Tropical Biomedicine 33(1): 170–179 (2016)

Serological evidence of exposure and possible *Taenia solium* larval infection in Orang Asli communities of Peninsular Malaysia

Sahu, P.S.[1*], Lim, Y.A.L.[2], Ngui, R.[2] and Mahmud, R.[2]
[1]Division of Pathology, School of Medicine, International Medical University, 57000 Kuala Lumpur, Malaysia
[2]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[*]Corresponding author e-mail: Priyadarshi@imu.edu.my
Received 20 April 2015; received in revised form 22 May 2015, accepted for publication 24 May 2015

Abstract. Orang Asli communities are known as aborigines of peninsular Malaysia who are underprivileged and also known to be carriers of many different parasitic infections. However, the possible burden of cysticercosis (caused by *Taenia solium* larvae or Cysticercus cellulosae) has never been explored in these communities. Objective of this study was to explore the seroprevalence of human cysticercosis among Orang Asli (subgroups: Semelai, Semai Pahang, Temuan, Orang Kuala, Temiar, Semai Perak, and Mah Meri) communities. In this preliminary study, both male and female of all age groups (1 to 68 years) were screened for *T. solium* larvae specific antibodies in sera employing a commercially procured IgG-ELISA kit. Sera from a total of 522 randomly chosen Orang Asli individuals were screened between July to December 2013. A total of 20 (3.8%) subjects were diagnosed positive for anti-Cysticercus antibodies (95% CI: 2.5% – 5.8%; χ^2=17.8; p<0.05). The prevalence of antibody positivity ranged between 0.9% (Semelai subgroup) to 9.9% (Orang Kuala subgroup). Statistical significance was observed between the low income status of family and seropositivity for cysticercosis (p=0.041) based on univariate analysis. Present study findings indicated that exposure to *T. solium* larval infection might have occurred in the aborigine communities from peninsular Malaysia. Results could only suggest that cysticercosis is an under recognized health problem here. Therefore a regional public health surveillance program might help verifying further the risk factors of *T. solium* cysticercosis targeting a larger population in both peninsular and East Malaysia.

INTRODUCTION

Human cysticercosis caused by larval *T. solium* (Cysticercus cellulosae) infection is known since many decades particularly in the tropical developing countries (Bern *et al.*, 1999). It is a significant public health problem in most of Asian continent (Willingham *et al.*, 2006; Xu *et al.*, 2010). Human acquires infection either exogenously by accidental ingestion of the parasite eggs through contaminated food/water or due to endogenous autoinfection as in the case of carriers of the adult worm in their intestine; by either means, eggs containing the hexacanth larvae are disseminated hematogenously to various organs in the body (viz., brain, eye, muscle etc.) which then develop into metacestode larvae or cysticerci. Although studies in the past have highlighted the underlying *T. solium* taeniasis/cysticercosis prevalence in Malaysia (Cross, 1988; Shekhar, 1991), there have been persisting assumptions that this region is non-endemic. The general impression of absence or low prevalence of the above infection here is thought to be due to strict regulations in pig farming practice particularly in peninsular Malaysia. However, the true estimate of the underlying

prevalence, associated risk factors as well as its transmission mechanisms in this country remains unexplored.

In recent years, a few case reports from Malaysia have been indicating an emergence of cysticercosis in this region (Chew *et al.*, 2001; Ibrahim *et al.*, 2003; Arasu *et al.*, 2005; Nor Zainura *et al.*, 2005; Hasan *et al.*, 2011). The most recent report of confirmed neurocysticercosis (NCC) was in a pediatric case who presented with seizure and underwent surgical excision of the live larva from the brain (Hasan *et al.*, 2011). The above case reports indicated that a possible inflow of immigrants being one of the main reasons for the recent occurrences of cases in Malaysia. However, anti-Cysticercus antibodies in sera has been previously demonstrated from one community based screening in a rural province of East Malaysia. This study indicated an evidence of exposure to the parasite in the local population (Noor Azian *et al.*, 2006). Hence, it is very hard to judge whether emergence of new cases are true emergence due to cross boundary travel and immigration of populations only or a re-emergence of incidences due to the underlying sub-endemicity of this infection among the native populations, in Malaysia which has not been explored.

In Malaysia, as in other developing countries, poverty has been known to exacerbate the health problems in people which include malnourishment, high incidences of infectious diseases, and also the persistent problem with intestinal parasitic infections as reported from earlier studies (Lim *et al.*, 2009; Ahmed *et al.*, 2012). High incidences of infectious diseases affecting the underprivileged communities particularly Orang Asli, the indigenous minority communities of peninsular Malaysia, include but not limited to tuberculosis, leprosy, malaria, and various intestinal parasitic infections (Lim *et al.*, 2009). Though recent improvements in socioeconomic status in Malaysia have shown positive impact on the reduction of intestinal parasitic infections in other communities however, this positive impact is being less significant in the Orang Asli communities (Lim *et al.*, 2009). In order to support the aims of the WHO's Millennium Development Goals for control of neglected tropical diseases (NTDs) including *Taenia solium* taeniasis/cysticercosis and other soil transmitted helminthes (STHs), the first step in addressing these issues regionally is by understanding the extent of the burden as well as to identify the key risk factors and the modes of their transmission to human population.

With the goal to explore the possible exposure to *T. solium* eggs and the underlying prevalence of cysticercosis in Orang Asli communities living in peninsular Malaysia, this study is to determine anti-*T. solium* larval antibodies in serum by employing a commercially procured ELISA kit. Some associated risk factors and possible modes of its transmission are discussed here.

MATERIALS AND METHODS

Study subjects

In this preliminary survey, a total of 522 Orang Asli from seven subgroups from different states of peninsular Malaysia were randomly selected for screening of anti-*T. solium* larval antibodies in their serum. The different subgroups of Orang Asli included: Orang Kuala (n=82); Mah Meri (n=16); Temuan (n=83); Semai Pahang (n=92); Semelai (n=108); Temiar (n=76); and Semai Perak (n=65). The demographic and baseline characteristic compositions of the studied population are presented as in Table 1. Majority of them were children under 13 years of age and hence majority did not have individual income. The household income was < 500 Malaysian Ringgit (MYR) per month in majority of cases. Only 3 subjects had secondary level education.

Specimen

Whole blood samples of the recruited study subjects (5ml from each) were collected aseptically and the separated sera were kept at -80°C till use. For children < 5 years of age, 2-3ml of venous blood was collected and processed as stated above.

Table 1. Demographic and baseline characteristics compositions of the studied population

Variable	No	(%)
Age		
Range	1-68 years	
Mean (± SD)	16.4 (± 13.4)	
Age groups (Years)		
1 to 4	10	1.9
5 to 6	7	1.3
7 to 12	367	1.3
13 to 17	20	3.8
18 and above	118	22.6
Age groups		
≤ 18 years	405	77.6
> 19 years	117	22.4
Gender		
Male	231	44.3
Female	291	55.7
Subgroups		
Semelai	108	20.7
Semai Pahang	92	17.6
Temuan	83	15.9
Orang Kuala	82	15.7
Temiar	76	14.6
Semai Perak	65	12.5
Mah Meri	16	3.1
Level of education		
No formal education	119	22.8
Primary school	400	76.6
Secondary school	3	0.6
Occupation categories		
Not working (i.e., student, housewife, etc.)	416	79.6
Jungle produce gatherers	45	8.6
Rubber tapper	50	9.6
Factory	11	2.1
Household income (RM/month)		
<MYR500 (< US$ 166.7)	341	65.3
>MYR500 (> US$ 166.7)	181	34.7
Total	**N=522**	

MYR- Malaysian Ringgit; US$- United States Dollar.

ELISA for detection of anti-*T. solium* larval antibodies in serum

A commercially procured ELISA Kit (*NovaTec Diagnostics, Germany*) was used in this screening study in order to detect the presence as well as to quantify anti-*T. solium* larval IgG antibodies in serum for diagnosis of cysticercosis. All the collected sera were tested following manufacturer's instruction. Briefly, antigen coated wells were incubated with 1:10 diluted patient serum (diluted with serum diluent buffer as provided in the kit). With every batch of samples, a negative control serum, a low positive control serum, and a high positive control serum (all supplied by the manufacturer) were also

tested for validity of the test. For each serum sample, the absorbance at 450 nm (OD_{450}) was measured and compared with that of the control sera. As per the manufacturer's specification based on the OD_{450} for low and high positive control sera, a sample was confirmed as positive for anti-Cysticercus IgG antibody when its absorbance was observed to be greater than that of the low positive control. A sample was considered as borderline when its absorbance was observed to be the same as that of the low positive control.

Statistical analysis

Univariate analysis was performed in order to identify the risk factors in association with the possible exposure to *T. solium* larval infection using SPSS 2010 statistical software.

RESULTS

The overall seroprevalence of cysticercosis among 522 Orang Asli was 3.8% (20 subjects) as determined by the IgG-ELISA. The overall results of the test seropositivity and the prevalence of cysticercosis in each sub group of Orang Asli populations in the decreasing order are presented in Table 2. The highest and lowest seroprevalence being recorded were of Orang Kuala and Semelai sub groups, respectively. There was no positive cases in the Temiar as well as Semai Perak sub groups.

Results of the univariate analysis for identifying risk factors in association with the possible exposure to *T. solium* larval infection are presented in Table 3. Statistically various demographic features such as age group, gender, educational level, and occupation were found non-significant ($p > 0.05$). However, a statistically significant ($p \leq 0.05$) association was observed between the household income and anti-*T. solium* larval antibodies in serum.

Out of total 448 individuals with a history of not-working, 405 were within paediatric age group. Rest 43 subjects were adults which did not have any work. So out of total 117 adult subjects, 74 individuals provided information who were employed as unskilled laborers in construction sites, factories, vegetable farms, oil palm and rubber plantations. There was no statistical difference found when we compared the seropositivity among the two groups based on employment (Table 3).

DISCUSSION

Though no information is available on the actual burden of *T. solium* cysticercosis in Malaysia, it is assumed that *T. solium* cysticercosis is not totally free from this region. Moreover, many hospitals do not perform a serological or any other laboratory based supportive diagnosis (*viz.*, histo-pathology) to verify the clinical suspicion of

Table 2. Seroprevalence of IgG antibodies to *T. solium larva* among the Orang Asli

Subgroup	ELISA for anti-*T. solium* larval IgG			95% CI	Chi-square test
	No. examined	No. positive	%		
Orang Kuala	82	8	9.8	5.0-18.1	
MahMeri	16	1	6.3	1.1-28.3	
Temuan	83	5	6.0	2.6-13.3	
Semai	92	5	5.4	2.3-12.1	
Pahang					
Semelai	108	1	0.9	0.2-5.1	
Temiar	76	0	0	0	
Semai Perak	65	0	0	0	
Total	**522**	**20**	**3.8**	**2.5-5.8**	$X^2=17.8$ $p<0.05$

Table 3. Univariate analysis of factors associated with seropositivity of *Taenia solium* cysticercosis among the studied population

Variables/Characteristics	N	Cysticercosis Seropositive		OR (95% CI)	*P* value
		no	%		
Gender					
Female	291	13	4.5	1.50 (0.57-3.81)	0.396
Male	231	7	3.0	1	
Age					
≤ 18 years	405	17	4.2	1.51 (0.53-4.35)	0.418
> 19 years	117	3	2.6	1	
Level of education					
No formal education	119	3	2.5	0.59 (0.17-2.04)	0.397
Formal education	403	17	4.2	1	
Occupation categories					
Working	74	2	2.7	0.67 (0.16-2.84)	0.585
Not working	448	18	4.0	1	
Household income (RM/month)					
< RM 500	181	11	6.1	2.38 (0.97-5.88)	0.041*
> RM 500	341	9	2.6	1	
Total	**522**				

N: Number examined; no: Number of subjects seropositive; Reference group marked as OR=1;
CI: Confidence interval; * statistically significant.

cysticercosis. In the present study, the overall prevalence of anti-Cysticercus antibodies in serum was estimated to be 3.8% indicating a possibility of an underlying sub-endemicity of this infectious disease in native communities of Malaysia. One of the previous reports highlighted 2.2% prevalence of anti-Cysticercus antibodies in sera from Sabah, Malaysia Borneo indicating evidence of exposure to the parasite in the local population (Noor Azian *et al.*, 2006). However, in the Sabah study, there was no information available on any specific community or sub-community. Also no risk factor analysis was done prior to this with respect to occurrences of cysticercosis in Malaysia.

In our study, the highest and lowest seroprevalence for possible cysticercosis was detected for Orang Kuala and Semelai sub groups, respectively. There was no positive cases in the Temiar as well as Semai Perak sub groups. In the present study, the association between household income and positive test for anti-*T. solium* larval antibodies in serum was found to be statistically significant. In this study, overall data showed majority of the study subjects having household income >500 MYR per month. Whereas majority of subjects who were seropositive for cysticercosis had household income <500 MYR per month. It shows clearly that poverty has a role in the underlying disease occurrences. Also none of them attained education up to the secondary level except for 3 cases. All others had obtained either primary level or no formal education.

In the present study out of total individuals with a no history of any employment, majority were within paediatric age group. Rest were adults which did not have any definite work. Those who were employed they were unskilled laborers in construction sites, factories, vegetable farms, oil palm and rubber plantations. Similar information was also obtained in case of the parents of the children included in our study (*data not shown*). So in either case the disease occurrences in a community can have a

social impact because epilepsy has major socioeconomic consequences for the individual patient and for society (Jennum *et al*., 2011). Hence, a possible underlying exposure to *T. solium* larval infection in the individuals with a positive antibody test is the matter of concern particularly when there is a chance of asymptomatic NCC in few among 20 such cases. Because NCC may lead to epilepsy in future which can affect their employment or earning.

Helminthic infections, among the most common NTDs, is known to be a major public health problem in Malaysia with a possible impact on the nutritional status and school attendance particularly of children in rural Malaysia (Ahmed *et al*., 2012). For school children, WHO has recommended mass treatment with praziquantel (PZQ) for controlling schistosomiasis as well as albendazole/mebendazole for soil transmitted and/or food borne helminthiases. However, there can be safety issues with mass anthelminthic treatment in children. There are incidences of children dying within days of treatment as part of mass treatment campaigns against schistosomiasis and/or fish-borne trematodiasis particularly in Southeast Asian countries (Ito *et al*., 2013). *T. solium* cysticercosis can also be a problem of school going children as other helminthic infections. But there have been no analyses of the cause of these sudden deaths. If these drug trial areas were also endemic for taeniasis/cysticercosis, then sudden deaths could have been due to underlying NCC by which the individuals succumb due to PZQ side effects particularly when given without a steroid (Wandra *et al*., 2011). Therefore it is necessary to keep in mind the necessity of ruling out possible underlying NCC in the individuals during mass treatment campaigns where there could be many asymptomatic cases in the target community (Wandra *et al*., 2011; Ito, 2013). In Malaysia, thus far no study has been conducted to screen for cysticercosis among the school children.

T. solium taeniasis/cysticercosis is mainly prevalent in areas where pigs range freely, sanitation is poor, human feces are used as fertilizer, education is low, and meat inspection is absent or inadequate, and thus is strongly associated with poverty. A wide prevalence of *T. solium* taeniasis and cysticercosis is being reported where it is considered to be emerging in small-holder farming communities in many under-developed areas of South East Asia (Willingham *et al*., 2010). Although economic analysis on pig farming methods have shown that the traditional production systems are wasteful and unprofitable, however, the prevailing production system using free-range pigs is apparently very resilient. Reports from Africa showed that the sustainability of the traditional sector is better than that of the intensive sector (Lekule and Kyvsgaard, 2003). The lower fixed cost of traditional pig production compared with intensive production is one of the best reasons for the above paradox. The scenario may be similar in many Association of Southeast Asian Nations (ASEAN) countries neighboring Malaysia (Xu *et al*., 2010; Wandra *et al*., 2011; Wandra *et al*., 2000; Rajshekhar *et al*., 2003; Suroso *et al*., 2006; Wandra *et al*., 2013). So there is a need of estimating the burden of possible infections transmitted through pigs by a thorough screening of the pig farming practices particularly in rural sectors in this region including Malaysia. Porcine serology usually provides an appropriate indicator of *T. solium* environmental contamination. Therefore, it has been recommended to be used in order to estimate the risk of infection by adapting control measures from other endemic regions as in Latin America (Garcia *et al*., 1999). However, there is no published report available on porcine studies in this region which may be due to various reasons including but not limited to the regional cultural issues.

The other possibility of source of transmission of infection to the local communities in Malaysia may be because of transmigration activities across the border. As burden of taeniasis is not known among the local population, the infection may be probably acquired from the immigrant workers who are food handlers. There was a report of confirmed NCC in a local born Malay woman who presented with headache,

and confusion; CT brain scan showed pathognomonic cysts with invaginated scolex and serological testing for cysticercosis was also strongly positive (Ibrahim *et al*., 2003). Besides going to Mecca for pilgrimage, this patient had not traveled overseas. Because Mecca is not known to be endemic for *T. solium* infection, acquiring the larval infection in this Malaysian woman remained unknown. However, it can only be hypothesized that one of the possible mean of acquiring the larval *T. solium* infection is through human to human transmission via contaminated food or water consumption which is most likely due to a large expatriate population from neighboring countries in Malaysia who works in restaurants across the country. Thus the above case showed the importance of screening for cysticercosis particularly among the food handlers working in Malaysia who have emigrated from *T. solium* endemic areas.

Human cysticercosis is recently being considered as an important cause of neurological disease worldwide; a growing number of cysticercosis cases have been reported in more developed countries as a result of increasing migration and tourism (Xu *et al*., 2010; Carpio, 2002; del la Garza *et al*., 2005; Kraft, 2007; Mamkin *et al*., 2007; Sorvillo *et al*., 2007). Previous seroprevalence studies indicated a varying rate of exposure to the parasite (ranging from 0.02 to 12.6%) across the Asian continent (Rajshekhar *et al*., 2003). In a series of cysticercosis cases in China, it has been reported that urban population to be more at risk than the rural population (Chaoshuang *et al*., 2008). While the whole of South East Asia is known to be endemic for *T. solium* taeniasis/cysticercosis, the true estimate of the underlying prevalence and the epidemiological information of this disease transmission in Malaysia remain limited. Being a Muslim majority country, pork consumption is prohibited for the Muslim populations here thus favour the possibility of non-occurrences of *T. solium* taeniasis in Muslims. However, there are also many non-Muslim communities who live within this multicultural country, and many of them are pork eaters.

Moreover the social situation in Malaysia has changed over the past few decades with massive influx of immigrants. Many are from various neighboring countries known to be endemic for *T. solium* taeniasis/ cysticercosis such as India, Indonesia, Philippines, Nepal, Sri Lanka and Thailand. The potentiality of transmission of *T. solium* infections mediated through human being is of concern here. Some of the immigrant workers are being employed as food handlers. There may be many illegal immigrants who do not undergo medical examinations prior to entry into the country as highlighted in a past report (Chew *et al*., 2001). In this report, a personal communication by the author with University of Malaya Medical Centre at Kuala Lumpur had showed that 0.05% of the registered immigrant workers in the year 2001 were positive for *T. solium/T. saginata* (Chew *et al*., 2001). Nevertheless if it was *T. solium*, these workers could be the source of transmission of *T. solium* eggs to the local population, including Muslims even though they do not consume pork. No such data is available so far in the recent years. So it is recommended that a compulsory screening for intestinal taeniasis besides other parasitic or infectious diseases is carried out in immigrant workers by the health authorities.

Hence, it may be difficult to conclude whether reporting of new cases of cysticercosis in Malaysia in the recent years are true emergence due to immigration of populations from endemic countries, or a re-emergence of cases due to the underlying low endemicity of this disease among the residents of this country including but not limited to the aborigines. With respect to the second possibility as stated above, results of a low seroprevalence in the present study may be due to inadequate exploration of the communities. With an increasing expatriate population particularly in recent years further studies in this field could potentially lead to the quantification of the real burden of this NTD in Malaysia.

Limitation in the present study was that, the antibody based test could only provide evidence of the exposure to the parasite larvae in recent past. There was no scope of monitoring the change in titer of the diagnostic

antibodies in their serum subsequently. No follow up was possible in this screening study to find a clinical confirmation considering the notable clinical features (*viz.*, seizures, headache, ocular complications etc) in those serologically positive cases. There may be limitations in establishing cysticercosis as the cause of underlying symptoms particularly in community settings as highlighted in a recent study (Cherian *et al.*, 2014). And there was no scope of analyzing any symptoms in our study subjects since they were apparently normal and the sampling was done in a random scale. So seroprevalence can be the only indicator of the possible exposure to the parasite as in the present study. There are many incongruities between immunological and molecular studies due to biased work as advocated by Ito (2013). In his view 'nothing is perfect'. Hence, indirect approaches using either immunological, or even molecular tools, are limited without confirmation from direct evidence of an infection particularly while studying cestode zoonoses. There are reports of serological cross reactions between cases with cysticercosis and other helminth infections in human viz., *Spirometra mansoni* sparganum excretory-secretory antigens cross-reactions with sera of patients with cysticercosis or paragonimiasis (Hu *et al.*, 2013). However, there was no scope of verifying these infections in the studied population. However, the ELISA used in the present study was a commercially procured diagnostic kit which did not have any such cross reaction information and the nature of the antigens coated in the plates was also not excretory-secretory. Therefore a dilemma of whether developing countries should develop their own diagnostic tests or rely on commercially available kits is also a notable concern to address in future studies as suggested previously (Ito, 2013).

CONCLUSION

Study findings indicated that exposure to *T. solium* larval infection might have occurred in the aborigine communities from peninsular Malaysia. Although the studied samples may not be a true representation of the whole community in this country, however, results could only suggest that cysticercosis is an under recognized health problem here. Therefore a regional public health surveillance program must verify further the risk factors targeting a larger population in both peninsular as well as East Malaysia.

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