



UNIVERSITI PUTRA MALAYSIA

**PREVALENCE AND RISK FACTORS OF CATTLE FASCIOLIASIS IN
SELECTED FARMS IN TAIPING, PERAK**

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FPV 2020 35**

**PREVALENCE AND RISK FACTORS OF CATTLE FASCIOLIASIS
IN SELECTED FARMS IN TAIPING, PERAK**

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE

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CERTIFICATION

It is hereby certified that we have read this project entitled “Prevalence and Risk Factors of Cattle Fascioliasis in Selected Farms in Taiping, Perak” by Fatin Nur Ain binti Kamarul Bahrin and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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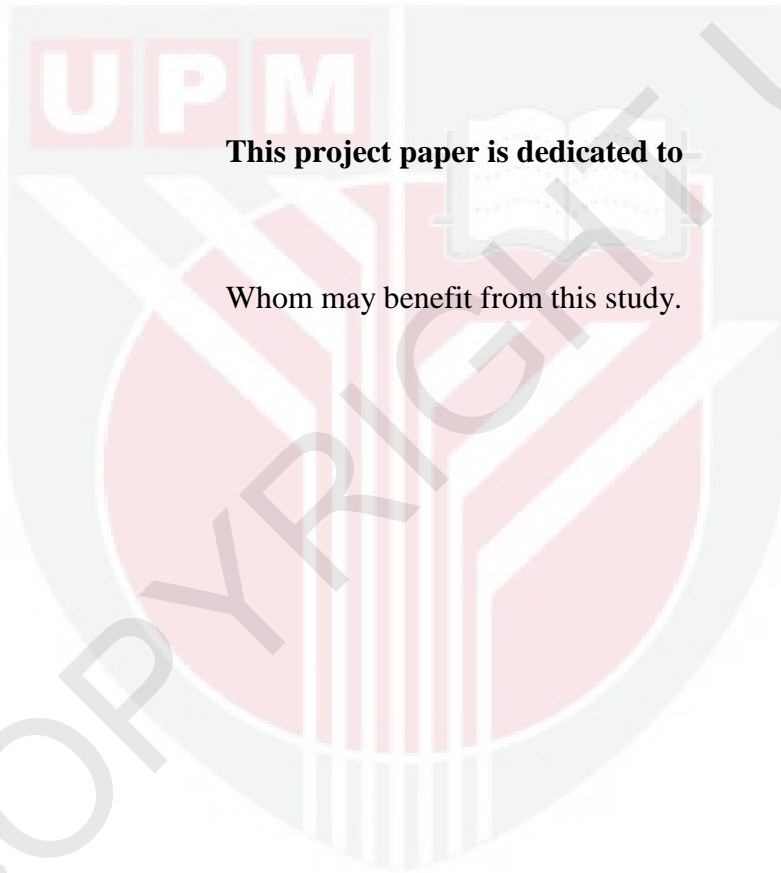
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DEDICATIONS

This project paper is dedicated to

Whom may benefit from this study.



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LIST OF ABBREVIATIONS

ver.	version
sp.	species
%	percent
°C	degree Celcius
ml	millilitre
mm	millimetre
=	equal to
&	and
<	less than
>	more than
≤	less than or equal to
≥	more than or equal to
DVS	Department of Veterinary Service
SFSM	Simple Faecal Sedimentation Method

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 –

Projek Tahun Akhir

**PREVALENS DAN FAKTOR RISIKO FASIOLIASIS PADA LEMBU DI
LADANG TERPILIH DI TAIPING, PERAK**

Oleh

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2020

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Fasioliasis dalam ruminan adalah penyakit zoonotik yang disebabkan oleh *Fasciola hepatica* dan *Fasciola gigantica*. Ia mengurangkan produksi ruminan dan menyebabkan hati yang berpenyakit perlu dikondem pada pemeriksaan di rumah sembelih. Kajian prevalens fasioliasis pada lembu dan faktor risiko berkaitan di Taiping, Perak adalah terhad. Oleh itu, kajian ini dijalankan untuk mengkaji kejadian fasioliasis pada lembu di ladang terpilih di Taiping, Perak dan faktor risiko berkaitan seperti jantina, umur, baka, skor keadaan badan, pengurusan ladang dan sejarah banjir. Sejumlah 35 ekor lembu daripada 4 ladang di Taiping telah dipilih secara

rambang menggunakan kaedah persampelan rawat mudah. Diagnosis fasioliasis pada lembu telah menggunakan dua kaedah iaitu Kaedah Pemendapan Najis Mudah (KPNM) dan Teknik Flukefinder®. Dalam perbandingan menggunakan dua kaedah tersebut, KPNM menunjukkan kejadian keseluruhan 2.86% (1/35) manakala Teknik Flukefinder® mengenalpasti dua sampel positif fasioliasis dan menunjukkan kejadian 5.71% (2/35). Keputusan daripada kajian ini menunjukkan tiada perbezaan yang ketara antara jantina, baka, skor keadaan badan, pengurusan ladang (separa intensif dan extensif), sejarah banjir dan fasioliasis. Walau bagaimanapun, haiwan daripada umur 1 tahun dan keatas mempunyai risiko yang lebih tinggi untuk mendapat fasioliasis berbanding haiwan kurang dari satu tahun. Dari kajian ini, keseluruhan prevalense fasioliasis pada lembu adalah rendah tetapi langkah berjaga-jaga dan kawalan perlu diambil kerana jangkitan fasioliasis adalah zoonotik.

KATA KUNCI: *fasioliasis, lembu, ruminan, faktor risiko, Taiping*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

**PREVALENCE AND RISK FACTORS OF CATTLE FASCIOLIASIS IN
SELECTED FARMS IN TAIPING, PERAK****By****FATIN NUR AIN KAMARUL BAHRIN****2020****Supervisor: Dr Nur Mahiza Md Isa****Co-supervisor: Prof Dr Saleha Abd Aziz****: Dr Fazila Saulol Hamid**

Fascioliasis in ruminant is a zoonotic disease mainly caused by *Fasciola hepatica* and *Fasciola gigantica*. It reduces ruminant productivity and cause the affected liver to be condemned in abattoir. The study on the prevalence of cattle fascioliasis and its associated risk factors in Taiping, Perak is limited. Therefore, this study was conducted to determine the prevalence of cattle fascioliasis in selected farms in Taiping, Perak, with its associated risk factors which were gender, age, breed, body condition score, management and flood history. A total of 35 cattle from four farms in Taiping were selected randomly using convenience faecal sampling. The diagnosis of fascioliasis in cattle was done using two methods which were Simple Faecal Sedimentation Method (SFSM) and Flukefinder® Technique. In the

comparison of the two different techniques, SFSM showed overall prevalence of 2.86% (1/35) while Flukefinder® technique detected two samples positive for fascioliasis and showed prevalence of 5.71% (2/35). Results from this study showed no significant difference of the association among gender, breed, body condition score, farm management (semi intensive and extensive), flood history to fascioliasis. Based on this study, the overall prevalence of cattle fascioliasis was low but, precautions must be taken as fascioliasis infection is zoonotic.

Keywords: *fascioliasis, cattle, ruminant, risk factors, Taiping,*

1.0 INRODUCTION

Ruminant can be infected by a number of parasites. These parasites are classified as protozoa, helminths, and ectoparasites. Parasites give a negative effects to animals which may compromise their health and welfare including pain, itchiness and discomfort. Blood sucking parasites cause blood loss which may lead to anaemia and death if substantial. Reduce productivity and susceptible to other diseases are also the negative impact of parasites manifestation and lead to economic losses. One of the causes of significant economic losses for the farmer is fascioliasis.

Fascioliasis is one of the major yet neglected parasitic diseases that are zoonotic and reduces productivity in ruminants. It is caused by trematodes that infects liver and associated organs (Isah,2019). An estimated 17 million people are infected and a further 180 million people at risk of infection. Thus, at the Third Global Meeting of the Partners for Parasite Control held at the World Health Organization (WHO), fascioliasis was included as an important helminthiasis affecting humans (Anonymous, 2004). In Malaysia, there was only one report of human fascioliasis (Bizini, 2018).

Animals infected with fascioliasis show either acute, subacute or chronic clinical signs. In acute and subacute infection, animals are anemic and jaundiced while death occurs several days after the onset of clinical signs. In chronic infection, animals show signs of appetite loss, weakness, weight loss, anemia, submandibular edema, blackish and soft stool and indigestion.

The most common flukes infecting ruminants are *Fasciola hepatica* (*F.hepatica*) and *Fasciola gigantica* (*F. gigantica*). In Malaysia, *F. gigantica* is the main cause of fascioliasis (Alia et al. 2020; Zainalabidin et al. 2015). The earliest study conducted by Yap et al. (1983) reported about 74% prevalence of fascioliasis in cattle in Selangor. Zainalabidin et al. (2015) found that the prevalence of fascioliasis in large ruminants slaughtered in abattoirs in Perak was 7.5% while Fazly (2015) reported 8% prevalence rate in Perak. The prevalence of fascioliasis in Terengganu farms was between 93 to 95% (Khadijah et al., 2015;2017). Masrin et al. (2015) reported that during the period of 2004 to 2013, the prevalence rate of 11.2% in Perak. In the Universiti Putra Malaysia (UPM) foster farms, the prevalence of fascioliasis was 10% (Aqilah, 2018). The prevalence of fascioliasis is associated with factors such as age, gender, breed, farm management, humidity and temperature in other countries (El-Tahawy et al.2017; Zhang et al. 2017; Alia et al. 2019). To date, there were a number of studies regarding the prevalence of fascioliasis in different region in Malaysia (Diyana et al. 2019; however, there is still lack of study regarding the risk factors associated with fascioliasis in Taiping, Perak.

Hypothesis

1. H01 : The prevalence of fascioliasis in cattle is not significant in selected farms in Taiping, Perak.

HA1 : The prevalence of fascioliasis in cattle is significant in selected farms in Taiping, Perak.

2. H02 : Age, gender, breed, farm management, humidity and temperature are not risk factors for fascioliasis in cattle.

HA2 : Age, gender, breed, farm management, humidity and temperature are risk factors for fascioliasis in cattle.

Objectives

1. To determine the prevalence of cattle fascioliasis in selected farms in Taiping, Perak.
2. To determine the risk factors associated with cattle fascioliasis in selected farms in Taiping, Perak.

2.0 LITERATURE REVIEW

2.1 Fascioliasis

Fascioliasis is an important disease in ruminants caused by two main liver flukes species *Fasciola hepatica* and *Fasciola gigantica* (Rita et al., 2017). The disease develop when the cattle ingests the encysted metacercariae of the *Fasciola* sp.(Najib et al., 2020). Liver flukes are flat trematodes with leaf-like shape. The size of *F. gigantica* is up to 75 mm by 15 mm which is bigger than *F. hepatica*, with body length of up to 30 mm by 15 mm when it reaches maturity (Global Health, 2019). It is large enough to be detected by naked eye. Both of the species causes the same effect on ruminants even though their sizes are different.

The Malaysian ruminant livestock sector consists of beef and dairy cattle, buffaloes, sheep and goats which contribute to about 12.1% of the total agricultural gross domestic product (GDP) in 2014 (Shanmugavelu, 2014). This low percentage is mainly due to many ruminant animals are reared in small-scale production; although progress can be seen in the recent years, however it is still unable to meet local demand (Mohamed, 2007). The lag in the Malaysian ruminant sector is caused by several factors such as lack of land resource, high feed price, cheaper import substitutes, poor private - sector involvement (Shanmugavelu, 2014), disease prevention and control (Mohamed, 2007) and lack of quality breeds, expertise and workforce (National Agro- Food Policy 2011- 2020).

In terms of disease prevention and control, infection of parasitic trematode (*Fasciola gigantica*) is often neglected due to the low prevalence risk (Fazly et.

al., 2015) and poor education exposure of parasitic infestation in Malaysian farmers. Since the ruminant sector is highly dependable on local feedstuff due to the low cost of feeding, lush pastures are often used for grazing cattle which poses a threat as infective trematode larvae thrive in this environment (Guerden et al., 2008; Chandrawathani et al., 2009). Since cattle is an herbivorous animal, infection of parasitic trematodes causes liver enlargement and pathological changes resulting in carcass condemnation at abattoirs leading to severe economic losses as the carcass is deemed unfit for market (Nyirenda et al., 2019).

Liver damage due to immature *F. gigantica* will cause the animal to become susceptible to Black disease caused by *Clostridium novyi* thus an increased mortality rate will occur before animal is slaughtered, causing substantial financial loss towards the farmer (Nyirenda et al., 2019). Additional economic losses may be associated with expenses on anthelmintic for treatment as the farmer with substantial losses will have to invest on anthelmintic drugs to prevent further infestation. Production efficiency of the farm will be reduced and negatively affect the whole production cycle of the farm as decreased milk production, poor weight gain and impaired fertility will occur, resulting a poor profit margin for farmers (Nyirenda et al., 2019).

2.2 Life Cycle of *Fasciola sp.*

Based on Global Health, Division of Parasitic Diseases and Malaria (2019), the life cycle of *Fasciola sp.* is complex that requires a suitable aquatic plants, an intermediate host for larval stage development, and the final host; for the flukes to become mature and reproduce as shown in Figure 1. The intermediate host is a snail in the family of Lymnaeidae in the genera *Lymnaea*, *Galba*, *Fossaria*, and *Pseudosuccinea*. The snail species may be different with respect to be intermediate host for *F. gigantica* or *F. hepatica*.

The route of transmission to ruminants or humans is via ingestion of contaminated food or water with the metacercariae. The life cycle starts with immature eggs released in the biliary duct are passed in the faeces or stool. Over two weeks in freshwater, the eggs become embryonated and release miracidia. In this stage, the miracidia will invade an intermediate host which is the snail. The miracidia will further develop into sporocysts, redia, and cercariae inside the snail. Once it reaches cercariae stage, it will be release freely in the fresh water and develops into metacercariae which is an infective stage and encysts to vegetation.

Animals that ingest the contaminated vegetation infested with the metacercariae of *Fasciola sp* will be infected. After ingestion of the metacercariae, they excyst in the duodenum as immature flukes and penetrate the intestinal wall into the peritoneal cavity. The immature flukes wander to the liver and reside in the biliary duct by penetrating the liver parenchyma to become adult flukes of different size which is longer in *F. gigantica* compared to *F. hepatica*. In humans, the maturation of immature flukes is much longer which takes about 3 to 4 month.

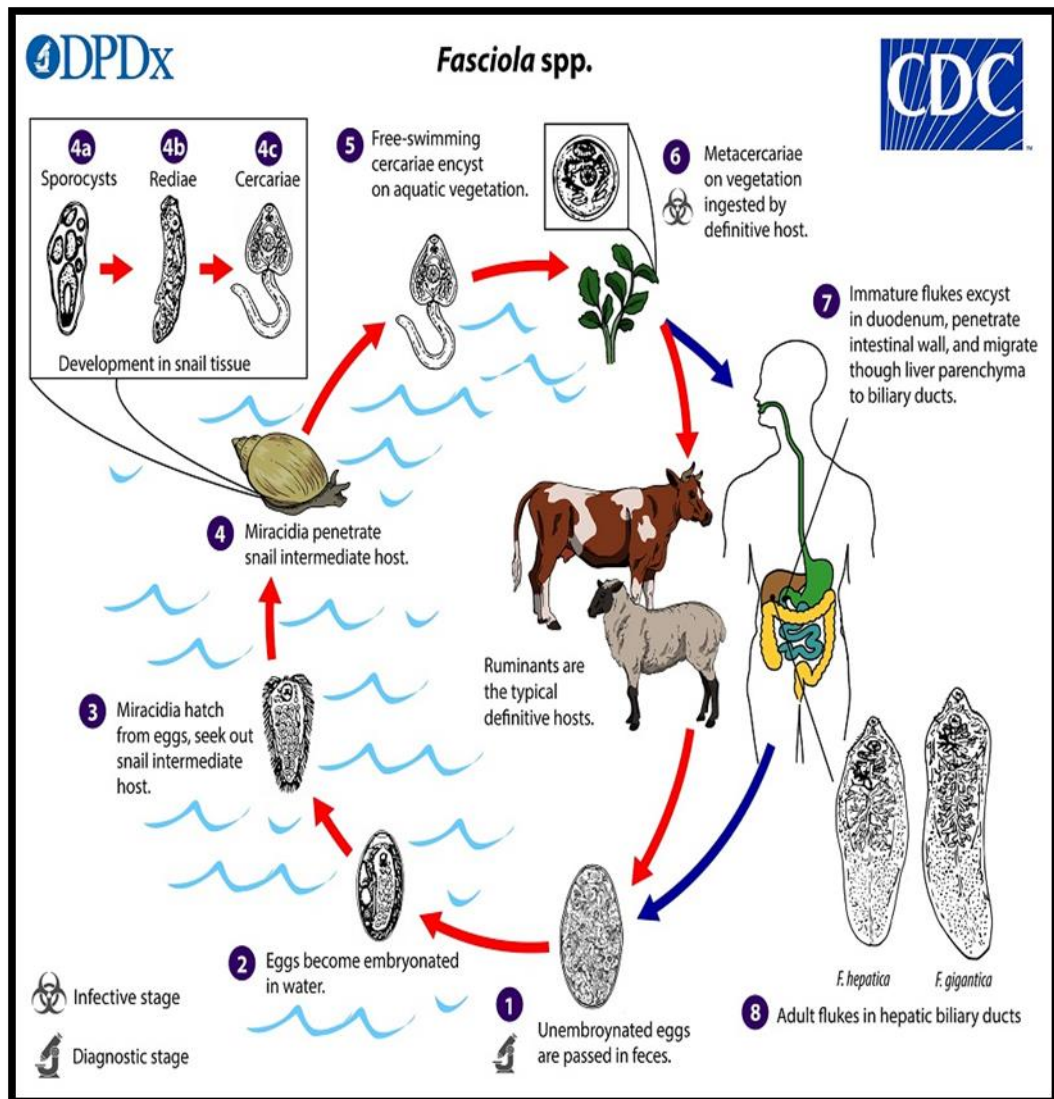


Figure 1: Life Cycle of *Fasciola* sp.

Global Health, Division of Parasitic Diseases and Malaria (2019)

2.3 Prevalence Status of Ruminant Fascioliasis in Malaysia

In Malaysia, there are a number of research regarding prevalence of fascioliasis in ruminants. Based on condemned liver samples, the prevalence of fascioliasis in cattle and buffaloes in Perak was 7.46% and 7.69%, respectively (Zainalabidin et al., 2015) while prevalence of 11.02% from faecal samples of ruminants was reported by Masrin et al. (2015) from year 2004 to 2013. In Kuala Terengganu, two studies showed prevalence of 95% (Khadijah et al., 2015) and 67% (Khadijah et al., 2017) of cattle fascioliasis. In a study conducted by Diyana et al. (2019), five selected Regional Veterinary Laboratories which are Johor Bahru, Johor (south); Kuantan, Pahang (east); Bukit Tengah, Pulau Pinang (north); Kota Bharu, Kelantan (east); and Sepang, Selangor (west) was 1.76% from year 2007 to 2017. A study on the prevalence of helminthiasis in cattle in Terengganu by Rita et al. (2017) revealed the prevalence of cattle fascioliasis based on faecal examination was 41%.

Saleha (1991) reported the prevalence of 34.4% of goat fascioliasis in certain states in Peninsular Malaysia (Perak, Kedah, Negeri Sembilan, Selangor, Melaka and Johor). According to Mursyidah et al. (2017), serological test that have been carried out on samples in 16 farms in Terengganu (Besut, Setiu, Kuala Terengganu, Hulu Terengganu, Marang, Dungun and Kemaman) revealed prevalence of 89% in goats and sheep.

2.4 Zoonotic Potentials of Fascioliasis

Both hepatic parasites; *F. gigantica* and *F. hepatica* are zoonotic which can infect humans (Zainalabidin et al., 2015). A study conducted by Groove (1990) was on the first ever documented fascioliasis in human in Berlin, Germany during an autopsy of a female in 1970. In Cuba, Uruguay and Argentina, human fascioliasis is common. Human can be infected by accidentally ingesting encysted metacercariae on raw fresh water green aquatic plant leaf such as watercress (*Nasturtium officinale*) (Saleha 1991, Kaya et al., 2011). Around one to three months after ingestion of the metacercariae, the hepatic phase begins which is the migration of immature liver flukes to biliary ducts by penetrating the liver parenchyma (Lim et al., 2007, Koç et al., 2009, Kabaalioğlu et al., 2000). Usually, the clinical signs of hepatic phase are liver function abnormality, fever, eosinophilia and abdominal pain (Aksoy, 2006) while biliary phase shows symptoms with or without cholangitis with intermittent right upper quadrant pain (Bektaş et al., 2010, Gulsen et al., 2006).

There are reported prevalence of human fascioliasis with 7.75% in 2015 (Quy et al., 2015) and 5.9% in 2016 (Nguyen et al., 2016) in Vietnam. In the North Africa (Ethiopia) (Nyindo et al., 2015), Middle East (Egypt, Iran, Iraq, Syria and Saudi Arabia) and South America (Peru and the Bolivian Altiplano) (Hilyer et al., 1992), the incidence rate of human fascioliasis was high. In Malaysia, there was a case of human fascioliasis in Tuaran, Sabah (Bizini, 2018). However, the prevalence of human fascioliasis in Malaysia remain unknown because the case is under reported (Najib et al., 2020).

2.5 Risk Factors Associated with Fascioliasis Infections

In Malaysia, variables like gender, age, deworming practice and management type were associated with bovine fascioliasis. A study conducted by Rita et al. (2017) found that gender and age of the animal was significantly associated with fascioliasis in selected farms in Terengganu. The female cattle were infected higher than male cattle, and cattle with age between 5 to 10 years were mostly being infected. While, in Sabah conducted by Nurshafiq (2019), deworming practice and management type are significantly associated with ruminant fascioliasis.

In other countries, there is association between fascioliasis in farm with gender, age, breed, type of farm, temperature and humidity. (El-Tahawy et al., 2017, Khan and Maqbool, 2017, Zhang et al., 2017). In another study by Kusumarini et al. (2020), there was a significant association between body condition score and fascioliasis.

The prevalence of cattle fascioliasis in different gender may differ because of sampling methods. Sampling in abattoir showed high prevalence in male because most beef cattle were male. Sampling in farms might show high prevalence in female due to more female in farms for reproductive purpose, milk production and others (Dawa et al., 2013; Khan et al., 2010; Zeleke et al., 2013).

Age of animal is one of the risk factors of fascioliasis. Based on studies conducted by Jaja et al. (2017), higher prevalence were reported in younger animal as compared to older animals. Young cattle graze pastures frequently thus, increase chances of being infected by *Fasciola* sp. The highest prevalence was reported in less than two years old age group (10.91%), and the lowest was more than three years old age groups (8.35%) (Tahawy et al., 2017). In contrast, based on another

study, 53% of prevalence was detected in adult cattle (2-3 years), and 78% had abnormalities in liver based on post-mortem and morphological findings (Kusumarini et al., 2020). This study showed the young and adult cattle can be potentially infected with fascioliasis.

Tahawy et al. (2017) reported that cattle with medium and poor conditions were associated with fascioliasis more than those with good body condition score. Fascioliasis was detected more in cattle with poor body condition score (Jaja et al, 2017). In the latest study, good body condition score showed highest prevalence which was 86%. The medium body condition score reported prevalence of 85% and lastly 3% of prevalence in poor body condition score (Kusumarini et al., 2020). This results indicated fascioliasis are chronic infection as poor BCS can be observe after a infected by *Fasciola* sp. (Kusumarini et al., 2020).

3.0 MATERIALS AND METHODS

3.1 Study Area

Four farms located in North Taiping, Perak are in Batu Kurau (4.978266500488239, 100.78927211222414) as shown in Figure 2. Batu Kurau is located 20km from Taiping city. The humidity in Batu Kurau is 95% while the temperature of the area is 28°C according to World Weather Online. Figure 3 shows the location of the selected farms in Perak.



Figure 2: Taiping and Batu Kurau location in Perak.



Figure 3: Selected farms in Taiping, Perak;

4°55'24"N, 100°47'32"E

4°56'55"N, 100°48'49"E

4°57'08"N, 100°47'18"E

4°56'25"N, 100°47'35"E

3.2 Sampling Procedure

Thirty five (35) fresh faecal samples were obtained via free catch from the groups of animal. All the samples were from cattle. Each faecal sample was placed separately in a clean plastic bag, labeled and stored at 4°C before analysis.

Other parameters; age, gender, body score, management, and deworming practice data were obtained by interviewing farmers based on a set of questionnaire. (*Appendix I*).



3.3 Sample Analysis

The analysis of the collected faecal samples was done in Parasitology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The characteristics of *Fasciola* spp. egg that can be detected microscopically are big, thin shell, yellow-stained in colour with operculum (Hussein et al., 2010). This must be differentiated from *Paramphistomum* spp. which is more elongated in shape, does not stained yellow and has a clear shell and distinct operculum (Hotessa and Kanko, 2020) (*Figure 10, 11, 12 & 13 in Appendix II*). There are two techniques which were used in this study for the detection and isolation of *Fasciola* spp. eggs from faeces which were Standard faecal sedimentation technique (SFSM) and Flukefinder® method (Richard Dixon, ID, US).

For SFSM, four grams of each faecal sample was mixed thoroughly with 50mL of water. Then, it was poured through a strainer to remove large particles into conical flask. Tap water was added into the conical flask and the suspension was then left to stand for 5 minutes to allow sedimentation. Then, two-third supernatant were discarded without disturbing the sediment. Tap water was added again for another two times or until until a clear supernatant was obtained. The sediment was placed in a petri dish, to which 3 drops of 1% methylene blue was added. Dissecting microscope was used to examine the sediment under 45x magnification.

Flukefinder® is a kit that has been used commercially with two separate sieves of approximately 125 nm and 30 nm mesh and 2 inch in diameter. For the procedure, two grams of each faecal sample was mixed with 30 ml of water and homogenized well. The suspension was then poured into the Flukefinder and rinsed with 100 ml of water. Larger materials retained by the large diameter sieve was discarded. Material

retained by the smaller sieve was back-washed and poured into a small beaker. The suspension was allowed to stand for two minutes before pouring the supernatant. The remaining solution was poured into a small petri dish. Lastly, 3 drops of methylene blue were added to the dish and the remaining material were examined under a 45x magnification of dissecting microscope. Eggs of *Fasciola species* were identified based on morphological characteristics (Valero et al. 2009), counted and recorded. The sieves were thoroughly cleaned and rinsed between samples.

3.4 Statistical Analysis

Prevalence of fascioliasis was calculated using the formula:

$$P(\%) = \frac{\text{Number of positive samples}}{\text{Total number of samples examined}} \times 100$$

Other parameters, namely, gender, age, body condition score, breed, management and farm's flood history were input into SPSS ver. 25 and the association was analysed using Chi-Square with 95% confidence interval.

4.0 RESULTS

4.1 Response Rate

During data collection, total of 35 animals were selected among the four farms. All four of the farmers had responded to the questionnaire. Therefore, the response rate was 100%.

4.2 Prevalence of Fascioliasis

Prevalence of fascioliasis using sedimentation technique:

$$P(\%) = \frac{1}{35} \times 100$$

$$P(\%) = 2.86\%$$

$$\text{Lower confidence interval} = 0.9100$$

$$\text{Upper confidence interval} = 1.0300$$

Prevalence of fascioliasis using Flukefinder® method

$$P(\%) = \frac{2}{35} \times 100$$

$$P(\%) = 5.71\%$$

$$\text{Lower confidence interval} = 0.8600$$

$$\text{Upper confidence interval} = 1.0200$$

(Table 14 in Appendix II)

4.3 Descriptive Analysis

4.3.1 Distribution of Cattle Fascioliasis Status

One sample (2.86%) was positive for fascioliasis from sedimentation technique (n = 35) while Flukefinder® technique revealed two samples (5.71%) were positive for fascioliasis as shown in Table 1.

Table 1: Distribution of cattle fascioliasis

NO.	Variables	Variable categories	N	Percentage (%)
1	Flukefinder®	Positive	2	5.71
		Negative	33	94.29
2	Sedimentation	Positive	1	2.86
		Negative	34	97.14

Statistical analysis in Table 2 below shows $P < 0.05$, there is significant in methods of detecting *Fasciola sp.* ova by using Flukefinder® and sedimentation technique.

Table 2: Chi-Square Tests of Flukefinder and SSFM Technique

Chi-Square Tests			
	Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square	16.985 ^a	1	.000
Continuity Correction ^b	3.747	1	.053
Likelihood Ratio	6.309	1	.012
Linear-by-Linear Association	16.500	1	.000
N of Valid Cases	35		

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is .06.

4.3.2 Distribution of Cattle Fascioliasis Status based on Farms

SFSM revealed only one out of four farms (25%) to be positive for fascioliasis. In Farm B, there is prevalence of fascioliasis with 20.00% as shown in Table 3. Flukefinder® technique revealed that two out of four farms (50%) are found to be positive for fascioliasis. In Farm B and C, there are prevalence of fascioliasis with 20.00% and 6.67% respectively as shown in Table 4.

Table 3: Farm * Sedimentation Crosstabulation

Farm * Sedimentation Crosstabulation						
Count						
		Sedimentation			Total	Prevalence
		Positive	Negative			
Farm	A	0	13	13	0.00%	
	B	1	4	5	20.00%	
	C	0	15	15	0.00%	
	D	0	2	2	0.00%	
Total		1	34	35	2.86%	

Table 4: Farm * FlukeFinder Crosstabulation

Farm * FlukeFinder Crosstabulation						
Count						
		FlukeFinder			Total	Prevalence
		Positive	Negative			
Farm	A	0	13	13	0.00%	
	B	1	4	5	20.00%	
	C	1	14	15	6.67%	
	D	0	2	2	0.00%	
Total		2	33	35	5.71%	

4.3.3 Distribution of Sampled Cattle

Table 5 below shows the distribution of associated factors of the sampled cattle. Most of the cattle were male (54.29%). Majority of the cattle were between 1-2 years old (71.43%). The cattle mostly were Kedah Kelantan breed (85.71%). Majority of the cattle had body score of 3 out of 5 (45.71%).

Table 5: Distribution of the Sampled Cattle (Gender, Age, Breed, Body Condition Score) (n = 35)

NO.	Variables	Variable categories	N	Percentage (%)	Mean \pm S.D
1	Gender	Male	19	54.29	1.46 \pm 0.51
		Female	16	45.71	
2	Age	< 1 year old	9	25.71	1.77 \pm 0.49
		1-2years old	25	71.43	
		> 2 years old	1	2.86	
3	Breed	KK	30	85.71	1.14 \pm 0.36
		KK Cross	5	14.29	
4	Body Condition Score	1	0	0.00	-
		2	13	37.14	
		3	16	45.71	
		4	6	17.14	
		5	0	0.00	

4.3.4 Distribution of Respondents' Farm Management Factors

Table 6 below shows the distribution of farm management factors of the cattle. All farms (100%) using natural water, scheduled deworming practice using ivermectin and never used pesticide/herbicide before. Most of the farm had history of flooding before (62.86%). Most of the cattle was managed semi intensively (51.43%).

Table 6: Distribution of Respondents' Farm Management Factors (Water source, Management, Flood history, Deworming practice, Pesticide/Herbicide use) (n = 35)

NO.	Variables	Variable categories	N	Percentage (%)	Mean±S.D
1	Water Source	Natural water	35	100.00	0.00±0.00
		Tap water	0	0.00	
2	Management	Intensive	0	0.00	2.49±0.51
		Semi-intensive	18	51.43	
		Extensive	17	48.57	
3	Flooding history	Never	13	37.14	0.63±0.49
		Flooded before	22	62.86	
4	Deworming practice	Never	0	0.00	1.00±0.00
		Scheduled	35	100.00	
5	Pesticide/Herbicide use	No	35	100.00	0.00±0.00
		Yes	0	0.00	

4.3.5 Comparison of Frequency of Positive and Negative Samples Resulting from the Faecal Sedimentation Technique and Flukefinder® technique

Table 7 below shows the positive and negative results of fascioliasis by using two different methods. SFSM detected one sample positive for fascioliasis at 2.86% (1/35) with 0.50 epg while Flukefinder® technique detected two positive samples for fascioliasis at 5.71% (2/35) with epg of 1.5 and 0.25. SFSM failed to detect 50% of the positive samples (detected only one positive sample); this showed that Flukefinder® technique was more sensitive. The egg per gram (epg) from Flukefinder® technique was higher at 1.5epg compared to 0.5epg by using SFSM.

Table 7: Comparison Frequency of Positive and Negative Samples Resulting from the Faecal Sedimentation Technique and Flukefinder® technique) (n = 35)

NO.	Variables	Variable categories	N	Percentage (%)
1	Faecal Sedimentation Technique	Positive	1	2.86%
		Negative	34	97.14%
2	Flukefinder® technique	Positive	2	5.71%
		Negative	33	94.29%

4.4 Chi Square Analysis

4.4.1 Association between Gender and Cattle Fascioliasis

Table 8 and Figure 4 show the association between gender and cattle fascioliasis. There was no significant difference [$p = 0.900$, $p > 0.05$] (Table 15 in Appendix II) in the fascioliasis prevalence between male and female animals.

Table 8: Association between gender and fascioliasis

Gender * FlukeFinder Crosstabulation

		FlukeFinder		Total
		Positive	Negative	
Gender	Male	1	18	19
	Female	1	15	16
Total		2	33	35

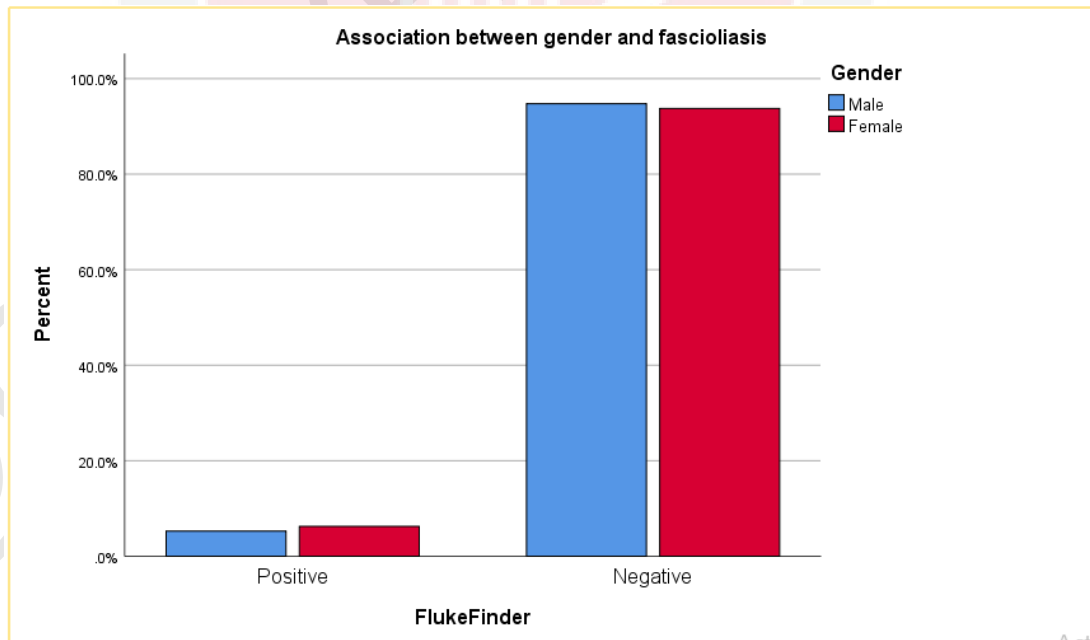


Figure 4: Association between gender and fascioliasis

4.4.2 Association between Age and Cattle Fascioliasis

Table 9 and Figure 5 show the association between age and fascioliasis. There was a significant difference [$p = 0.000$, $p \leq 0.05$] (Table 16 in Appendix II) in the fascioliasis prevalence among age groups of cattle.

Table 9: Association between age and fascioliasis

Age * FlukeFinder Crosstabulation

		FlukeFinder		Total
		Positive	Negative	
Age	< 1 years old	0	9	9
	1 to 5 years old	1	24	25
	>5 years old	1	0	1
Total		2	33	35

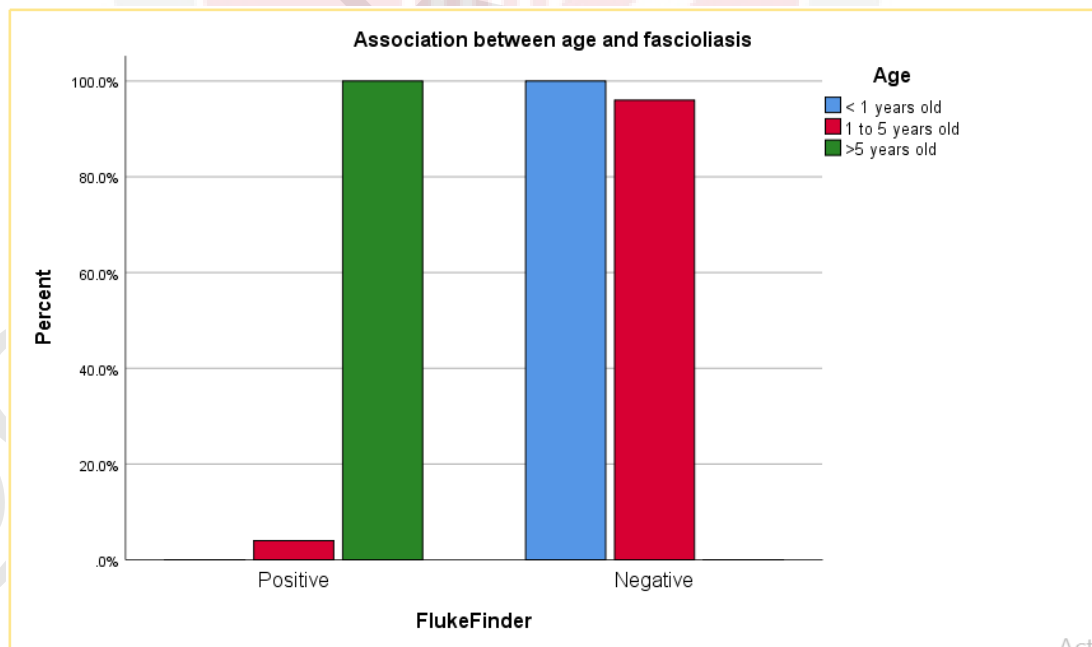


Figure 5: Association between age and fascioliasis

4.4.3 Association between Breed and Cattle Fascioliasis

Table 10 and Figure 6 show the association between breed and fascioliasis. There was no significant difference [$p = 0.137$, $p \geq 0.05$] (Table 17 in Appendix II) in the fascioliasis prevalence between both breed.

Table 10: Association between breed and fascioliasis

Breed * FlukeFinder Crosstabulation

		FlukeFinder		Total
		Positive	Negative	
Breed	Kedah	1	29	30
	Kelantan			
	KK Cross	1	4	5
Total		2	33	35

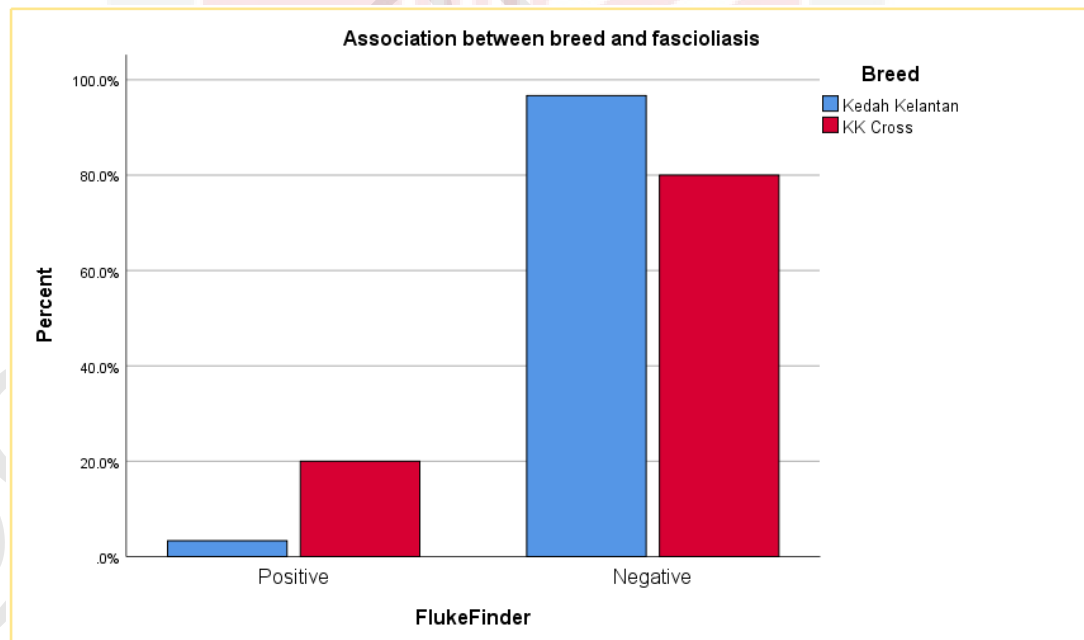


Figure 6: Association between breed and fascioliasis

4.4.4 Association between Body Condition Score and Cattle Fascioliasis

Table 11 and Figure 7 show the association between body condition score and fascioliasis. There was no significant difference [$p = 0.792$, $p \geq 0.05$] (see Table 18 in Appendix II) in the fascioliasis prevalence between body condition score.

Table 11: Association between body condition score and fascioliasis

BCS * FlukeFinder Crosstabulation

		FlukeFinder		Total
		Positive	Negative	
BCS	2	1	12	13
	3	1	15	16
	4	0	6	6
Total		2	33	35

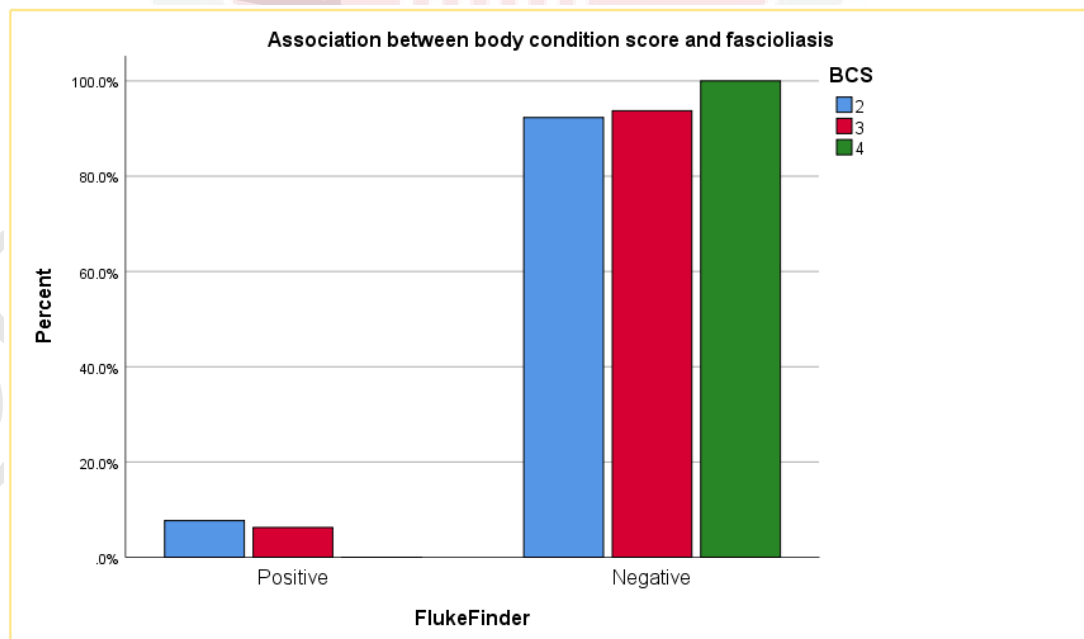


Figure 7: Association between body condition score and fascioliasis

4.4.5 Association between Farm Management and Fascioliasis

Table 12 and Figure 8 show the association between farm management and fascioliasis. There was no significant difference [$p = 0.967$, $p \geq 0.05$] (see Table 19 in Appendix II) in the fascioliasis prevalence between semi-intensive and intensive farm managements.

Table 12: Association between farm management and fascioliasis

FlukeFinder * Farm Management Crosstabulation

		Management		Total
		Semi Intensive	Extensive	
FlukeFinder	Positive	1	1	2
	Negative	17	16	33
Total		18	17	35

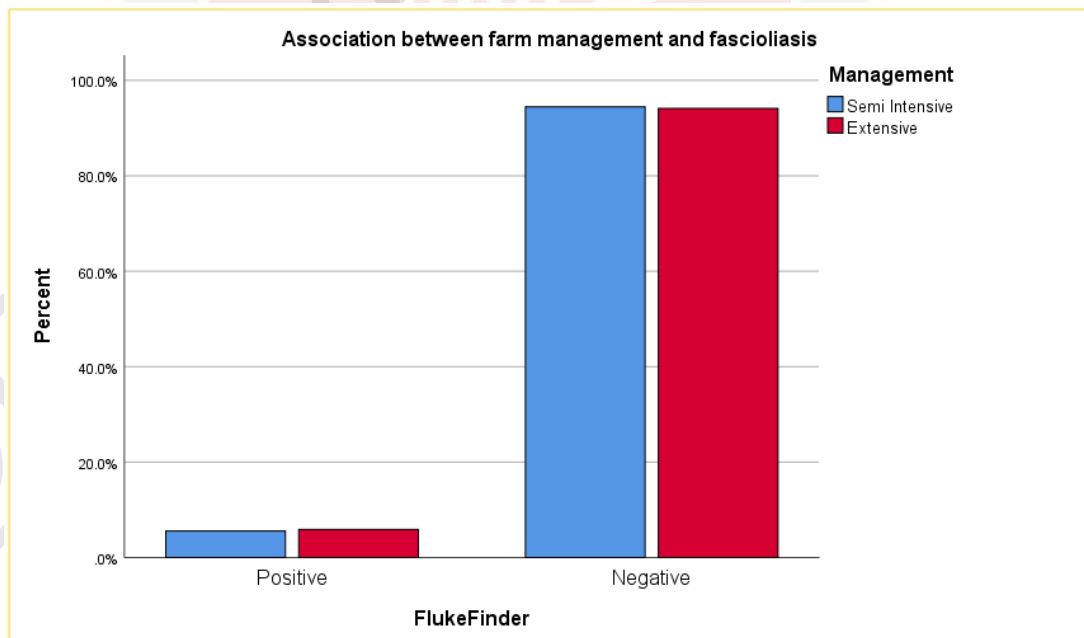


Figure 8: Association between farm management and fascioliasis

4.4.6 Association between Flood History and Fascioliasis

Table 13 and Figure 9 show the association between flood history and fascioliasis. There was no significant difference [$p = 0.263$, $p \geq 0.05$] (Table 20 in Appendix II) in the fascioliasis prevalence between farm that had history of flooding and that did not.

Table 13: Association between flood history and fascioliasis

Flooded * FlukeFinder Crosstabulation

		FlukeFinder		Total
		Positive	Negative	
Flooded	Never Flooded	0	13	13
	Flooded before	2	20	22
Total		2	33	35

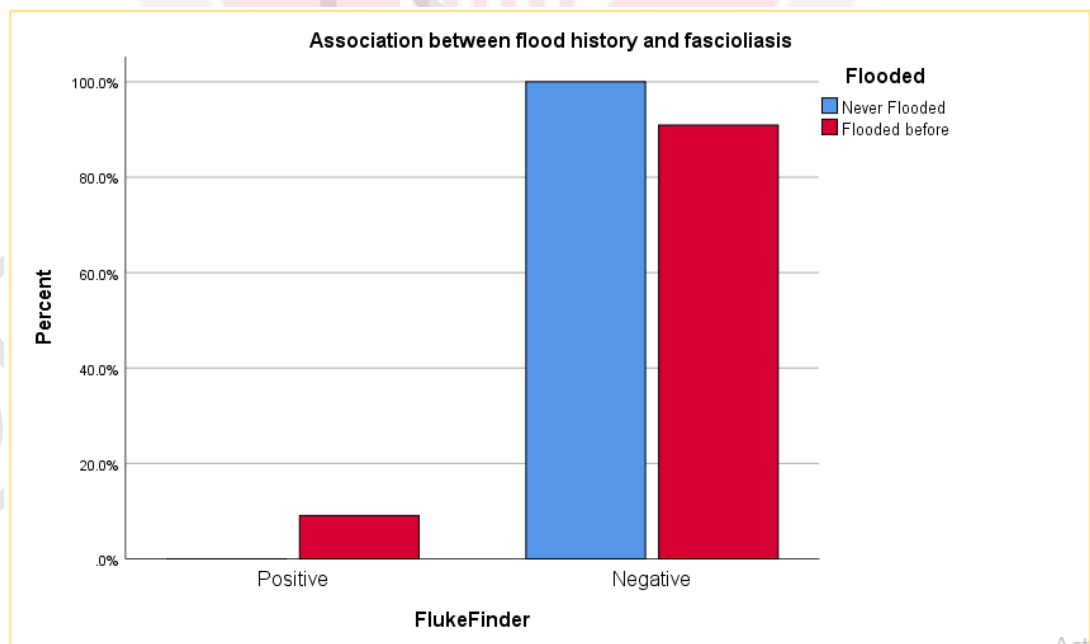


Figure 9: Association between flood history and fascioliasis

5.0 DISCUSSION

This study investigated the prevalence and risk factors of cattle fascioliasis in selected 4four farms in Taiping, Perak. Based on this study, there were fascioliasis in cattle which showed an overall prevalence of 2.86% (1/35) using Faecal Simple Sedimentation Method (FSSM) and 5.71% (2/35) using Flukefinder® technique. Two out of four farms had cattle positive for fascioliasis with prevalence of 20% and 6.67% respectively. There were 5.26% of male and 6.25% of female cattle which were positive with fascioliasis. The results also shows 4% of cattle of age between 1 to 5 years old and 100% of cattle of age of 5 years above were positive for fascioliasis. There were 3.33% of Kedah Kelantan breed and 20% of KK Cross breed positive for fascioliasis. For body condition score of 2 and 3, the prevalence of fasioliasis were 7.69% and 6.25% respectively. The results shows 5.56% of cattle managed semi intensively and 5.88% of cattle managed extensively were positive for fascioliasis. Lastly, 9.09% of cattle in farms that had history of flooding prior to sampling were positive for fascioliasis.

The prevalence rate was higher when detected by Flukefinder® technique compared to FSSM. Previous study revealed Flukefinder® technique detected more positive samples (27/51) compared to FSSM (11/51) (Kleiman et al., 2005). Due to sample processing method by SFSM, the eggs could have been lost causing false negative results. The results from this study suggested that using Flukefinder® technique was a more reliable diagnostic method compared to FSSM which could underestimate the prevalence of cattle fascioliasis and the egg output.

This study shows relatively low prevalence of cattle fascioliasis in Perak compared to several studies that had been conducted. Masrin et al. (2015) reported during the period of 2004 to 2013, the prevalence rate was 11.2% in Perak. Zainalabidin et al. (2015) found the prevalence of fascioliasis in large ruminants slaughtered in abattoirs in Perak was 7.5% while Fazly (2015) reported 8% prevalence rate in Perak. The low number of fluke infection can cause false negative results as detection of few numbers of eggs might be difficult and missed during diagnostic procedure. In addition, the low number of samples collected was inadequate resulting in low prevalence of cattle fascioliasis.

The prevalence of cattle fascioliasis in between male and female animals may differed in studies because of sampling methods. Sampling in abattoir may show high prevalence in male because most beef cattle were male. Sampling in farms may show high prevalence in female due to more female in farms for reproductive purpose, milk production and others (Dawa et al., 2013; Khan et al., 2010; Zeleke et al., 2013). Results in this study showed no association between male and female cattle fascioliasis, [$p = 0.900$, $p > 0.05$].

As for the age of animal with fascioliasis, this study showed there was a significant difference. Most of the cattle positive for fascioliasis were in one to five years old group based on this result. Tahawy et al. (2017) found that the highest prevalence was reported in age group of more than 5 years (10.91%), and the lowest was in age groups of more than three years (8.35%). In contrast, based on another study, 53% of prevalence was detected in adult cattle (2-3 years), and 78% had abnormalities in liver based on post-mortem and morphological findings

(Kusumarini et al., 2020). This study showed the young and adult cattle can be potentially infected with fascioliasis.

Next, there are no association between breed of cattle to fascioliasis. The results showed both breeds, KK and KK crosses were equally infected by fascioliasis. This findings is supported by a study conducted by Yeneneh et al. (2012) who found no difference among cattle breed with fascioliasis. In contrast, according to Mochankana and Robertson (2018), other countries had reported of high prevalence in traditional / local breed compared to *Bos Taurus* breeds.

There was no association between body condition score (BCS) and fascioliasis based on this study. This is may be due to limited sample size. Tahawy et al. (2017) reported that cattle with medium and poor body condition were associated with fascioliasis than those with good body condition score. Fascioliasis was higher in cattle with poor body condition score (Jaja et al, 2017). According to Tulu and Gebeyehu (2018), animals with poor BCS has more chances of being infected with fascioliasis compared to those with good BCS. Poor BCS animals are more susceptible if they have concurrent infection or chronic disease. However, in a latest study, good body condition score showed the highest prevalence at 86%; while the medium body condition score reported prevalence of 85% and lastly 3% of prevalence in poor body condition score (Kusumarini et al., 2020). This may indicate cattle in poor body condition score was experiencing chronic infections which caused weight loss. For a good body condition score, the cattle may experience acute infections.. In this study, fascioliasis was detected in cattle with poor, medium and good body condition score.

Also, in this study, there was no significant association between farm management and flooding history with fascioliasis. This result might be due to the selected farms did not practice intensive farming. None or low prevalence of fascioliasis is expected in cattle under intensive farming as there was no exposure to water bodies and free grazing near the river that might be contaminated with encysted metacercariae. Increase in prevalence of fascioliasis was expected in extensive or semi intensive farming and with history of flooding as supported in one study conducted by Takeuchi-Storm et al. (2017). The study found high prevalence of fascioliasis with animal exposed to wet grazing area. In this study, the prevalence is low may be because of low sample size and location of the farm is not near to water bodies.

6.0 CONCLUSION

Based on this research, the overall prevalence of cattle fascioliasis was low in selected farms in Taiping, Perak which were 2.86% (1/35) using FSSM and 5.71% (2/35) using Flukefinder® technique. This study showed a significant difference in the association between age and fascioliasis. However, there were no significant difference in the association between gender, breed, body condition score, farm management (semi intensive and extensive) and flooding history with fascioliasis. The overall prevalence of cattle fascioliasis was low; however, farmers must be given awareness about this disease and precaution must be taken considering the zoonotic potential of fascioliasis.

7.0 RECOMMENDATION

For the next research, the sample size and number of farms need to be increased to make the results more reliable. Fluke detection in condemned liver in abattoir and more extensive study regarding risk factors of fascioliasis in other region are recommended to determine the status prevalence and risk factors of fascioliasis in Malaysia.

8.0 REFERENCES

Alia J.N.D, Lokman, I. H., Fazila, N. S. H., et al., (2019). A Retrospective Study on Bovine Fascioliasis in Veterinary Regional Laboratories in Peninsular Malaysia. *Journal of Parasitology Research*, vol. 2019, Article ID 7903682, 5 pages.

Alia, J. N. D., Mahiza, N. M. I., Latiffah, H., Fazila, N. S. H., Lokman, I. H., Hazfalinda, H. H., Chandrawathani, P., Ibitoye, E. B., and Juriah, K. (2020). Occurrence, Morphometric, and Molecular Investigation of Cattle and Buffalo Liver Adult Fluke in Peninsular Malaysia Main Abattoirs. *Journal of Parasitology Research*, Article ID 5436846, 6 pages.

Anonymous, E. (2004). Thinking beyond deworming. *The Lancet*. 364(9450), 1993–1994.

Bizini, F. (2018). Fascioliasis in Tuaran, Sabah: A Case Report. *Borneo Journal of Medical Sciences (BJMS)*, 35. Retrieved from <https://jurcon.ums.edu.my/ojums/index.php/bjms/article/view/1382>

Dawa D., Abattoir M., Mebrahtu G., Beka K. (2013). Prevalence and economic significance of fasciolosis in cattle slaughtered at Dire Dawa Municipal Abattoir, Ethiopia. *Journal of Veterinary Advances*, 3: 319–324.

El-Tahawy, A.S., Bazh, E.K. and Khalafalla, R.E. (2017). Epidemiology of bovine fascioliasis in the Nile Delta region of Egypt: its prevalence, evaluation of risk factors, and its economic significance. *Vet World*. 10(10), 1241–1249. doi: 10.14202/vetworld.2017.1241-1249.

Hussein, A. N., Hassan, I. M., & Khalifa, R. M. (2010). Development and hatching mechanism of Fasciola eggs, light and scanning electron microscopic studies. *Saudi journal of biological sciences*, 17(3), 247–251. <https://doi.org/10.1016/j.sjbs.2010.04.010>

Hotessa A.S. and Kanko D.K. (2020). Review on Paramphistomosis. *Advances in Biological Research*, 14(4): 184-192. doi: 10.5829/idosi.abr.2020.184.192

Jaja I.F., Mushonga B., Green E., and Muchenje V. (2017) Seasonal prevalence, body condition score and risk factors of bovine fasciolosis in South Africa. *Veterinary and Animal Science*, 4: 1-7. <https://doi.org/10.1016/j.vas.2017.06.001>. Retrieved from: <http://www.sciencedirect.com/science/article/pii/S2451943X17300418>)

Khadijah, S., Izzudin, A.H., Rita, N., Veronica, S., Aida, H. And Wahab, A.R. (2015). Endo and ectoparasite infections in two cattle farms located in Kuala Terengganu, Peninsular Malaysia. *Asian Journal of Agricultural and Food Sciences*. 3, 667-674.

Khadijah, S., Rita, N. and Mursyidah, A.K. (2017). The prevalence of helminthiasis in cattle, Terengganu, Peninsular Malaysia. *Tropical Biomedicine*. 34(2), 324–331.

Khan M.N., Sajid M.S., Khan M.K., Iqbal Z., Hussain A. (2010). Gastrointestinal helminthiasis: Prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. *Parasitology Research*, 107: 787–794.

Kusumarini S.R., Permata F.S., Widyaputri T., and Prasetyo D. (2020). Prevalence of fasciolosis emphasis on age, origin, body condition and post mortem by geographic information systems on sacrificial examination in Malang District – East Java. *Journal of Physics: Conference Series*, 1-7. doi:10.1088/1742-6596/1430/1/012025

Masrin A., Magendren S., Chandrawathani P., and NurainIrzierah I. (2015) Current Status Of Fascioliasis in Ruminants: Cases Diagnosed from 2004 to 2013 in VRI, Ipoh. Presented at 51st MSPTM Annual Scientific Conference, Kuala Lumpur, (POSTER). Retrieved from <http://research.dvs.gov.my/opac/article.php?id=90>

Mursyidah A.K., Khadijah S., and Rita N. (2017). *Fasciola* and *Paramphistomum* infections in small ruminants (sheep and goat) in Terengganu. *Malaysian J Vet Res*. 2017, 8(2):8–12.

Mochankana, M. and Robertson, Ian. (2018). Cross-sectional prevalence of *Fasciola gigantica* infections in beef cattle in Botswana. *Tropical Animal Health and Production*. 50. 10.1007/s11250-018-1568-9.

Rita N., Khairi M., and Saad K. (2017). The prevalence of helminthiasis in cattle, Terengganu, Peninsular Malaysia. *Trop Biomed*, 34(2):324–331.

Saleha AA. (1991). Liver fluke disease (fascioliasis): epidemiology, economic impact and public health significance. *Southeast Asian J Trop Med Public Health*, 361-4. PMID: 1822928.

Siti Aqilah Z.N., Rozaihan M., Maizatul Akmal M., Nazim Razali K., Abd Rashid A.R., Mohd Jefri N. and Nur Mahiza M.I (2018). Prevalence of liver and rumen flukes in foster farms, Universiti Putra Malaysia. 54th Annual Seminar Malaysian Society of Parasitology and Tropical Medicine, Bangsar, Kuala Lumpur.

Takeuchi-Storm, N., Denwood, M., Hansen, T.V.A. *et al.* (2017). Farm-level risk factors for *Fasciola hepatica* infection in Danish dairy cattle as evaluated by two diagnostic methods. *Parasites Vectors*, 10: 555
<https://doi.org/10.1186/s13071-017-2504-y>

Tulu D. and Gebeyehu S. (2018). Study of Prevalence and Associated Risk Factors of Bovine Fasciolosis in Jimma Horro District of Kellem Wollega Zone, Western Ethiopia. *Journal of Arch Vet Sci Med*, 1 (1): 009-018
doi:10.26502/avsm.002

Yap, T.C., Mohna, S.S., Gill, H.S.(1983). A study on the causes of condemnation of carcasses and organs of livestock slaughtered at the Shah Alam abattoir. *Kajian Vet* 1983. 15, 1-10.

Zainalabidin, F. A., Azmi, M. S., Bakri, W. N., Sathaya, G., & Ismail, M. I. (2015). Screening for Zoonotic Fascioliasis in Slaughtered Large Ruminants in Abattoirs in Perak, Malaysia. *Tropical Life Sciences Rresearch*, 26(2), 121–124.

Zelege G., Menkir S., Desta M. (2013). Original article Prevalence of ovine fasciolosis and its economic significance in Basona worana district, central Ethiopia. *Scientific Journal of Zoology*, 2: 81–94.

Zhang, X.X., Feng, S.Y., Ma, J.G., Zheng, W.B., Yin, M.Y, Qin, S.Y., et al. (2017). Seroprevalence and risk factors of fascioliasis in yaks, bos grunniens, from three counties of Gansu province, China. *Korean J Parasitol*. 55(1), 89–93.
doi: 10.3347/kjp.2017.55.1.89.

APPENDICES

Appendix I

Questionnaire on prevalence and risk factors of cattle fascioliasis in selected farms in Taiping, Perak

1.) Owner's name : _____

2.) Farm's name : _____

3.) Farm's address : _____

4.) Farm's age : _____

5.) Farm management : Intensive Semi-intensive Extensive

- Herd size : Male Female

- Animal age : < 1 year 1-2 years > 2 years

6.) Breed : _____

7.) Body Condition Score : 1 _____ 2 _____ 3 _____ 4 _____ 5 _____

8.) Water source : _____

9.) Flood history : Yes No

10.) Deworming practice : Yes No

11.) Pesticide/herbicide used : Yes No

12.) History of fascioliasis before : Yes No

13.) Presence of snails : Yes No

Appendix II

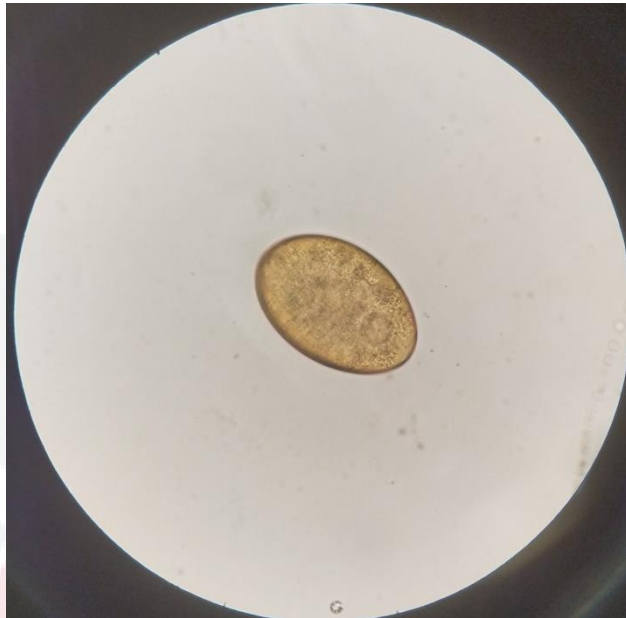


Figure 10: Fasciola sp. egg under 10x magnification using compound microscope



Figure 41: Fasciola sp. egg under 40x magnification using compound microscope

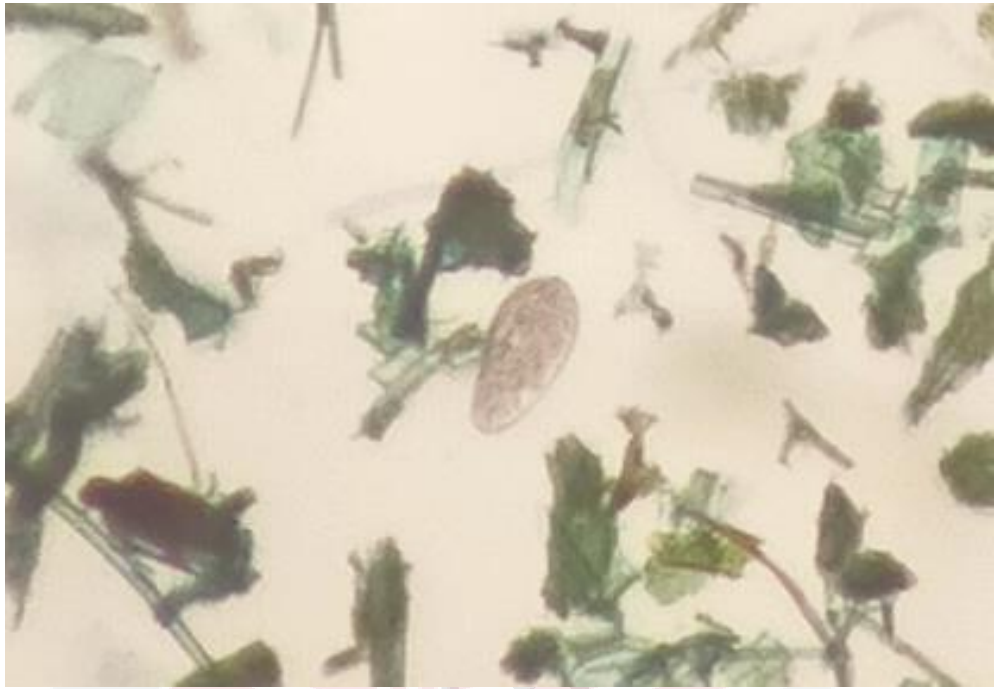


Figure 52: Paramphistomum sp. egg under 45x magnification dissecting microscope



Figure 63: Paramphistomum sp. egg and Fasciola spp. egg under 45x magnification dissecting microscope

Descriptives			Statistic	Std. Error
FlukeFinder	Mean		.94	.040
	95% Confidence Interval for Mean	Lower Bound	.86	
		Upper Bound	1.02	
	5% Trimmed Mean		.99	
	Median		1.00	
	Variance		.055	
	Std. Deviation		.236	
	Minimum		0	
	Maximum		1	
	Range		1	
	Interquartile Range		0	
	Skewness		-3.989	.398
	Kurtosis		14.752	.778
	Sedimentation	Mean		.97
95% Confidence Interval for Mean		Lower Bound	.91	
		Upper Bound	1.03	
5% Trimmed Mean			1.00	
Median			1.00	
Variance			.029	
Std. Deviation			.169	
Minimum			0	
Maximum			1	
Range			1	
Interquartile Range			0	
Skewness			-5.916	.398
Kurtosis			35.000	.778

Table 14: Prevalence of fascioliasis

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.016 ^a	1	.900		
Continuity Correction ^b	.000	1	1.000		
Likelihood Ratio	.016	1	.900		
Fisher's Exact Test				1.000	.713
Linear-by-Linear Association	.015	1	.902		
N of Valid Cases	35				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .91.

b. Computed only for a 2x2 table

Table 15: Chi-Square test between gender and fascioliasis

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	17.182 ^a	2	.000
Likelihood Ratio	6.935	2	.031
Linear-by-Linear Association	4.685	1	.030
N of Valid Cases	35		

a. 4 cells (66.7%) have expected count less than 5. The minimum expected count is .06.

Table 16: Chi-Square test between age and fascioliasis

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.210 ^a	1	.137		
Continuity Correction ^b	.199	1	.656		
Likelihood Ratio	1.560	1	.212		
Fisher's Exact Test				.269	.269
Linear-by-Linear Association	2.146	1	.143		
N of Valid Cases	35				

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is .29.

b. Computed only for a 2x2 table

Table 17: Chi-Square test between breed and fascioliasis

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.467 ^a	2	.792
Likelihood Ratio	.800	2	.670
Linear-by-Linear Association	.369	1	.544
N of Valid Cases	35		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is .34.

Table 18: Chi-Square test between body condition score and fascioliasis

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.002 ^a	1	.967		
Continuity Correction ^b	.000	1	1.000		
Likelihood Ratio	.002	1	.967		
Fisher's Exact Test				1.000	.743
Linear-by-Linear Association	.002	1	.967		
N of Valid Cases	35				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .97.

b. Computed only for a 2x2 table

Table 19: Chi-Square test between farm management and fascioliasis

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.253 ^a	1	.263		
Continuity Correction ^b	.134	1	.714		
Likelihood Ratio	1.928	1	.165		
Fisher's Exact Test				.519	.388
Linear-by-Linear Association	1.218	1	.270		
N of Valid Cases	35				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .74.

b. Computed only for a 2x2 table

Table 20: Chi-Square test between flooding history and fascioliasis