



UNIVERSITI PUTRA MALAYSIA

***EPIDEMIOLOGY OF BOVID FASCIOSIS IN LARUT AND MATANG,
PERAK, MALAYSIA***

NAIM CHE KAMARUDDIN

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**EPIDEMIOLOGY OF BOVID FASCIOSIS IN LARUT AND MATANG,
PERAK, MALAYSIA**

By

NAIM CHE KAMARUDDIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

January 2022

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EPIDEMIOLOGY OF BOVID FASCIOSIS IN LARUT AND MATANG, PERAK, MALAYSIA

By

NAIM CHE KAMARUDDIN

January 2022

Chair : Nur Mahiza Md Isa, PhD
Faculty : Veterinary Medicine

Fasciolosis is considered as an emerging neglected tropical disease that is threatening ruminant productivity and is zoonotic. In previous study, the Taiping abattoir showed the highest fasciolosis occurrence compared to the other investigated main abattoirs in Peninsular Malaysia, which could impede the government's initiatives to promote ruminant production in the region. Therefore, this study intended to describe the epidemiology of bovid fasciolosis in Larut and Matang (Taiping), Perak (LM) to initiate the effort to control fasciolosis which could drive to economic loss. A total of 371 faecal samples from bovid (dairy cattle, beef cattle, buffalo) from 23 selected farms were examined in this cross-sectional study from February until August 2020. Animals and farms detail were recorded, and a questionnaire has been completed by the farmers to collect data of risk factors during the farm visit. The coproscopical examination of faecal samples to record the *Fasciola* faecal egg count (fec) using Flukefinder® sedimentation was performed and the co-infection with Paramphistomes was observed. The correlation of *Fasciola* fec with Paramphistomes fec in co-infected bovinds and also with *Fasciola* coproantigen concentration were correlated using Spearman correlation test. Heatmap density was used to interpolate the selected risk factors to visualise the fasciolosis exposure areas in LM. For the result, overall prevalence of bovid fasciolosis in LM was observed 36.9% (n=137/371). A total of 65.2% visited farms (n=15/23) having at least one case of bovid fasciolosis. Significant risk factors (p<0.05) for bovid fasciolosis in LM were buffalo (OR=9.5), bovid age more than three years (OR=5.5), body condition score lower than 5 (OR=1.2-14.9), larger grazing range (OR=1.3), more than one ruminant species in the farm (OR=2.0-2.1), extensive housing system (OR=4.0), increasing farm age (OR=1.2), and co-infection with Paramphistomes (OR=1.4). *Fasciola* and Paramphistomes fec in co-infected bovinds was having a significant moderate positive correlation (r=0.36, p<0.01). Besides, *Fasciola* fec to *Fasciola* coproantigen concentration was also having significant moderate positive correlation (r=0.55, p<0.01). The exposure areas to fasciolosis in this study showed higher exposure in the northern LM compare to the central LM. Thereby, this study revealed moderate prevalence of bovid fasciolosis in LM and factors of host intrinsic, farm management,

and co-infection with Paramphistomes are significantly associated with bovid fasciolosis in LM. The positive correlation of *Fasciola* and Paramphistomes in co-infected bovid could provide the predictive basis for bovid fasciolosis from the Paramphistomes occurrences. Besides, *Fasciola* coproantigen concentration could be useful in monitoring *Fasciola* eggs shedding to initiate targeted treatment of fasciolosis, however need further study for the application of coproantigen ELISA for this insight. The areas of high fasciolosis exposure in LM is beneficial to conduct control programmes to sustain the bovid production and welfare. The outcomes of this study are advantageous towards the understanding of the epidemiology of local bovid fasciolosis which is important in veterinary and public health.

Keywords: prevalence, fasciolosis exposure, risk factors, ruminant parasite, exposure map, livestock.

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

EPIDEMIOLOGI FASCIOLOSIS BOVID DI LARUT DAN MATANG, PERAK, MALAYSIA

Oleh

NAIM CHE KAMARUDDIN

Januari 2022

Pengerusi : Nur Mahiza Md Isa, PhD
Fakulti : Perubatan Veterinar

Fasciolosis dianggap sebagai penyakit tropika yang terabai yang mengancam produktiviti ruminan dan bersifat zoonotik. Dalam kajian lepas, rumah sembelih Taiping menunjukkan kejadian fasciolosis yang tertinggi berbanding dengan rumah sembelih utama lain yang diselidik di Semenanjung Malaysia, yang boleh menghalang inisiatif kerajaan untuk meningkatkan pengeluaran ruminan di rantau ini. Oleh itu, kajian ini bertujuan untuk menghuraikan epidemiologi fasciolosis bovid di Larut dan Matang (Taiping), Perak (LM) bagi memulakan usaha mengawal fasciolosis yang boleh menyebabkan kerugian ekonomi. Sebanyak 371 sampel najis daripada bovid (lembu tenusu, lembu pedaging, kerbau) dari 23 ladang terpilih telah diperiksa dalam kajian keratan rentas ini dari Februari hingga Ogos 2020. Perincian haiwan dan ladang telah direkodkan, dan soal selidik telah dilengkapkan oleh penternak untuk mengumpul data faktor risiko semasa lawatan ladang. Pemeriksaan koproskopik sampel najis untuk merekodkan kiraan telur najis (fec) *Fasciola* menggunakan pemendapan Flukefinder® telah dilakukan dan jangkitan bersama Paramphistome telah diperhatikan. Korelasi *Fasciola* fec dengan Paramphistomes fec dalam bovid yang dijangkiti bersama dan juga dengan kepekatan koproantigen *Fasciola* telah dikorelasikan menggunakan ujian korelasi Spearman. Ketumpatan peta haba digunakan untuk menginterpolasi faktor risiko yang dipilih untuk menggambarkan kawasan terdedah fasciolosis di LM. Keputusannya, prevalens keseluruhan fasciolosis bovid di LM diperhatikan 36.9% (n=137/371). Sejumlah 65.2% ladang dilawat (n=15/23) mempunyai sekurang-kurangnya satu kes fasciolosis bovid. Faktor risiko yang signifikan ($p < 0.05$) untuk fasciolosis bovid di LM ialah kerbau (OR=9.5), umur bovid lebih daripada tiga tahun (OR=5.5), skor keadaan badan lebih rendah daripada 5 (OR=1.2-14.9), julat kawasan ragut yang lebih besar (OR=1.3), lebih daripada satu spesis ruminan dalam ladang (OR=2.0-2.1), sistem perumahan yang luas (OR=4.0), umur ladang yang semakin meningkat (OR=1.2), dan jangkitan bersama Paramphistome (OR=1.4). *Fasciola* dan Paramphistome fec dalam Bovid yang dijangkiti bersama mempunyai korelasi positif sederhana yang signifikan ($r=0.36$, $p < 0.01$). Selain itu, kepekatan *Fasciola* fec dan koproantigen *Fasciola* juga mempunyai korelasi positif sederhana yang signifikan ($r=0.55$, $p < 0.01$). Kawasan

terdedah kepada fasciolosis dalam kajian ini menunjukkan pendedahan yang lebih tinggi di utara LM berbanding di pusat LM. Dengan itu, kajian ini mendedahkan prevalens sederhana fasciolosis bovid di LM dan faktor intrinsik perumah, pengurusan ladang, dan jangkitan bersama dengan Paramphistomes merupakan signifikan dikaitkan dengan fasciolosis bovid di LM. Korelasi positif *Fasciola* dan Paramphistomes dalam bovid yang dijangkiti bersama boleh memberikan asas ramalan untuk fasciolosis bovid daripada kejadian Paramphistome. Selain itu, kepekatan koproantigen *Fasciola* berguna dalam memantau pengeluaran telur *Fasciola* untuk memulakan rawatan fasciolosis yang disasarkan, namun memerlukan kajian lanjut bagi penggunaan ELISA koproantigen untuk pendapatan ini. Kawasan terdedah fasciolosis yang tinggi di LM berguna untuk menjalankan program kawalan untuk mengekalkan pengeluaran dan kebajikan bovid. Hasil kajian ini berfaedah ke arah pemahaman epidemiologi fasciolosis bovid tempatan yang penting dalam kesihatan veterinar dan awam.

Kata kunci: prevalens, pendedahan fasciolosis, faktor risiko, parasit ruminan, peta dedahan, ternakan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Nur Mahiza binti Md Isa, PhD

Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Nur Fazila binti Saulol Hamid, PhD

Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Zulfa Hanan binti Ashaari, PhD

Senior Lecturer
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Member)

Ferdius @ Ferdaus binti Mohamat Yusuff, PhD

Senior Lecturer
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 August 2022

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Signature : _____
Name of Chairman of
Supervisory Committee : Nur Mahiza Md Isa

Signature : _____
Name of Member of
Supervisory Committee : Nur Fazila Saulol Hamid

Signature : _____
Name of Member of
Supervisory Committee : Zulfa Hanan Ashaari

Signature : _____
Name of Member of
Supervisory Committee : Ferdius @ Ferdaus Mohamat Yusuff

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LIST OF ABBREVIATIONS

AIC	Akaike's information criterion
ANOVA	Analysis of variance
BCS	Body condition score
CO1	Cytochrome oxidase subunit 1
COWS	Control of Worms Sustainably
EPG / epg	egg per gram
ESA	Excrete/secrete antigen
<i>F. gigantica</i>	<i>Fasciola gigantica</i>
<i>F. hepatica</i>	<i>Fasciola hepatica</i>
FCathL	<i>Fasciola</i> cathepsin-L
FEC / fec	faecal egg count
FECRT	faecal egg count reduction test
FFEC	<i>Fasciola</i> faecal egg count
GLDH	Glutamate dehydrogenase
IH	Intermediate host
ITS2	Internal transcribed spacer 2
ITT sheep	Indonesian thin tail sheep
<i>L. auricularia</i>	<i>Lymnaea Auricularia</i>
<i>L. rubiginosa</i>	<i>Lymnaea rubiginosa</i>
<i>L. swinhoei</i>	<i>Lymnaea swinhoei</i>
<i>L. viridis</i>	<i>Lymnaea viridis</i>
mAb	Monoclonal antibody
MCH	Mean corpuscular haemoglobin

MLR	Multiple linear regression
n	Sub-total number of populations
N	Total number of populations
n _{adj}	Adjusted sub-total number of populations
Nad1	NADH dehydrogenase subunit 1
NEJ	Newly excysted juvenile
NTD	Neglected tropical disease
OD	Optical density
OR	Odd ratio
PCV	Packed cell volume
r	Coefficient of correlation
RBC	Erythrocyte counts
SCOPS	Sustainable Control of Parasites
SD	Standard deviation
SDH	Sorbitol dehydrogenase
Th	T helper
VIF	Variance inflation factor
VRI	Veterinary Research Institute
VRL	Veterinary Regional Laboratories
WHO	World Health Organization
WPI	Weeks post-infection

CHAPTER 1

INTRODUCTION

1.1 Background

Fasciolosis is a significant food borne parasitic disease to domestic ruminants and capable to infect human through the consumption of plants or water which containing infective *Fasciola metacercariae* (Saleha, 1991). This disease is an emerging neglected tropical disease (NTD) towards human public health with the estimation of 180 million human are at risk of fasciolosis worldwide (Cwiklinski et al., 2016). Fasciolosis is considered as an endemic disease to ruminant livestock in Malaysia due to the climate that is optimal for the snails proliferation. The freshwater snail is known to play an important role as intermediate host and suitable for the development of *Fasciola cercariae*.

Two species of Fasciolid parasite, namely *Fasciola hepatica* and *F. gigantica* can parasitise the herbivorous mammals through ingestion of the infective *Fasciola metacercariae*. However, the researchers in several countries like Vietnam, Thailand and China revealed that there is a newly discovered *Fasciola*, which is “*Fasciola*-hybrid” due to the unusual events from the areas of *F. hepatica* and *F. gigantica* co-exist (Nguyen et al., 2018). Although, in Malaysia, *F. gigantica* is the only *Fasciola* found in this country (Diyana et al., 2020).

Fasciolosis is a significant problem to ruminant livestock because *Fasciola* is causing morbidity and mortality to the infected animals, hence leads to the economic loss. Estimation of round 3.2 billion USD loss is recorded worldwide annually, due to fasciolosis in livestock (Mehmood et al., 2017). This is associated with the decrease in the animal production (i.e. milk and meat), liver condemnation, and fatality apart from morbidity and mortality (Saleha, 1991). Morbidity in infected ruminant is associated with the reduction of weights, fertility rate, and weaker immune system (Mehmood et al., 2017). Whilst, mortality in infected sheep was associated with chronic liver damage accumulated over several years if without successful treatment (Munita et al., 2019).

Infected ruminant usually results to liver condemnation during the inspection from abattoirs. However, the economic loss due to the liver condemnation is considerable (Shinggu et al., 2019) and demonstrated by the body weight reduction and the narrowing of growth rate (Shinggu et al., 2019). Due to this reason, the *Fasciola*-infected animal will not result in maximum profitability (Shinggu et al., 2019). Fasciolosis is more prevalent in developing country, including Malaysia compared to the developed countries, mainly because of the husbandry management practices, farmers’ knowledge on the livestock diseases and the availability of appropriate control programme (Mehmood et al., 2017; Shahudin et al., 2018).

1.2 Problem statements and relevance of the study

Malaysia is a country that is committed in promoting the food security in the country. Thus, around RM99.5 billion of gross domestic product is allocated for the agricultural sector; and the livestock production contributes of 14.9% of the agricultural sector. Subsequently, this makes the third largest agricultural industry after the oil palm plantation and other cultivations (Malaysian Department of Statistics, 2019). In line with the conceptualisation of national Shared Prosperity Vision 2021 to 2030, the sixth strategic thrust in the first strategy is; to recognise the potential economic areas for regional development based on each states in Malaysia. One of the key activities for the economic growth is by strengthening the livestock sector, mainly in Perlis, Kedah, Perak, Selangor, Negeri Sembilan, Sarawak, and Sabah (Malaysian Ministry of Economic Affairs, 2019). However, there are challenges as ruminant livestock are potentially exposed to parasites, thus limiting the livestock quality, security, and safety products in Malaysian agricultural industry (Azima Laili et al., 2020).

Perak is one of the states identified for strengthening the livestock sector in the national Shared Prosperity Vision 2021 to 2030 (*Wawasan Kemakmuran Bersama 2021 sehingga 2030*). However, a survey was done in the Larut and Matang (Taiping), Perak abattoir; revealed that Larut and Matang (Taiping) showed the highest bovid fasciolosis occurrence compare to other main abattoirs in Peninsular Malaysia (Diyana et al., 2020). In addition, Larut and Matang (Taiping) town is receives the highest rainfall in Peninsular Malaysia with mean of over 4,000mm annually (The Strait Times, 1959; Malaysian Department of Meteorology, 2020), which is a suitable site for *Fasciola* to thrive (Mochankana and Robertson, 2018; John et al., 2019). Following this finding, it is crucial to investigate further on the fasciolosis prevalence in Larut and Matang (Taiping). In addition, the risk factors of the disease are also needed to be determined for the local bovid fasciolosis epidemiology.

Recently, co-infection of *Fasciola* and Paramphistomes occurrence has been increasingly reported by several investigations which might provide significant understanding on fasciolosis epidemiology (Sargison et al., 2016; Huson et al., 2017; Naranjo-Lucena et al., 2018; Munita et al., 2019). Therefore, it is important to see the correlation between these two parasite species in Larut and Matang (Taiping) as the interaction of co-parasitism might influence the disease dynamics among the co-infected host (Huson et al., 2017).

A recent coproantigen ELISA was developed by Mezo et al. (2004) and has been consider as a sensitive diagnostic in detecting early active *Fasciola* infection (four to seven weeks before patency) (Valero et al., 2009). The diagnostic is more practical in a large scale of fasciolosis surveillance compare to the conventional faecal sedimentation (Mezo et al., 2004; Brockwell et al., 2013; Kajugu et al., 2015). However, the association of *Fasciola* faecal egg count (FFEC) with the *Fasciola* coproantigen concentration through coproantigen ELISA is needed, to observe the application of this diagnostic in detecting the fasciolosis predominantly by determining the animals which excreting high FFEC. Therefore, it is important to evaluate the correlation of *Fasciola* coproantigen concentration to *Fasciola* faecal egg count (FFEC).

Moreover, determination of exposure areas is beneficial to observe the spatial distribution of *Fasciola* which were increasingly applied in UK for fasciolosis forecasting (Rapsch et al., 2008; Novobilsky et al., 2014). Hence, the identification of exposure areas of bovid fasciolosis is important to ease in planning control measures to sustain the livestock production in Larut and Matang (Taiping). Besides, could be used as a tool to alert the zoonotic potential to the public.

1.3 Scope of the study

This study focuses on describing the epidemiology of bovid fasciolosis in Larut and Matang (Taiping), Perak, Malaysia. In developing parasite control programme to limit the negative impact to ruminant production, the prevalence and significant risk factors in the region must be described first. This study is a cross-sectional study of bovid fasciolosis which has been conducted in randomly selected farms as to identify the risk factors that may drive the infection which subsequently extent the exposure or transmission to susceptible hosts. The main factors investigated in present study were the host intrinsic and farm management system employed in the selected farms, whilst the occurrence of Paramphistomes through the detection of eggs was also noted as the co-infection of *Fasciola* and Paramphistomes was common from the faecal samples of the study. The host intrinsic factors include the ruminant species, sex, age group, and body condition score; while the farm management factors include the water source, mixed species farming, housing system, grazing range, and number of bovid per farm; and also, the positivity of co-infection with Paramphistomes which were analysed through the logistic regression model to identify the significant risk factors of bovid fasciolosis in this study. Owing to the common occurrence of *Fasciola* co-infected with Paramphistomes, correlation analysis was done for further evaluation of Paramphistomes co-infection as the risk factor for bovid fasciolosis. In particular, this study evaluated the use of coproantigen ELISA to monitor the *Fasciola* faecal egg count disperse to the environment through correlation analysis which providing basis on the potential use of coproantigen ELISA in controlling fasciolosis. Exposure mapping of bovid fasciolosis was visualised in this study to locate the exposure areas in Larut and Matang (Taiping). Findings in this study may be utilised to formulate the hypotheses in future studies to improve the understanding of the epidemiology of local bovid fasciolosis.

1.4 Main aim and objectives

The main aim of this study was to describe the epidemiology of bovid fasciolosis in Larut and Matang (Taiping) Perak, Malaysia. Thus, to achieve the goals, this study focuses on five (5) specific objectives as below:

1. To determine the bovid fasciolosis prevalence of selected farms in Larut and Matang (Taiping), Perak by faecal examinations.
2. To identify the risk factors associated with bovid fasciolosis in Larut and Matang (Taiping) by questionnaire survey.
3. To correlate the *Fasciola* and Paramphistomes faecal egg count in co-infected bovid.
4. To correlate the *Fasciola* faecal egg count and *Fasciola* coproantigen concentration through optical density of coproantigen ELISA.
5. To map the exposure areas of bovid fasciolosis in Larut and Matang (Taiping) through heatmap density based on *Fasciola* exposures (prevalence, *Fasciola* faecal egg count) and farm management (herd amount and grazing area) factors.

1.5 Study hypotheses

1. High fasciolosis prevalence is present from bovid population in Larut and Matang (Taiping) from the descriptive analysis of this study.
2. The factors of host intrinsic, farm management, and co-infection with Paramphistomes are significant risk factors associated with bovid fasciolosis in Larut and Matang (Taiping) through the logistic regression model of data obtained in this study.
3. There is significant positive correlation of the *Fasciola* faecal egg count with Paramphistomes faecal egg count in the co-infected bovid through the correlation test
4. There is significant positive correlation of *Fasciola* coproantigen concentration of ELISA optical density (OD) with *Fasciola* faecal egg count through the correlation test.
5. The exposure areas of Larut and Matang (Taiping)) to fasciolosis are reflected to the sampling areas from this study which have a high index of combined selected risk factors from the bovid fasciolosis.

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