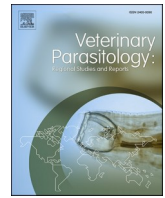




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Original Article



## Prevalence and risk factors of fasciolosis in a bovine population from farms in Taiping, Malaysia

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### ABSTRACT

Fasciolosis is a zoonotic disease, considered an emerging neglected tropical disease threatening ruminant productivity and causing economic losses. Controlling fasciolosis is challenging due to the complex life cycle of *Fasciola*, which involves snail intermediate hosts. The high rainfall status in Taiping makes it an optimal region for snail abundance, which increases the opportunity to complete *Fasciola*'s life cycle. Previous studies showed that liver condemnation caused by fasciolosis was highly prevalent in the Taiping abattoir compared to other investigated main abattoirs of Peninsular Malaysia. Therefore, the present study determined the prevalence of bovine fasciolosis and risk factors in farms from Larut and Matang (Taiping), Malaysia. Sampling was carried out from February until August 2020. In this cross-sectional study, a total of 371 fecal samples from bovines (dairy cattle, beef cattle, buffalo) were examined from 23 farms selected based on location, farmer consent, and history of anthelmintic usage. Animal's intrinsic and farm management details were recorded, and interview sessions were conducted with farmers to collect information on the potential risk factors. Individual fecal samples were examined for the presence of *Fasciola* egg using Flukefinder® sedimentation. There was moderate prevalence of bovine fasciolosis in Taiping (36.9%,  $n = 137/371$ ). Significant risk factors ( $p < 0.05$ ) were observed, which include buffalo group (OR = 9.5, 95% CI: 9.44–9.55), age of >3 years (OR = 5.5, 95% CI: 5.43–5.57), thinner animals with body condition score of 1 to 4 (OR = 1.2–14.9, 95% CI: 1.09–15.08), and larger grazing area (OR = 1.3, 95% CI: 1.30–1.31). Additional risk factors include the presence of more than one ruminant species in the same farm (OR = 2.0–2.1, 95% CI: 2.00–2.22), extensive housing system (OR = 4.0, 95% CI: 3.77–4.23), farm age (OR = 1.2, 95% CI: 1.20–1.21), and also co-infection with Paramphistomes (OR = 1.4, 95% CI: 1.10–1.71). The present study underscores the importance of local bovine fasciolosis epidemiology, which could be used to conduct future veterinary and public health programmes to inform effective parasitic management aimed at reducing the prevalence of fasciolosis.

### 1. Introduction

Ruminant products are in high demand in Malaysia, and the livestock farming industry is becoming increasingly popular in the region (Shahudin et al., 2018). This is consistent with the Malaysian government's goal to strengthen the domestic livestock industry and reduce Malaysia's reliance on imports of ruminant products. Nonetheless, the challenges

arising from diminished animal productivity and the condemnation of fibrotic livers due to parasitic infections, such as fasciolosis, have significant implications for food security (Takeuchi-Storm et al., 2017). In the pursuit of sustainable livestock production, the prevention and management of parasitic diseases hold paramount importance. This requires the implementation of continuous surveillance in farms to facilitate targeted interventions, particularly in controlling fasciolosis within

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bovine production (Howell et al., 2015).

In Malaysia, ruminant fasciolosis has been reported in a region neighboring Taiping, Kelantan, with a prevalence of 14.6% ( $n = 45/308$ ) through coproscopic analysis (Ahmad-Najib et al., 2021). Despite the relatively low on-farm fasciolosis prevalence, it serves as an estimate for Taiping. A survey across the main abattoirs in the Malaysian Peninsular revealed that Taiping had the highest occurrence of fibrotic liver condemnations due to *Fasciola* infection (Diyana et al., 2020). While there is currently no specific study linking this observation to local climate and environmental factors in Taiping, including hot-humid temperatures, high humidity, and substantial rainfall (The Strait Times, 1959; Malaysian Department of Meteorology, 2020), the mentioned conditions are known to create a favorable environment for the complete life cycle of the *Fasciola* liver fluke (Duthaler et al., 2010; Howell et al., 2015). This emphasizes the potential causative role of high humidity and rainfall in fostering conditions conducive to the prevalence of fasciolosis in the region (Duthaler et al., 2010; Howell et al., 2015). Given the favorable abiotic conditions for *Fasciola* development and the high occurrence of fasciolosis in Taiping based on the abattoir's comparison, it is imperative to conduct a comprehensive assessment of the prospective fasciolosis status in the area. Encompassing on-farm studies and an evaluation of herd-level prevalence would provide a deeper understanding of the dynamics and distribution of *Fasciola* infection within the population.

Moreover, besides the environmental factors, it is essential to evaluate host intrinsic factors and farm management as risk factors (Howell et al., 2015; Shinggu et al., 2019). These factors are of particular significance due to regional variations in farming practices, which are strongly influenced by local policies (Shahudin et al., 2018). Studies investigating the prevalence of fasciolosis and regional risk factors play a crucial role in assessing the local epidemiology of the disease, ultimately aiding in identifying effective control programs. Therefore, the objective of the present study was to determine the prevalence of bovine fasciolosis and its associated risk factors on farms in Taiping, Malaysia.

## 2. Materials and methods

### 2.1. Study design

The present study was a cross-sectional study following the guidelines from STROBE-Vet (Strengthening the Reporting of Observational Studies in Epidemiology - Veterinary) in veterinary medicine (Sargeant et al., 2016).

The present study gathered information on ruminant farms in Larut and Matang areas (Taiping) such as the locality, variety of livestock, animal count, and health and farm management practices from the local Department of Veterinary Services. Verbal consent was sought from farm owners to participate in the study. Only those farmers who consented and had not provided anthelmintic treatment to their animals for at least six months prior to the sample collection were randomly selected for a physical farm visit. This six-month criterion was imposed to align with the complete life cycle of *Fasciola*, thereby reducing the likelihood of false negatives in the assessment of fasciolosis. Within these selected farms, the present study exclusively sampled the bovine group, encompassing dairy cattle, beef cattle, or buffalo, due to the observed common prevalence of fasciolosis in this group (Naim et al., 2021). Moreover, considering the significance of the bovine livestock population, which constitutes 61% of Malaysia's total livestock population in comparison to other small ruminant productions like sheep and goats (Malaysian Department of Statistics, 2019), this bovine group was defined as the study population for the present study. Animals of any age and sex within the bovine group were included in the present study.

The determination of the total sample size for collecting fecal samples across the bovine population in the present study followed the guidelines outlined by Thrusfield (2007). Given the limited data on bovine on-farm fasciolosis prevalence in Taiping and the varied

prevalence in other regions of Malaysia, an expected prevalence of 50% was chosen for the maximum sample size estimation. A 95% confidence level and 5% margin of error were selected to ensure the desired precision, guaranteeing that the probability of randomly drawn samples falls within the range of two standard errors from the prevalence of the sampled bovine population.

Considering the total bovine population in Taiping, recorded as 8374 in the 2018 annual livestock census conducted by the Malaysian Department of Veterinary Services, a minimum of 367 fecal samples across the bovine population in Larut and Matang (Taiping) were deemed necessary for the present study. The samples were collected from a combined group of bovine, including both cattle and buffaloes, without segregating them into two separate groups in this study. This approach was introduced to provide overall prevalence estimates for the bovine group due to the homogeneity in risk factors from previous studies. The present study ensured a minimum representation of at least 10% from each species in every farm to enhance the robustness of the risk factor analysis, following the recommendation by Conray (2016). This approach has been shown to yield accurate results within the investigated population.

### 2.2. Study sites and ruminant farms

The Larut and Matang areas (Taiping) region (4.8519°N, 100.7416°E) is covered by rainforest and mangrove forest by 12.38%, and the main agricultural land use is for oil palm (68.26 km<sup>2</sup>) and rubber cultivation (66.34 km<sup>2</sup>). The domestic ruminant livestock population in this region was estimated to be approximately 13,985, which includes buffaloes (1408), beef cattle (6748), dairy cattle (218), goats (5342), and sheep (269) serving as food consumption to about 16% of the total human population in Perak (Malaysian Department of Veterinary Services, 2020; Malaysian Department of Statistics, 2019). Briefly, the bovine population in Taiping makes up around 59.9% ( $n = 8374/13,985$ ) of the total ruminant population in this region.

A total of 23 farms (farm A-W) participated in the present study. All the farms' locations were recorded according to the latitude, longitude, and altitude taken from the Google Earth application. The farms were dispersed from Taiping town or urban areas to rural areas in the northern part, predominantly in Larut and Matang (LM) districts. Cross-sectional sampling was conducted from February until August 2020, following the monsoon season, with no notable changes observed during the sampling period. Fecal sample collection and farm visits were done in the morning to mid-day (before 1 P.M.). In farms that practice extensive housing systems, integrated animal husbandry in plantations is a common practice to allow the ruminants to graze freely in the matured palm oil estate before being caged in the housing during the night time.

### 2.3. Fecal sample collection

Rectal-fecal sample collection from bovine was carried out during the farm visit. Estimation of  $\pm 5$  g or a spoonful of fecal samples from each bovine were collected using clinical rubber gloves. Each glove was labelled with a unique identification number referring to the farm and animal tag number. Information on bovine host details such as ruminant type, sex, age group, and body condition scoring (BCS) was recorded. The BCS (1 to 5) was determined using a chart as suggested by Edmonson et al. (1989). Briefly, a BCS 1 indicates emaciation, characterized by prominent bones and a lack of muscle mass. A BCS of 2 signifies thinness, with noticeable ribs and limited muscle development. In contrast, a BCS of 3 reflects a moderate or ideal condition, where the ribcage is visible but not prominent, and there is a balanced muscle mass. A BCS of 4 suggests moderate fat cover, making ribs challenging to feel due to a layer of fat. Lastly, a BCS of 5 denotes a fat or overweight condition characterized by significant fat covering the ribs and spine and visible fat deposits. The age groups of the bovine were categorized into

three groups based on the records provided by the farmers: (1) age group A, representing bovines aged 6 months to 1 year old, (2) age group B, representing bovines aged 1 to 3 years, and (3) age group C, representing bovines older than 3 years. Collected fecal samples were stored in dark, 4 °C icebox to avoid embryonation and hatching before coproscopical diagnosis. Samples were then brought to the Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, UPM, for diagnosis within 14 days of sample collection to prevent sample degeneration.

2.4. Interviews with the farmers

Data on farm management practices and farmers' backgrounds with regard to fasciolosis were collected using both closed-ended and open-ended structured questionnaires. The questionnaire encompassed two major sections (farming practices and farmers' knowledge, attitudes, and practices) concerning the epidemiology of bovine fasciolosis. Sub-sections on aspects of farming practices involved the water source used on the farm, the number and type of ruminant species kept, the housing system, the estimated grazing area, and the total count of individual animals. Additionally, the subsections on the farmers include the age, education level, ability to identify adult *Fasciola* fluke, knowledge of *Fasciola* mode of transmission, pathogenesis, and control measures, which were recorded to assess their understanding of fasciolosis. The interviews were conducted in the Malay language.

2.5. Coproscopical procedure and *Fasciola* identification

To determine the fasciolosis occurrence, the animals were considered positive if *Fasciola* eggs were present in their fecal samples, determined through Flukefinder® (Richard Dixon, ID, USA) sedimentation. Coproscopical assay using Flukefinder® was used to diagnose the presence of *Fasciola* and any other co-infecting helminths such as Paramphistomes. Egg count was quantified to measure the fluke egg burden. Flukefinder® sedimentation was opted for due to the higher sensitivity and specificity compared to other sedimentation methods (Faria et al., 2008; Elelu et al., 2016). Each bovine's 2 g fecal sample was mixed with 30 mL treated tap water and filtered through Flukefinder®. The Flukefinder® was rinsed to ensure complete removal of any adhered helminth eggs. Sediment in the Flukefinder® was transferred to a vial, settled for at least two minutes, and then transferred to a gridded petri dish. Three drops of 10% methylene blue were added, and the dish was observed under a stereomicroscope at 25× magnification, following a zig-zag pattern. Eggs per gram (epg) were calculated by dividing the total number of helminth eggs detected by 2 g. To prevent cross-contamination, thorough washing of the Flukefinder® was done between samples.

The identification of *Fasciola* egg was based on the observation of the egg shape, coloration, and operculum morphology. The *Fasciola* egg is the largest helminth egg with golden-yellowish color and an operculated thin shell (Hussein et al., 2010). Other eggs were identified using the standard key (Mifsut and Ballarin, 2015).

2.6. Prevalence map visualization

The regional map of Taiping was obtained from the Google Earth website in KML file format and exported into Qgis version 3.10 (Geographic Information System) to map the fasciolosis prevalence from investigated farms. Locations of the farms were categorized regionally for the northern and central of Larut and Matang (LM) in Taiping based on the differences in the ecological and socio-economic settings. Prevalence of farm-level fasciolosis was first pin-pointed in a raster formatted map categorized into high (>50%), moderate (16%–50%), and low (<16%) based on the results of the present study.

2.7. Statistical analysis

Raw data were tabulated in Excel format (Ms Office) with thorough inspection during data entry to prevent any transcriptional errors and missing data. The datasets were then imported to R statistical software version 1.3.1073 for further analysis.

The “EpiR package” in R (Stevenson, 2013) was used in the descriptive analysis of the data obtained in the present study, which includes the estimation of bovine fasciolosis occurrence in each visited farm (confidence intervals at 95%). The bovine host intrinsic, farm management, and farmer's background were described in percentage, minimum/maximum value, and the mean/standard deviation (SD), respectively.

Multivariable logistic regression model (glm function – R) has opted to study the relationship between the risk factors of bovine fasciolosis in farms in Taiping with the presence of *Fasciola* egg. The included factors were selected using a backward selection to reduce overfitting to avoid random errors in the model. Additionally, the backward selection was used to increase the logistic regression model's predictive power and goodness of fit. Eliminating any factors out of the model was carried out by comparing the ANOVA and odd ratio (OR) to prevent large value changes. Pearson's chi-square was used to detect collinearities among the factors to reduce the uncertainty of the model. Final model was chosen based on R-squared value (close to the value of 1 for good predictive value) and smaller Akaike's information criterion (AIC) (deviance/quality measurement of each model to analyze amount of information lost in the factor selection) of each model (Narisetty, 2020). Since population heterogeneity must be considered when assessing a factor's ability to the outcome, a good model is crucial in establishing the OR of each significant factor. The reference category with the lowest likelihood of acquiring fasciolosis was used to calculate the odd ratio for each categorical risk factor (Marston, 2010).

3. Results

Key findings of the farm-level occurrence of bovine fasciolosis from 23 farms in Larut and Matang, Taiping (LM), obtained through the Flukefinder® sedimentation method are summarized in Table 1. Of the

Table 1  
Farm-level occurrence of bovine fasciolosis in Taiping, Malaysia.

Farm	Herd size	Number of samples collected	Number of positive samples	Fasciolosis frequency (%)	C.I. (95%)
A	70	7	3	42.9	9.9–81.6
B	100	38	25	65.8	48.6–80.4
C	20	10	0	0.0	–
D	20	11	3	27.3	6.0–61.0
E	60	17	4	23.5	6.8–49.9
F	14	3	0	0.0	–
G	40	16	8	50.0	24.7–75.3
H	50	22	22	100.0	84.6–100.0
I	300	45	14	31.1	18.2–46.6
J	13	7	5	71.4	29.0–96.3
K	50	9	0	0.0	–
L	300	47	26	55.3	40.1–69.9
M	20	9	4	44.4	13.7–78.8
N	25	12	1	8.3	0.2–38.5
O	26	13	8	61.5	31.6–86.1
P	42	16	10	62.5	35.4–84.8
Q	100	15	0	0.0	–
R	100	13	0	0.0	–
S	10	5	1	20.0	0.5–71.6
T	50	15	0	0.0	–
U	2	2	0	0.0	–
V	14	12	3	25.0	5.5–57.2
W	40	27	0	0.0	–
OVERALL	1466	371	137	36.9	32.0–42.1

C.I.: Confidence interval.

23 farms, 15 farms (65.2%,  $n = 15/23$ ) had at least one animal infected with *Fasciola*, with the occurrence of fasciolosis from each farm ranging from 8.3% to 100% of the examined animals. In addition, out of 15 *Fasciola*-positive farms, 7 farms (46.7%,  $n = 7/15$ ) were recorded with 50% or more fasciolosis occurrence, indicating a high risk of fasciolosis in Taiping. Therefore, the overall individual prevalence of bovine fasciolosis in Taiping was 36.9% ( $n = 137/371$ ).

Table 2 is a descriptive analysis of the occurrences of bovine fasciolosis according to risk factors such as host intrinsic factors, farm management practices, and farmer background. Beef cattle was the most examined bovine species group (69.5%,  $n = 258/371$ ) followed by dairy cattle (17.8%,  $n = 66/371$ ), and buffalo (12.7%,  $n = 47/371$ ). For the bovine species group, dairy cattle had the highest fasciolosis prevalence (78.7%,  $n = 37/66$ ), compared to buffalo (42.4%,  $n = 28/47$ ) and beef cattle (27.9%,  $n = 72/258$ ). More samples were collected from female animals (54.7%,  $n = 203/371$ ) than male (45.3%,  $n = 168/371$ ) with the fasciolosis prevalence of 42.9% ( $n = 87/203$ ) and 29.8% ( $n = 50/168$ ) respectively. The estimated age of most animals was between 1 and 3 years old (67.9%,  $n = 252/371$ ) as most of them would be slaughtered before reaching at older age based on the interview with the local farmers. The body condition score (BCS) of examined animals in the present study was predominantly presented by the BCS-3 (54.2%,  $n = 201/371$ ).

Table 3 presents the percentage of *Fasciola*-positive farms based on the farm characteristics. Most farms in Larut and Matang (Taiping) were family-owned and passed through generations. The animals were allowed to free-graze nearby oil palm plantations up to 40 km<sup>2</sup>. Most

**Table 2**  
Occurrence of bovine fasciolosis according to risk factor.

Factors	Number of samples	Number of positive samples	Fasciolosis frequency (%)	p-value
<b>HOST INTRINSIC</b> ( $n = 371$ )				
Ruminant type				<0.001
Beef cattle	258	72	27.9	
Dairy cattle	66	37	78.7	
Buffalo	47	28	42.4	
Sex				0.01
Male	168	50	29.8	
Female	203	87	42.9	
Age group				<0.001
<1 year	35	2	5.7	
1-3 years	252	91	36.1	
>3 years	84	44	52.4	
Body condition score (BCS)				<0.001
1/5	16	16	100.0	
2/5	86	50	58.1	
3/5	201	65	32.3	
4/5	47	5	10.6	
5/5	21	1	4.8	
<b>FARM</b>				
<b>MANAGEMENT</b> ( $n = 371$ )				
Water source				<0.001
Treated	31	1	3.2	
Natural	143	39	27.3	
Both	197	97	49.2	
Mixed species farming				0.27
Single	179	59	33.0	
Double	115	45	39.1	
Triple	77	33	42.9	
Housing system				<0.001
Extensive	226	75	33.2	
Semi-extensive	114	61	53.5	
Intensive	31	1	3.2	

p-value derived from Z-proportional test indicating the statistical significance between each factor group.

**Table 3**  
Percentage of *Fasciola*-positive farms based on farm characteristics.

Farm characteristics	Number of farms	Number of positive farms	Percentage (%)
<b>FARM MANAGEMENT</b> ( $n = 23$ )			
Water source			
Treated	3	1	33.3
Natural	10	6	60.0
Both	10	8	80.0
Mixed species farming			
Single	12	7	58.3
Double	7	5	71.4
Triple	4	3	75
Housing system			
Extensive	15	9	60.0
Semi-extensive	5	5	100.0
Intensive	3	1	33.3
Grazing range (Km <sup>2</sup> )*	0–40 ( $\bar{x}$ : 13.8, SD: 13.5)	–	–
Number of bovine per farm*	2–300 ( $\bar{x}$ : 113.2, SD: 110.6)	–	–
<b>FARMER</b> ( $n = 23$ )			
Farmer's age*	25–76 ( $\bar{x}$ : 49.1, SD: 9.4)	–	–
Farm age*	1–52 ( $\bar{x}$ : 18.9, SD: 16.2)	–	–
Farmer's education level			
Primary/absent	10	7	70.0
Secondary	10	6	60.0
Higher	3	2	66.7
Able to identify <i>Fasciola</i>			
Yes	11	8	72.7
No	12	7	58.3
Aware of <i>Fasciola</i> transmission mode			
Yes	4	3	75
No	19	12	63.2
Aware of effect of fasciolosis			
Yes	10	6	60.0
No	13	9	69.2
Aware of <i>Fasciola</i> control measure			
Yes	3	2	66.7
No	20	13	65.0

\* Continuous factors.

farms practice traditional farming management with extensive housing systems (65.2%,  $n = 15/23$ ). Only three farms (13.0%,  $n = 3/23$ ) utilised treated water in the farm, while the other farms used the nearest rivers to feed their animals. Mixed ruminant species farming was observed in 11 farms (47.8%,  $n = 11/23$ ), with 7 farms practicing double species farming (30.4%,  $n = 7/23$ ) and 4 farms practicing triple species farming (17.4%,  $n = 4/23$ ). Fasciolosis occurrence was observed to increase with the number of species groups present on the farm. The number of bovine individuals in each visited farm ranged from 2 to 300 animals. Additionally, the age range of the farmers from the 23 visited farms were from 25 to 76 years old, with the oldest farm being in operation for 52 years. Most farmers were found to lack knowledge of fasciolosis such as the disease identification, transmission mode, effects of infection, and the pre-requisite control measures.

A multivariable logistic regression analysis of the investigated bovine fasciolosis risk factors is presented in Table 4. Significant associated risk factors for fasciolosis were buffalo species ( $p < 0.01$ ), bovine age of more than three years ( $p < 0.01$ ), body condition scores lower



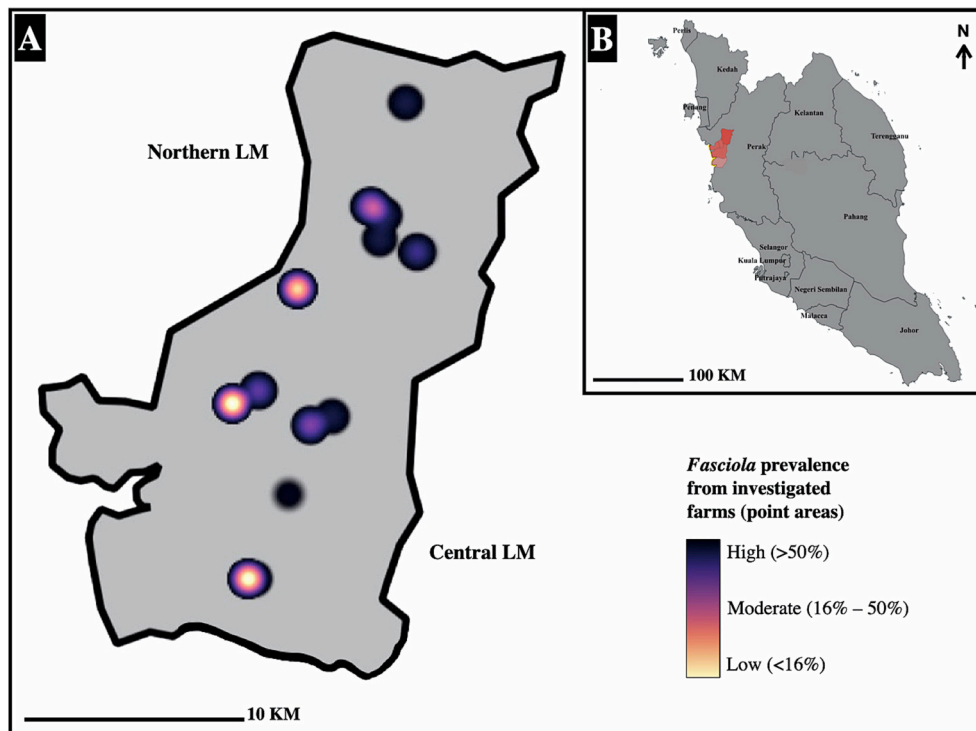
**Table 4**  
Logistic regression analysis of the risk factors of bovine fasciolosis in Taiping, Malaysia.

Risk factor	Factor family type	Sub-categories	Estimate value	Standard error	Odd ratio (95% CI)	p-value
Intercept			-0.81	2.28		0.72
Ruminant species	Categorical	Buffalo	2.25	0.53	9.5 (9.44–9.55)	<0.01*
	(ref: Beef cattle)	Dairy cattle	0.67	0.54		0.22
Sex	Categorical	Male	-0.21	0.31		0.51
	(ref: Female)					
Age group	Categorical	B (1–3)	1.27	0.66		0.05
	(ref: A (<1 year))	C (>3)	1.71	0.70	5.5 (5.43–5.57)	0.01*
Body condition score	Categorical	1	-0.80	1.79	14.9 (14.72–15.08)	<0.01*
	(ref: 5)	2	-2.42	1.0	6.0 (5.90–6.10)	<0.01*
		3	-3.62	1.0	1.7 (1.60–1.80)	<0.01*
		4	-4.62	1.06	1.2 (1.09–1.31)	<0.01*
Grazing range	Numerical	-	-0.07	0.02	1.3 (1.30–1.31)	<0.01*
Herd amount	Numerical	-	-0.003	0.002		0.22
Species group	Categorical	Double	0.61	0.3	2.0 (2.00–2.03)	0.05*
	(ref: Single)	Triple	0.64	1.13	2.1 (2.00–2.22)	0.05*
Water source	Categorical	Treated only	0.05	0.05		0.97
	(ref: Natural only)	Both	1.29	1.29		0.38
Housing system	Categorical	Extensive	-5.34	2.25	4.0 (3.77–4.23)	0.02*
	(ref: Intensive)	Semi	0.11	0.49		0.83
Farm age	Numerical	-	-0.05	0.02	1.2 (1.20–1.21)	<0.01*
Farmer age	Numerical	-	0.04	0.02		0.10
Farmer education	Categorical	Secondary	0.48	0.61		0.43
	(ref: primary)	Higher	-0.54	0.78		0.49
Co-infection with Paramphistomes	Binary	-	12.14	2.98	1.4 (1.10–1.71)	<0.01*

\* Statistically significant, ref = reference group.

than score-5 ( $p < 0.01$ ), larger grazing range ( $p < 0.01$ ), the presence of more than one species group in same farm ( $p < 0.05$ ), extensive housing system ( $p < 0.05$ ), farm age ( $p < 0.01$ ), and co-infection with Paramphistomes ( $p < 0.01$ ). The odds of buffalo species associated with bovine fasciolosis in Taiping was 9.5 times greater than the beef cattle, which showed to be the strongest associated risk factor among others. Bovine age group of more than three years old showed 5.5 times more risk for fasciolosis as compared to the younger animal. In addition, body

condition scores of BCS-4, BCS-3, BCS-2, and BCS-1 were found to have 1.2, 1.7, 6.0, and 14.9 times higher likelihood of having fasciolosis respectively. Increasing  $\text{Km}^2$  of grazing areas poses 1.3 times higher chances of fasciolosis. In addition, farms with double or triple ruminant species group were observed to have 2.0 and 2.1 times higher chances of fasciolosis respectively. Furthermore, extensive housing system showed 4.0 times to fasciolosis while the farm age with an odd of 1.2 times has higher chances of fasciolosis. Interestingly, bovine which were co-



**Fig. 1.** Map (a) shows the investigated farms in Larut and Matang (LM) with the *Fasciola* prevalence in gradient color from black (high) to light yellow (low), and (b) shows the LM location in Peninsular Malaysia (red color). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

infected with Paramphistomes showed odds of 1.4 times to be infected with *Fasciola*.

Fig. 1 depicts the fasciolosis prevalence in investigated farms in Larut and Matang, Taiping (LM). The gradient color separation in farms were determined based on the prevalence levels, denoting low, moderate, and high fasciolosis prevalence. A noticeable cluster in higher fasciolosis prevalence was evident in the northern LM compared to the central LM. The farm-level bovine fasciolosis prevalence points on the map highlighting moderate and high prevalence in the northern LM and varied prevalence levels in the central LM.

#### 4. Discussion

A moderate prevalence of bovine fasciolosis in Taiping (36.9%,  $n = 137/371$ ) was determined using Flukefinder® sedimentation. Earlier studies undertaken in other areas in Perak state, Malaysia reported a lower and higher fasciolosis prevalence than the present study which were 69.2% (Rajamanickam et al., 1987) and 7.5% (Fazly-Ann et al., 2015) in cattle and 7.7% in buffalo (Rajamanickam et al., 1987). This may result from variations in study designs, including the compilation of data from different sources like sedimentation or slaughterhouse records, or a combination of both (Rajamanickam et al., 1987; Fazly-Ann et al., 2015). Other local studies conducted in the Terengganu state with comparable study designs reported higher coproscopical fasciolosis prevalence rates of 78.0% (Khadijah et al., 2017) and 94.5% (Khadijah et al., 2015) respectively. It is worth noting that these studies had smaller sample sizes compared to the present study and were conducted in locations prone to floods during the monsoon season. The ecological and climatic conditions after the wet season can facilitate the growth of *Fasciola* intermediate host (Olkeba et al., 2020). Moreover, the fasciolosis prevalence in the present study was consistent with a similar study conducted in an equatorial climate in Colombia. The Colombian study reported a moderate fasciolosis prevalence of 20.5%, determined through fecal sedimentation (Pinilla et al., 2020). Various factors, including climate, environmental conditions, diagnostic methods, and the diversity of definitive hosts, contribute to the regional variability of bovine fasciolosis occurrence (Charlier et al., 2011; Howell et al., 2015; Mochankana and Robertson, 2018; Villa-Mancera and Reynoso-Palomar, 2019). Therefore, the findings in the present study emphasize the importance of ongoing surveillance for fasciolosis occurrence in Taiping, Malaysia.

In addition, the present study showed that buffalo species group had 9.5 times higher risk of fasciolosis ( $p < 0.01$ ) as compared to the beef cattle. This may be due to the grazing behavior of bubaline animals that usually graze in flat prairie areas which hold run-off water. Therefore, it is exposed to the habitat for the *Fasciola* intermediate host, the freshwater snail (Mahato and Harrison, 2005). Prevalence of fasciolosis in buffalo was also higher than cattle in another study investigating the differences in occurrence among species (Khan et al., 2009). Water buffalo is economically important in developing countries especially in Southeast Asia and an important main definitive host for *F. gigantica* in this region as shown in Indonesia (Nurhidayah et al., 2020). The *F. gigantica* occurrences in buffalo species is common in tropical and subtropical regions (Phalee et al., 2015; Yuan et al., 2016). However, large ruminants, such as buffalo, present a significant challenge in fasciolosis monitoring due to the absence of apparent clinical signs, leading to potential neglect in detection (Shi et al., 2013).

From previous study, host sex plays as a significant factor in ruminant fasciolosis (Mochankana and Robertson, 2018; Nurhidayah et al., 2020). However, in the present study, sex was not identified as a significant risk factor. The higher fasciolosis prevalence in female ruminants is attributed to a higher ratio of females examined in the studies compared to males. The finding was consistent with those of earlier studies that employed similar sample size proportion of both sexes in the tropical region and showed no association between fasciolosis occurrences and sex (Magaji et al., 2014; Shinggu et al., 2019; Japa et al.,

2020). The lack of a sex-related association in the present study could be attributed to equal exposure to fasciolosis for both sexes (Japa et al., 2020), reinforcing the need for further investigation into the complexities of host-sex-related susceptibility to fasciolosis.

The present study also showed that age is a significant risk factor for bovine fasciolosis and was evident in all host age groups in Taiping. The prevalence of fasciolosis increases with age, with older animals being 5.5 times prone to the infection. This is due to the longer period of exposure to *Fasciola*-contaminated pasture in older animals (Mochankana and Robertson, 2018; Shinggu et al., 2019; Nurhidayah et al., 2020). *Fasciola* has been known to persist in infecting the host for up to 20 years, depending on the host species and other internal factors (Moazeni and Ahmadi, 2016). Consequently, *Fasciola* fluke burden in the older host is prolonged and sheds eggs, which sustain the infection. While acknowledging the potential for anthelmintic treatment to introduce a confounding factor, the sampled animals in the present study remained untreated with any anthelmintic for a minimum of 6 months before sampling, thereby ensuring equal presentation across all age groups. During the sample collection for the present study, older animals were observed roaming freely while younger animals were kept in the housing. Additionally, the farmers provide feedlots to the calves; therefore, the younger age group was less frequently exposed to *Fasciola* metacercariae. In contrast, a study that involved farm management that was not stratified by animal age showed no significant association between age and fasciolosis, as the animals of all ages were equally exposed to fasciolosis (Shinggu et al., 2019).

The body condition score (BCS) of less than score-5 (thinner animals) is significantly associated with fasciolosis in the present study. From the coproscopical examination, the present study revealed that 100% ( $n = 16/16$ ) of BCS-1 animals were detected with *Fasciola* eggs, indicating a high occurrence of fasciolosis in poorer BCS compared to BCS-5. Fasciolosis was associated with lower BCS, such as BCS-1 and BCS-2, with odds of 14.9 and 6.0 times. Animals with BCS-3 and BCS-4 showed lower odd ratios to be detected with *Fasciola*, with 1.7 and 1.2 times, respectively. The migration phase of newly excysted juvenile (NEJ) fluke through the liver parenchyma is linked to the acute infection highly associated with host metabolic disturbance (Aragaw and Tilahun, 2019). Therefore, this explains the weight reduction in animals infected with *Fasciola*. Subsequently, the *Fasciola* infection in livestock prompts the loss of appetite and anemic, which leads to lower BCS (Aragaw and Tilahun, 2019).

It was observed that the management of the farms in Taiping varies according to the farming traditions and local policies or incentives from the authorities. The likelihood of exposure to metacercariae increases as the livestock are reared using extensive housing systems and allowed to roam freely. Due to this reason, the extensive housing system was found to be significantly associated with bovine fasciolosis in Taiping ( $p < 0.05$ , OR = 4.0). In addition, fasciolosis exposure increases in farms with extensive housing systems and larger grazing areas. Although climate and environmental factors pose around 21% of ruminant fasciolosis occurrences (McCann et al., 2010), good agricultural practices and hygiene on farms could potentially minimize the exposure of animals to *Fasciola* infective metacercariae (Howell et al., 2015).

The present study observed that rearing multiple ruminant species on the same farms was significantly associated with bovine fasciolosis ( $p < 0.01$ ). Observations from the present study indicate that mixed farming practices are common in Malaysia, with 47.8% of the investigated farms, including cattle, buffaloes, sheep, and goats, within the same farm compound. Compared with farms rearing a single ruminant species, farms with two ruminant species had a 2.0 times higher likelihood of having fasciolosis, and farms with three ruminant species showed a slightly higher risk with 2.1 times. The increased number of definitive host reservoirs would demonstrate varying susceptibility and exposure rates across the animals, which could contribute to this finding (Hochachka and Dhondt, 2000). Following the infection, the incubation period and clinical signs vary among host species, rendering challenges

in the targeted treatment and prolonged disease period in animals (Hochachka and Dhondt, 2000). Fasciolosis was found to have a strong positive correlation with mixed or multiple ruminant species farming (Khan et al., 2009).

Contrary to the findings of a study on fasciolosis in sheep, which reported no correlation between rearing multiple species and an increased risk of fasciolosis (Naim et al., 2021), it is important to highlight that their investigation solely focused on sheep as the host, neglecting other species coexisting on farms. In contrast to the present study, high farm density or herd size did not emerge as significant risk factors for bovine fasciolosis in Taiping, particularly in farms housing only one species. Furthermore, it is crucial to acknowledge that controlling fasciolosis by manipulating herd size is impractical (Takeuchi-Storm et al., 2017). These robust findings underscore the significance of considering a diverse range of species present on farms to understand their respective roles in the epidemiology of fasciolosis.

The present study reveals a significant association between farm age and bovine fasciolosis ( $p < 0.01$ ), with each year of farm age increasing the odds of fasciolosis by 1.2 times. This compelling finding suggests that the longer farms have been in operation, the higher the likelihood of ruminants being affected by fasciolosis. This observation may be attributed to the ongoing release of *Fasciola* eggs into the environment through animal feces, leading to the persistence of *Fasciola* eggs in the environment. It increases the animals' exposure to fasciolosis throughout the year (Jaja et al., 2017; Aragaw and Tilahun, 2019). In conjunction with this significant finding, it is concerning that over 50% of the farmers demonstrated a lack of awareness regarding fasciolosis in general. This lack of knowledge among the farmers raises a cause for concern, as it may lead to negligence in implementing effective fasciolosis control measures, consequently contributing to the higher prevalence of the disease in older farms. Most Malaysian farmers implement control measures based on business profits and costings (Shahudin et al., 2018; Suit-B et al., 2020). Limited knowledge of diseases serves as a factor in the widening gap between awareness and control practice, leading to the persistence of disease incidence (Suit-B et al., 2020).

The present study revealed a statistically significant association ( $p < 0.01$ ) between Paramphistomes co-infection and *Fasciola*-infected bovine, with a notable odds ratio of 1.4. This finding suggests an increased likelihood of encountering Paramphistomes in bovine that are concurrently infected with *Fasciola*. It is important to note that this association may signify a potential risk factor, indicating a correlation between the presence of *Fasciola* and the elevated probability of Paramphistome infection in the studied population. Furthermore, co-infection of *Fasciola* and Paramphistomes has been commonly reported in tropical regions (Khadijah et al., 2017; Aragaw and Tilahun, 2019) and is emerging in the temperate areas such as Europe (Naranjo-Lucena et al., 2018). In co-infected definitive hosts, the number of Paramphistomes eggs was higher than *Fasciola* eggs, showing that the interaction between these helminths may impact the occurrence of fasciolosis (Keyyu et al., 2005). Moreover, both helminths share similar biological characteristics and life cycles, attributed to the capability of infecting similar intermediate hosts of the parasite, the freshwater snail species (Abrous et al., 1999; Madsen and Hung, 2015). A previous study reported successful cercarial shedding by snails co-infected with *Fasciola* and Paramphistomes (Abrous et al., 1999). Determining the transmission dynamics that lead to the abundance and dispersion of fasciolosis relies on the interaction and competition between these helminths in the intermediate and the definitive host (Poulin et al., 2016). However, comprehensive analyses and further research are imperative to elucidate the intricacies of this association, as well as to discern the broader implications and underlying mechanisms of co-infection. Additionally, caution is advised in interpreting these results solely based on egg counts, as egg count may not linearly correlate with the overall burden or clinical effects of co-infection.

This present study also showed that northern Larut and Matang,

Taiping (LM) have a higher fasciolosis prevalence than central LM. The visualization of the prevalence map put forth the possible zoonotic transmission as Taiping town, located in central LM is a human-populated area of >109,000 population (Malaysian Department of Statistics, 2019). To date, there is no human fasciolosis reported from this region. Ideally, the exposure map could be further improved by integrating land use data, especially to identify possible areas for snail intermediate host habitat; however, such data was not openly available during the sample collection of the present study. Despite being derived from a relatively small number of investigated farms, the generated map holds value for visualizing the prevalence of *Fasciola* in regional settings. It visually represents the exposure risk based on the present study's findings. Despite the constraints in non-sampled areas, the map offers relevant insights into the distribution of *Fasciola* within the studied region.

Consequently, reports on the snail intermediate host in Malaysia are inadequate. However, the snail intermediate host depends on the environmental and climatic factors resulting in population abundance. Therefore, the microclimatic variation in different regions affects the transmission pattern of fasciolosis. For example, rainfall and temperature are the main factors for cercarial development in the snail (Villa-Mancera and Reynoso-Palomar, 2019), which then will develop into infective metacercariae and reflect a higher risk of fasciolosis outbreak (Mochankana and Robertson, 2018). During the survey of the present study, Lymnaeid snail was found in only one farm located in central LM (Farm O). However, findings on snail intermediate host in Taiping is insufficient to be discussed in the present study.

Future studies could strengthen the present study's findings by addressing its limitations. Specifically, despite *Fasciola* species molecular identification, providing detailed descriptions and characterizations of helminth egg morphology, such as egg size measurements, would be crucial for precise species identification in the local region. Second, although the freshwater snail *Lymnaea* sp. is a common *Fasciola* intermediate host in Southeast Asia (Madsen and Hung, 2015), the distribution of *Lymnaea* snails was not found in the investigated farms. Therefore, conducting an analysis of eDNA from environmental samples and integrating the data with Geographic Information System (GIS) may necessitate further investigations into identifying the spatial and temporal presence and density of the intermediate host of *Fasciola* (Jones et al., 2018). Third, *Fasciola* co-infection with other helminths, such as Paramphistomes, should be further assessed as it could alter the genetic variation, implicating host-parasite interaction (Seppala and Jokela, 2016). Finally, for predicting infection risk in mapping, more factors, including climatic and environmental should be included in future studies to model a more progressive refinement of the risk prediction of fasciolosis (Charlier et al., 2011). Nevertheless, these limitations of the present study can be used to formulate hypotheses for future epidemiological studies.

## 5. Conclusions

In conclusion, the present study presents a cross-sectional investigation of bovine fasciolosis in Taiping through fecal examination, showing moderate prevalence. Thus, moderate bovine fasciolosis prevalence could reflect on the exposure risk to both humans and livestock, underpinning its importance in both veterinary and public health in this region. The logistic regression model in the present study shows that the host intrinsic (buffalo species group, animal age more than three years old, body condition score less than score-5), farm management (larger grazing area, more than one species in the same farm, extensive housing system, older farm), and co-infection with Paramphistomes were significant risk factors for bovine fasciolosis in Taiping, Perak. The exposure map constructed in the present study is useful for future refinement of fasciolosis studies and for the authorities to initiate control strategies. Thereupon, managing and preventing fasciolosis requires many strategies and collective efforts depending on the regional settings. Taken

together, the present study fills the limited knowledge of fasciolosis epidemiology in Malaysia and highlights future research needs, especially in marginalized and developing countries with high fasciolosis prevalence.

### Ethical statement

The work described in this study involved the use of non-experimental animal and non-invasive sampling was performed. The fecal samples collected were non-invasive and non-painful procedures following the clearance number UPM/IACUC/AUP-007/2019. All respondents were adults who had been given informed consent and were aware of the nature of this study. The socio-demographic data and farmer questionnaire were referred to approval number JKEUPM-2022-181.

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### CRediT authorship contribution statement

**Naim Che-Kamaruddin:** Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nur Fazila Saulol Hamid:** Validation, Supervision. **Lokman Hakim Idris:** Validation, Resources. **Ferdous Mohamat Yusuff:** Validation, Supervision. **Zulfa Hanan Ashaari:** Validation, Supervision. **Hasmawati Yahaya:** Writing – review & editing. **Norhidayu Sahimin:** Writing – review & editing, Funding acquisition. **Nur Mahiza Md Isa:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

### Declaration of competing interest

The authors declared that they have no competing interests in the publication of this manuscript.

### Data availability

The raw datasets supporting this article's findings are available from NCK and NMI upon reasonable request. Please send a request to [naimchekamaruddin@gmail.com](mailto:naimchekamaruddin@gmail.com) and [nurmahiza@upm.edu.my](mailto:nurmahiza@upm.edu.my) to request access to the data.

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