

## High seroprevalence of anti-*Fasciola* antibody among cattle farm workers and dwellers in Kelantan

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**Abstract.** Human fascioliasis is a public health problem particularly in areas where ruminants are raised. The aims of this study were to determine the seroprevalence of anti-*Fasciola* antibody and the associated risk factors among cattle farm workers and dwellers in Kelantan. A total of 90 blood samples were collected in this cross-sectional study. A set of validated questionnaire was used to obtain information on socio-demographic profiles and dietary habits of participants. The sera were subjected to enzyme linked immunosorbent assay (ELISA) for the detection of anti-*Fasciola* IgG antibody. The association between seropositivity and the significant risk factors were determined via logistic regression. From the result, serological screening revealed 60 (67%) participants positive for anti-*Fasciola* IgG antibody. The factors found to be significantly associated with seropositivity against anti-*Fasciola* IgG antibody were the age group of 18 years old and above with calculated odds ratio of 3.2 times ( $p=0.032$ ) and the duration of farming activities of more than 5 years with calculated odds ratio of 2.6 times ( $p=0.036$ ). In conclusion, *Fasciola* infection is prevalent among cattle farm workers and dwellers in Kelantan.

### INTRODUCTION

Fascioliasis is an important zoonotic parasitic disease that causes serious health problems to human worldwide. It is believed to affect 17 million people worldwide with 91 million individuals at risk for the infection (Tolan, 2011). The causative agents for the infection are mainly trematodes of *Fasciola hepatica* and it is mostly found in all continents (Mas-Coma *et al.*, 2005). However, *Fasciola gigantica* is the predominant species in South East Asia and Africa (Adarosy *et al.*, 2013). Human fascioliasis is among the neglected tropical diseases in developing countries and has increased significantly over the past 20 years as a consequence of environmental and climate changes (Mas-Coma *et al.*, 2018). Fascioliasis, particularly in children is a public health problem in regions with

intensive ruminant farming (Rodriguez-Ulloa *et al.*, 2018).

The main route of *Fasciola* transmission is via the consumption of water and aquatic vegetables such as lettuce and water cress infected with the cystic stage of metacercaria (Walker *et al.*, 2008). Ruminants, such as cattle, sheep, goats and buffaloes, are the definitive host in the life cycle of *Fasciola* species. Human is the accidental host and the risk of the infection is higher among individuals involved in raising the ruminants. As cattle farming activities are increasingly popular among farmers in Malaysia and also Kelantan, they are at higher risk of occupational exposure to *Fasciola* infection.

Infection in human may lead to serious hepatic consequences due to pathological changes in the liver cells and tissues during the early migrating stage of the flukes.

Symptomatic fascioliasis includes high fever, diarrhoea, abdominal pain, anorexia and malaise; however, they are undifferentiated from those caused by other forms of endemic infections. The standard laboratory diagnosis of infection is through microscopic examination of *Fasciola* eggs in the faecal samples. Indirect method such as serological detection of antibody against the parasite is increasingly popular due to its high specificity and sensitivity as compared to the coprological examination of eggs (Caban-Hernandez *et al.*, 2014; Tran *et al.*, 2019).

Although several studies have reported prevalence of the infection among ruminants, its occurrence in human is still underreported in Malaysia. To date, one human case of fascioliasis has been reported in our country (Naresh *et al.*, 2006). A plausible reason for the rare occurrence of human fascioliasis in Malaysia is the absence of extensive evaluation of the disease. More than half of human cases are sub-clinical leading to a low disease suspicion among the primary care practitioners. The availability of information on the prevalence of human fascioliasis and the risk factors are very limited and fragmented. It might be viewed as a less important disease by the public health sector in comparison with other parasitic diseases due to the scarcity of epidemiological data and case reports implicating disease morbidity and mortality. Therefore, this study aimed to determine the seroprevalence of anti-*Fasciola* antibody and the associated risk factors among farm workers and dwellers in Kelantan.

## METHODS

### Study design and settings

This cross-sectional study was carried out in Kelantan, Malaysia. Purposive sampling method was employed to recruit participants from ten districts (Table 1) in the state from December 2017 to September 2018 (Table 1). According to single proportion sample size estimation, the minimum sample size required for the study is 80, based on the estimated prevalence of 11.9%, desired margin of error of 7.5%, confidence level of 95% and dropout of 10% (Hussein and Khalifa, 2010; Carnevale *et al.*, 2013).

### Survey

A set of questionnaire on fascioliasis risk factors was designed and validated. The questionnaire consists of two parts; Part A: socio-demographic profiles and Part B: dietary habits. Face-to-face interview was conducted upon informed consent in obtaining survey information.

### Serum collection

Three mL of blood sample were collected from each participant into a plain blood tube. The blood was then incubated one hour at room temperature prior to centrifugation for 10 minutes at  $1000 \times g$ . Serum was collected and kept at  $-80^{\circ}\text{C}$ .

### Detection of anti-*Fasciola* antibody

*Fasciola* serology was performed using *Fasciola* IgG ELISA Test (Corrtez Diagnostics Inc, USA) according to the manufacturer's

Table 1. Number of farms and number of farmers or dwellers per district

District	Number of farms	Number of farmers screened
Kota Bharu	2	10
Pasir Mas	6	9
Gua Musang	7	13
Kuala Krai	4	10
Jeli	2	4
Machang	4	5
Bachok	2	10
Tanah Merah	2	2
Tumpat	2	7
Pasir Puteh	1	20
<b>Total</b>	<b>32</b>	<b>90</b>

protocol. The reported positive and negative agreements of the kit were 100%. Briefly, the serum samples were diluted 1:100 in dilution buffer and incubated in 96 wells microplate for 10 minutes at 21°C +/- 3°C (Maciel *et al.*, 2018). Negative and positive sera provided by the company were used as negative and positive controls respectively. After incubation, each well was washed for 5 minutes 3 times with 300 µL washing buffer. Then 100 µL of enzyme conjugate was added and the plate was incubated for 5 minutes at 21°C +/- 3°C. After incubation, the plate was washed again before incubation with 100 µL of chromogen tetramethylbenzidine (TMB) for 5 minutes. Then 50 µL of stop solution was added to each well and the optical density (OD) was measured at 450 nm using a microplate reader (Bio-Rad, USA). Absorbance reading greater than 0.1 OD units indicates the presence of anti-*Fasciola* IgG antibody. Absorbance reading less than 0.1 OD units was interpreted as a negative result indicating that the individual has no detectable level of antibody.

### Statistical analysis

Data were recorded and analyzed using SPSS version 24.0 (IBM Corporation). The seroprevalence of anti-*Fasciola* antibody among participants was presented in frequency and percentage. The association between individual variable in the survey data (i.e. socio-demographic profiles and dietary habits) and seropositivity of anti-*Fasciola* antibody was determined using Pearson's chi-square ( $\chi^2$ ) and Fisher Exact test. Interactions among survey variables showing significant association with seropositivity of anti-*Fasciola* antibody were examined using binary logistic regression analysis. The interactions were presented in odds ratio and 95% confidence intervals (CI) of the odds ratio. The association between variables is considered statistically significant if the *p*-value is less than 0.05.

### Ethical statement

The present study protocol was reviewed and approved by the Human Ethics Com-

mittee (JEPeM) of Universiti Sains Malaysia (ref. no. USM/JEPeM/16090282). The study protocol was explained to the participants prior to obtaining the informed consent.

## RESULTS

### Socio-demographic profiles and dietary habits

A total of 90 farm workers and dwellers, predominantly males, voluntarily participated in this study. The age ranged from 10 to 76 years old and the majority were adult (Table 2). Other sociodemographic and dietary habit profiles are shown in Table 2.

### Seroprevalence of anti-*Fasciola* antibody

Of the 90 sera analyzed, 60 were positive for anti-*Fasciola* IgG antibody. The seroprevalence of anti-*Fasciola* antibody in this study was 67%. Thirteen participants showed OD values of more than 0.6 (Figure 1). Among the ten districts in Kelantan, Gua Musang showed the highest number of seropositive farm workers and dwellers while Kuala Krai and Tanah Merah showed the lowest number of seropositive cases (Figure 2).

### Factors associated with seropositivity of anti-*Fasciola* antibody

The cattle farm workers and dwellers that showed seropositivity for anti-*Fasciola* antibody and those who showed negative results were compared to identify the potential risk factors of the infection. The two groups did not differ significantly by gender, education levels, monthly household income, water source, consumption of raw vegetables grown using water from farm, consumption of washed fruits and vegetables and consumption of raw vegetables taken from river. However, there was a significant association between seropositivity and the age groups ( $p = 0.032$ ) and the duration of working in the farm ( $p = 0.036$ ) (Table 2). Binary logistic regression analysis showed that those aged 18 years or older are having about 3 times higher odds of positive serological findings, as compared to those

Table 2. Factors associated with seropositivity of anti-*Fasciola* antibody among cattle farmers and dwellers in Kelantan

Attribute	N (%)	Fasciola Serology		$\chi^2$	p value
		Positive n (%)	Negative n (%)		
Gender				0.000	1.000
Male	69 (77%)	46 (67%)	23 (33%)		
Female	21 (23%)	14 (67%)	7 (33%)		
Age (years)				4.599	0.032*
17 and below	16 (22%)	7 (44%)	9 (56%)		
18 and above	74 (78%)	53 (72%)	21 (28%)		
Education				2.645	0.266
Primary school	16 (18%)	8 (50%)	8 (50%)		
Secondary school	44 (49%)	30 (68%)	14 (32%)		
University	30 (33%)	22 (73%)	8 (27%)		
Monthly household income				5.570	0.062
Less than RM2000	44 (49%)	30 (68%)	14 (32%)		
RM2000–RM5000	16 (18%)	14 (87%)	2 (13%)		
More than RM5000	30 (33%)	16 (53%)	14 (47%)		
Duration of working (years)				4.410	0.036*
Less than 5	40 (44%)	22 (55%)	18 (45%)		
5 and above	50 (56%)	38 (76%)	12 (24%)		
Drinking water				–	–
Treated	100 (100%)	60 (67%)	16 (33%)		
Untreated	0 (0%)	0 (0%)	0 (0%)		
Consumption of raw vegetables grown using untreated water				0.274	0.714
Yes	8 (9%)	6 (75%)	2 (25%)		
No	82 (91%)	54 (66%)	28 (34%)		
Consumption of raw vegetables taken from river				3.878	0.097
Yes	52 (58%)	31 (60%)	21 (40%)		
No	38 (42%)	29 (76%)	9 (24%)		
Consumption of washed fruits and vegetables				0.433	0.511
Yes	64 (71%)	44 (69%)	20 (31%)		
No	26 (29%)	16 (62%)	10 (38%)		
Consumption of fully cooked food				1.023	0.551
Yes	88 (98%)	58 (66%)	30 (34%)		
No	2 (2%)	2 (100%)	0 (0%)		

$\chi^2$  Pearson's chi-square test.

\* Significant difference ( $p < 0.05$ ).

aged 17 years or younger (OR: 3.245, 95% CI: 1.070, 9.841,  $p = 0.038$ ) (Table 3). On the other hand, those who worked for more than five years revealed 2.591 times higher odds of positive serological findings, as compared to those who worked less than five years (OR: 2.591, 95% CI: 1.054, 6.370,  $p = 0.038$ ).

## DISCUSSION

This study describes the first serological screening of human fascioliasis in Malaysia. The seroprevalence of anti-*Fasciola* antibody in this study was 67%. The underlying reasons of the high seroprevalence could be due to,

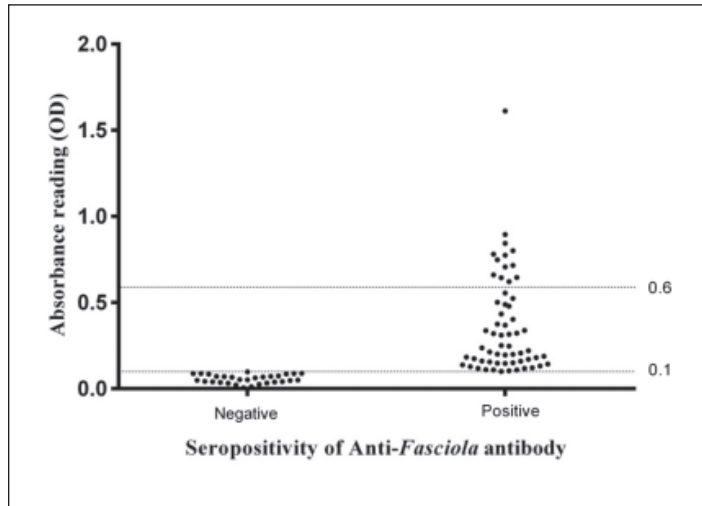


Figure 1. The optical density of anti-*Fasciola* IgG antibody.  
 Note: the cut off OD value suggested by the manufacturer of the commercial kit is 0.1; the cut off OD value suggested by Tran *et al.* (2019) is 0.6.

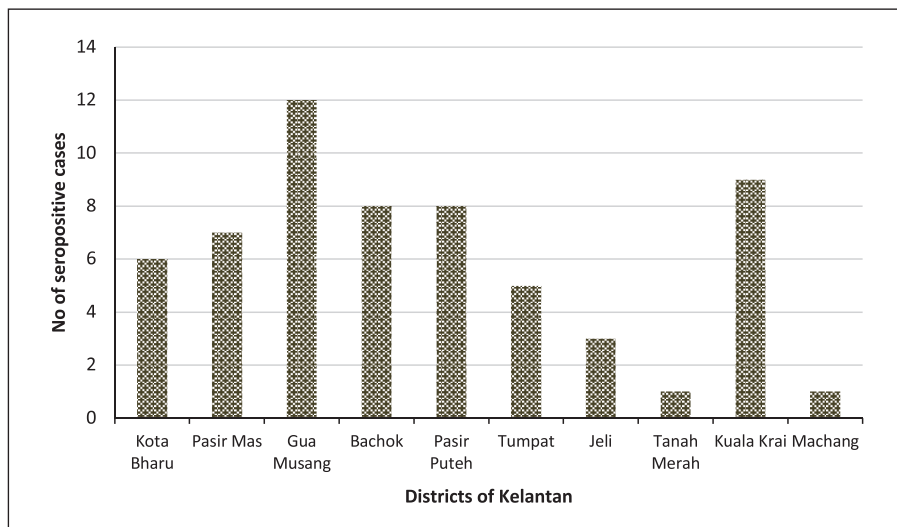


Figure 2. Distribution of seropositivity of anti-*Fasciola* antibody among cattle farmers and dwellers in districts of Kelantan.

Table 3. Binary logistic regression analysis of seropositivity of anti-*Fasciola* antibody among cattle farmers and dwellers in Kelantan

Variable	Odds ratio	95% CI	<i>p</i> value
Age (0= 17 years old and below; 1= 18 years old and above)	3.245	1.070, 9.841	0.038*
Duration of working (0= less than five years; 1= more than five years)	2.591	1.054, 6.370	0.038*

\* Significant difference ( $p < 0.05$ ).

firstly, intermittent exposure of the farmers and dwellers to *Fasciola* infection throughout their lives on the farm; (Rodriguez-Ulloa *et al.*, 2018). Secondly, the serological test which is designed for the detection of *Fasciola* spp provides interpretation for various types of exposure, resulting in high number of seropositive cases (Sarkari and Khabisi, 2017). It is also important to note that absorbance values obtained from the serological test mostly showed very low titer of antibody. Since cut off values for interpreting positive results varies according to different geographical areas (Tran *et al.*, 2019), thus, determination of cut off values for *Fasciola* infection in Malaysia is needed to increase the specificity of the serological tests.

The seroprevalence of anti-*Fasciola* antibody varies according to districts in Kelantan. The highest number of seropositive cases was in Gua Musang [92% (12/13)] where consumption of raw vegetables such as watercress and water spinach is common. These aquatic plants usually grow in watery areas, typical habitats for metacercariae to encyst on (Mas-Coma *et al.*, 2018). The consumption of these raw vegetables is most often implicated with *Fasciola* infection (Soliman, 2008; Cengiz *et al.*, 2015). On contrary, this study showed no association between consumption of raw vegetables and seropositivity of anti-*Fasciola* antibody.

In the present study, the significant associated factors with seropositivity of anti-*Fasciola* antibody were the age of participants and the duration of farming. Cattle farmers aged 18 years or older were found to be at greater risk of *Fasciola* infection compared to those aged 17 years or younger. This finding was in accordance with previous study in Turkey which showed higher prevalence of fascioliasis among patients aged 36 years or older than in those aged 35 years or younger (Cengiz *et al.*, 2015). In contrary, studies in Iran showed no significant association between seropositivity of fascioliasis and age groups (Bozorgomid *et al.*, 2017; Heydarian *et al.*, 2017). Participants who dwelled for more than five years were more likely to have *Fasciola* infection. Even though they have the farming experience,

they are oblivious or have little knowledge about the risk of infection which require a comprehensive health education program to increase awareness of this threat.

In this study, there was no significant association between *Fasciola* serology and survey variables i.e. farmers gender, education level and monthly household income. These findings were in accordance with previous studies in Vietnam and Latin America (Sarkari *et al.*, 2012; Quy *et al.*, 2015; Maciel *et al.*, 2018; Rodriguez-Ulloa *et al.*, 2018). Interestingly, studies in Turkey showed females, whom carried out most of the household's task by the river, had higher risk to *Fasciola* infection than males (Cengiz *et al.*, 2015).

Serological detection of anti-*Fasciola* antibody does not always correlate with the existence of current infection. Exposure to the parasite will produce antibody which remains for at least five months to two years (Sarkari and Khabisi, 2017). Presence of antibody also does not have correlation with chronic infection. Antibody can be detected during migration of the juvenile flukes (Mas-Coma *et al.*, 2005). Seropositivity may suggest acute and chronic infections as well as previous exposure to the parasite (Afshan *et al.*, 2013). Therefore, combination of serological and coprological examination is required to confirm the diagnosis.

## CONCLUSION

In this study we found a high seroprevalence of anti-*Fasciola* antibody among cattle farm workers and dwellers in Kelantan. This study provides evidence on the significant associations between *Fasciola* spp. infections and factors such as age and duration of exposure. Most farm workers and dwellers aged more than 18 years old and are oblivious of the threat of *Fasciola* spp. lurking in the farm and by spending longer duration in the farm areas increased the risk of *Fasciola* infection. Therefore, extensive surveys are necessary to implement control and preventive measures among the community at risk.



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