aspiculuris tetraptera* (red arrow) is identified under 100x magnification.	39
10	Presence of pinworms. A - Presence of ova (black arrow) within the gravid female of *Syphacia obvelata* under 100x magnification. Note the shape of the ova is flattened on one side; B - The ellipsoid-shaped ova (red arrow) of *Aspiculuris tetraptera* observed from faecal floatation technique under 400x magnification	40
11	Comparison of pinworm features. A - A gravid female of *Syphacia obvelata* at 100X. B - Subtle cervical alae and round oesophageal bulb (black arrow). C - Long and pointed tail (red arrow) with obvious vulvar opening (yellow arrow). D - A gravid female *Aspiculuris tetraptera* at 100X was observed. E - Characterized by oval oesophageal bulb (black arrow). F- Conical tail (red arrow) observed.	41
12	Presence of *Syphacia muris* ova under a light microscope with x400 magnification	43

13	Prevalence of helminths and ova; *Syphacia spp.* and *Aspiculuris tetraptera*, ectoparasites and blood parasite in laboratory rodents (N=48)	45
14	Design of experiment to assess the level of parasitic infection of different stocking densities and between regulated and non-regulated environment in BALB/c mice.	51
15	Setting of open-top cages for the study of stocking density	53
16	Setting of cages for the study of environmental condition.	55
17	Detection of *Syphacia obvelata* ova via perianal tape test from Week 0 (age 4 to 5 weeks) to Week 4 (age 8 to 9 weeks) in groups of different stocking densities of 3, 6 and 9 mice per cage. (N = 54 mice, n = 18 mice per stocking density group).	59
18	Detection of endoparasite in selected BALB/c mice (N=18 mice; n=6 mice per group) placed in different stocking densities using gastrointestinal examination.	61
19	Post-hoc test on relationship between stocking density and parasitic infection in BALB/c mice using gastrointestinal examination	62
20	Detection of *Syphacia obvelata* ova via perianal tape test from Week 0 (age 4 to 5 weeks) to Week 4 (age 8 to 9 weeks) in groups of regulated and non-regulated environment (N = 54 mice, n = 18 mice per stocking density group).	65
21	Detection of endoparasite in BALB/c mice placed in different environmental condition using gastrointestinal examination.	68
22	Post-hoc test on relationship between environmental condition and parasitic infection in BALB/c mice using gastrointestinal examination.	71
23	Layout of breeding unit and facility at Premise 1 (A) and Premise 2 (B).	78
24	Presence of *Heterakis spumosa*. A - Evidence of *Heterakis spumosa* ova at 100x magnification (red circles). B - *Heterakis spumosa* ova is oval shape	81

	surrounded by a thick mamillated shell at 400x magnification.	
25	Presence of common fur mite; *Myocoptes musculinus* under 40x magnification.	82
26	Presence of lice; *Polyplax serrata*. A - Egg of *Polyplax serrata* lice on the base of hair shaft in a fur pluck test under 400x magnification. B - An adult lice of *Polyplax serrata* species under 400x magnification.	83
27	Presence of *Chirodiscoides caviae* mite. A - Presence of *Chirodiscoides caviae* eggs on the hair shaft under 100x magnification. B - Evidence of a female adult mite at 400x magnification.	84
28	Comparison of prevalence of parasites in ICR mice between Premise 1 and Premise 2	85
29	Comparison of prevalence of parasites in Sprague Dawley rats between Premise 1 and Premise 2	86
A	Double-door system before entering animal holding room	112
B	Interior of mice holding room	112
C	Open-top cages	113
D	Individually-ventilated cages	113
E	Presence of two mites with different appearance at 100x magnification. Mite is oval-shaped with shorter III and IV limbs while mite is elongated in shape	114
F	Presence of mite with 3 pairs of limbs measuring 196 µm long by 102 µm wide at 400x magnification	114
G	Presence of mite measuring 222 µm long by 106 µm wide at 400x magnification	115
H	Presence of fragmented mite at 400x magnification	115
I	Close-up of mite with triangular gnathosoma, long and pointed chelicerae and setae at each limb at 400x magnification	116

LIST OF ABBREVIATIONS

%	Percentage
^{o}C	Celcius
^{o}F	Fahrenheit
µ	Micro
µL	Microliter
µm	Micrometre
ANOVA	Analysis of variance
ARU	Animal Resource Unit
BALB/c	Balb Albino
BW	Body weight
cm^2	Square centimetre
CRL	Charles River Laboratories
dB	Decibel
df	Degree of freedom
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
F	The ratio of two variances
F1	Filial 1
FPV	Faculty of Veterinary Medicine
HAR	Harlan
HEPA	High-Efficiency Particulate Air
HVAC	Heating, ventilation and air conditioning
IACUC	Institutional Animal Care and Use Committee
ICR	Institute of Cancer Research

IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-17	Interleukin-17
ILAR	Institute for Laboratory Animal Research
IVC	Individually ventilated cages
kcal	Kilocalorie
kJ	Kilojoule
LCT	Lower critical temperature
LED	Light-emitting diode
m^2	Square metre
MANOVA	Multiple analysis of variance
mL	Millilitre
ME	Metabolizable energy
MS	Mean square
n	Size of the sample from each population
N	Total sample size
NaCl	Sodium chloride
UPM	Universiti Putra Malaysia
OTC	Open top cages
P	Probability
PCR	Polymerase chain reaction
S.G.	Specific gravity
SD	Sprague Dawley
SPF	Specific pathogen-free

SPSS	Statistical Package for the Social Sciences
ss	Sum of square
TH2	T-helper 2 cell
TNZ	Thermoneutral zone

CHAPTER 1

INTRODUCTION

The laboratory mice (*Mus musculus*) and laboratory rat (*Rattus norvegicus*) are the most commonly utilised animal models in research due to its well-adapting features, relatively docile nature, and its close resemblance to those of human characteristics (Melina, 2010). The laboratory mice and rat also act as standard models when targeting for effective reproducible results. However, they are seldom investigated for parasitic infection before being selected in use for testing. Conventional animals may be infected by ectoparasites and endoparasites that can influence the interpretation of results if the parasites went undetected and exist in high burden (Baker, 2007; Pritchett, 2007). Identification of the parasites can be performed in live or dead animals through various diagnostic techniques (Parkinson et al., 2011). Therefore, knowledge of diagnostics techniques relevant to parasitic infection is imperative in obtaining a reliable source of laboratory animal that fit for research use.

Notifiable ectoparasites and endoparasites have been documented in the laboratory rodents (Medeiros, 2012). Pinworms belonging to the family of Oxyurids are helminths of major importance to the laboratory mice are the; *Syphacia obvelata (S. obvelata)* and *Aspiculuris tetraptera (A. tetraptera)* and for the laboratory rat; *Syphacia muris (S.muris).* The pinworms can be differentiated by morphological differences of the ova and adult worms (Baker, 2007; Pritchett, 2007; Taffs, 1976). Transmission of the parasites occurs by the faecal-oral route through ingestion of embryonated eggs shed in the faeces (Perec-Matysiak et al., 2006). Clinical symptoms are rare unless with a heavy infestation of worms that may result in dehydration, pruritus at the perianal region, enteritis, impaction or rectal prolapse (Baker, 2007; Medeiros, 2012). A study conducted in various institutions detected approximately 75% of the laboratory mice were infected with *S. obvelata* while 60% of them were infected with *A. tetraptera* (Carty, 2008). Another study based in Turkey (Beyhan et al., 2010) even revealed that prevalence of pinworms in rats can go up to 100% for *S. muris*, 53.6% for *A. tetraptera*, and 46.4% for *S. obevelata*. The prevalence of the parasites exhibits the importance of a proper biosecurity program with frequent health screening and necessary treatments from the animal facilities.

The most common ectoparasites found in the laboratory mice are the fur mites; *Myocoptes musculinis (M. musculinis)* and *Myobia musculi (M. musculi)*. *Myocoptes musculinis* can be differentiated from *M. musculi* by the characteristics of the third and fourth pair of legs. Transmission of the ectoparasites occurs by direct contact with an infected host. A low infestation of the ectoparasites is usually subclinical however heavy infestation may alter host behaviour and physiology that can cause variability of research results (Baker, 2007). Clinical symptoms that may be present include pruritus, alopecia,

scabbing and irritation. The most common ectoparasites found in the laboratory rats are the *Notoedres muris (N. muris)* that closely resembles the *Sarcoptes* spp. but it can be differentiated by its female adult that lacks heavy dorsal spines, cones, and triangular scales (Wall & Shearer, 1997). Transmission occurs by direct contact with the host. The Notoedric mange causes pruritus and papular, crusting dermatitis at the pinnae, nose, tail, and limbs (Robert J. Flynn, 1973). Blood parasites are rarely reported in laboratory rodents but mentionable blood parasites in mice include the *Plasmodium* spp., *Hepatozoon* spp., and *Haemabartonella* spp (Sirois, 2015) and the T*rypanosoma* spp. in both mice and rats (Baker, 2007; Sirois, 2015).

Various diagnostic techniques have been performed in identifying parasites. Direct examination of the gastrointestinal contents has been described as the 'gold standard' for pinworm detection (Dole et al., 2011; Feldman & Bowman, 2007). The perianal tape test method is commonly used for detection of the *Syphacia spp.* (Baker, 2007; Eguiluz et al., 2001; Sasa, 1962; Taffs, 1976), whereas, the faecal floatation method is commonly used to detect *A. tetraptera* infection (Baker, 2007; Phillipson, 1974; Taffs, 1976). Various methods have also been described for detection of ectoparasites such as by tape impression, fur pluck and carcass immersion. For detection of blood parasites such as *Plasmodium spp., Trypanosoma spp.,* and *Haemabartonella spp.*, the thin and thick blood smear methods have been described (Baker, 2007).

Laboratory animal facilities should provide an environment setting, and practice management that is well suited for the species or strains of animals maintained. Their physical, physiological, and behavioural needs should be taken into account to allow them to grow and elicit natural behaviour that appropriate for their health and well-being (National Research Council (US) Committee, 1991). There is unanimity on the optimal cage space for rodents despite numerous research (Foltz et al., 2007). Recommendation of cage sizes is usually made based on the animals' weight and the stocking density (Gonder & Laber, 2007). However, this might not be the scenario in real-life instances especially in animal facilities with limited spaces. High stocking density has been associated with reduced circulating antibodies (Vessey, 1964) and minimised resistance to infection in mice (Brayton & Brain, 1974; Peterson et al., 1991). The regulation of the environment is also imperative in an animal facility because several studies have revealed that exposure of the laboratory animals towards fluctuations or extremes of the environment can lead to changes in behaviour, physiology and morphology of the animals that affect their well-being and interferes with the performance and outcome of the research (Gordon, 1990, 1993; Pennycuik, 1967). Recommendations of ambient temperature, relative humidity, ventilation and light cycle have been made by animal welfare regulations and minimising stress is emphasised in order to ensure the welfare of the animals are protected.

Due to limited documentation on the effects of different management methods implemented at conventional animal facilities on the parasitic infection of laboratory animals, the objectives of this study are to identify the presence of

common parasitic infection in laboratory rodents, to assess the level of parasitic infection of different stocking densities and between the regulated and non-regulated environment in laboratory mice, and to compare parasitic infection between two different animal facilities in laboratory rodents. Obtaining laboratory animals from a reliable source is imperative when its intended use is for research and development. It is crucial to delve through the animal health status before it can be used as an experimental model. This is important to ensure the reliability and validation of the research results. Contamination level of the animal facilities may also be assessed by the parasitic loads of the animals sheltered. Thus, appropriate control and preventive measures of the transmission of diseases can be made early before animals are used for research purposes.

1.1 Justification

External and internal parasites have been known to confound research findings that post great risk to validation of the study. Conventional animal facilities in Malaysia also have varying management that contribute to vague parasitic infection in laboratory mice used for research study. Thus, the study was conducted to answer these research questions:

1. Are parasites commonly found in laboratory mice and rats?
2. Does different conditions in the environment affect the parasitic infection in laboratory mice and rats?
3. Does variation in management of facilities contribute to the parasitic infection in laboratory mice used for research study?

1.2 Objectives

Therefore, the objectives of this study are to:

1. To identify the presence of parasitic infection in laboratory mice and rats
2. To assess parasitic infection of contrast management methods; stocking density and environmental condition in BALB/c mice.
3. To compare parasitic infection between two conventional animal facilities in laboratory mice and rats

1.3 Hypothesis

H_o: There are no difference in parasitic infection in the laboratory mice and rats between facilities and management methods.
H_a: There are difference of parasitic infection in the laboratory mice between facilities and management methods.

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