SEROPREVALENCE OF CYSTICERCOSIS IN A RURAL VILLAGE OF RANAU, SABAH, MALAYSIA

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Abstract. The objective of this study was to determine exposure to cysticercosis among a rural population in a selected village in Ranau, Sabah, Malaysia. A total of 135 serum samples were analyzed. The result showed that the seroprevalence of cysticercosis antibodies was 2.2%. There was no significant difference in the seroprevalence among age groups (p=0.307). Even though there was a slightly higher antibody titer in males compared to females, the difference was not significant (p=0.400). The results indicate evidence of exposure to cysticercosis in this rural population.

INTRODUCTION

Cysticercosis is caused by the tapeworm larvae of *Taenia solium* and is closely associated with pigs (Miyazaki, 1991). Cysticercosis is widespread throughout the world, and is especially common in Central and South America, Africa, Southeast Asia, Korea, China and the former Soviet Union. The prevalence is marked in those areas where people like to consume raw pork and insufficiently cooked pork or where the breeding of pigs is improper. The infection is also related to the economic and social conditions of the affected community, which include the use of night soil for agriculture, inadequate sanitation, a low standard of living, ignorance of the spread of the disease, religious rites which use contaminated water, and migration of the population. Cysticercosis is endemic in several developing countries in Asia, Africa and Latin America. It often attacks the human nervous system and is frequently responsible for hospitalization due to epilepsy, intracranial hypertension or meningitis, in endemic areas (Gomes *et al*, 2002; Rajbhandari, 2004).

Studies by Carrique-Mas *et al* (2001) showed that the seroprevalence among a rural population in Bolivia was 22% in humans and 37.4% in pigs. The risk factors for human infection included older age groups, the absence of sanitary facilities, poor formal education and an inability to recognize infected pork. Cysticercosis is more common in adults than in children, and its seropositivity rate increases with age (Garcia and Bruckner, 1993). In a Mexican village, it was shown that the overall seroprevalence of cysticercosis was 10.8%; the seropositivity increased with age and reached a maximum in subjects aged 46-55 years (Sarti *et al*, 1992). In Vietnam, Willingham *et al* (2003) reported that over the previous 15 years, more men were treated for cysticercosis with most patients being young to middle-aged adults.

The Institute for Medical Research (IMR), Kuala Lumpur provides serological diagnostic testing for cysticercosis. A total of 7 and 15 samples were received in 2003 and 2004, respectively, for the diagnosis of cysticercosis. The positivity rates (by ELISA) were 29% and 14.3%, respectively. However, there has been no report on the seroprevalence of the infection in Malaysia, to indicate the endemicity of the infection. Ranau is a rural district of Sabah where the population in some villages has the habit of keeping domesticated wild boars under their houses, giving rise to the risk of cysticercosis infection among this population. Therefore the objective of this study was to determine the exposure of this rural population to cysticercosis.

MATERIALS AND METHODS

Serum samples

The sample size was calculated based on an expected cysticercosis seroprevalence of 1.65% (Sutisna *et al*, 1999) and maximum

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seroprevalence rate of 4%. Using Epi-Info Version 6, the sample size required for this study at a 95% confidence level was 101 samples. One hundred and thirty five serum samples were randomly selected from a collection of sera collected from a rural village in Pinawantai, Ranau during a malaria survey in 1996. The sera had been kept at -20°C undisturbed by frequent freezing and thawing until used for this study. The ELISA test kit, Cysticercosis Kit (Cypress Diagnostics, Inc), was used to detect the antibodies. The test was performed according to the manufacturer's instruction.

ELISA- cysticercosis

A 10 μl serum sample was diluted with 990 μl sample buffer and vortexed for several minutes. Fifty microliters were transferred to duplicate ELISA plates and incubated for 15 minutes at room temperature. The plate was then washed 5 times with washing buffer followed by adding conjugate into each well and incubated again for 15 minutes. After washing the plate, 50 μl of substrate and 50 μl of chromogen solution were added and incubated in the dark for 15 minutes. The reaction was stopped by adding 50 μl of stopping solution. The optical absorbance was then read using an ELISA Reader (Dynatech) at 450 nm. Positive and negative controls were provided by the manufacturer. The individual optical density (OD) of the sample was expressed as the corrected OD using the following formula:

$$\text{Corrected OD} = \frac{\text{Positive control OD } 1^{st} \text{ plate}}{\text{Positive control OD of current plate}} \times \text{OD of the sample}$$

Statistical analysis

Data was analyzed using SPSS® version 10. Differences in proportion were tested using chi-square. For continuous data, the Mann-Whitney, Kruskall-Wallis test and Spearman rank correlation was used where appropriate.

RESULTS

Proportion of serum samples by age groups and gender

The 135 serum samples were classified into 8 age groups. Most of the samples (51.8%) were from adolescents and children below 20 years of age (Table 1), which were typical of that rural population. There were slightly more samples from females (53.3%) compared to males (46.7%).

Frequency of distribution of antibody titers

Fig 1 shows the frequency distribution of anti-cysticercal antibody titers (OD) as measured by ELISA. The OD ranged from 0.007 to 1.398,

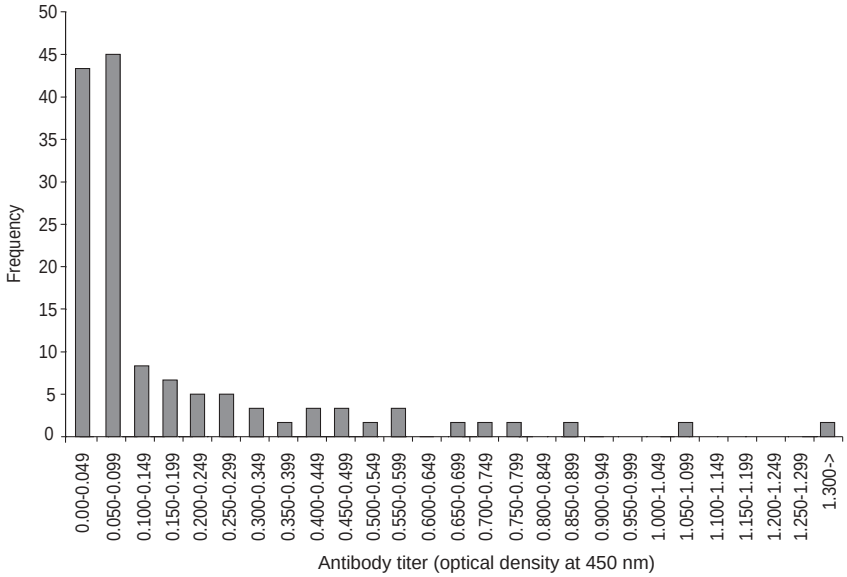

Fig 1–Frequency distribution of anti-cysticercal antibodies among rural villagers in Ranau, Sabah, Malaysia.

Table 1
Proportion of the serum sample by age group.

Age group	Frequency	Percent
0-4	11	8.2
5-9	28	20.7
10-19	31	23.0
20-29	15	11.1
30-39	14	10.4
40-49	17	12.6
50-59	13	9.6
60->	6	4.4
Total	135	100.0

Table 2
Anti-cysticercal antibodies by age group.

Age group	Antibody titer		p-value
	Mean ± SD	Median ± SD	
0-4	0.063 ±0.034	0.052 ±0.055	(Kruskal-Wallis test) p=0.307
5-9	0.160 ±0.202	0.060 ±0.147	
10-19	0.115 ±0.159	0.058 ±0.048	
20-29	0.212 ±0.372	0.053 ±0.269	
30-39	0.150 ±0.223	0.063 ±0.143	
40-49	0.258 ±0.273	0.218 ±0.386	
50-59	0.158 ±0.169	0.055 ±0.219	
60->	0.386 ±0.482	0.190 ±0.624	

with a median of 0.065 and a mean titer of 0.169±0.242 SD. The majority (87.0%) of the serum samples had a very low OD of less than 0.100. Nine samples (6.7%) had a moderate OD of between 0.500 to 0.999, while 3 samples had a high OD of more than 1.000. If the cut-off point value for the ELISA positivity is taken arbitrarily as mean ±3SD (OD=0.889), the seroprevalence of cysticercosis in this village was 2.2% (3/135).

Antibody titers by age group and gender

Table 2 shows the mean ± SD and the median antibody titers by age group. There was no significant difference in the antibody titers among the age groups (p=0.307). There was also no significant correlation between age and antibody titers (rs= 0.16, p=0.064). All three positive cases were adults, (23-68 years old), two of them were males. There was a slightly higher mean antibody titer in the males (0.194 ± 0.256) compared to the females (0.147 ± 0.218), but the difference was not significant (p=0.400).

DISCUSSION

Rajshekhar *et al* (2003), in a review paper about *T. solium* taeniasis and cysticercosis in Asia described that the disease is endemic in Asian countries, such as India, China, Indonesia, Thailand, Korea, Taiwan and Nepal. Cysticercosis is the cause of epilepsy in up to 50% of Indian patients presenting with partial seizures. It is also a cause of epilepsy in Indonesia, Vietnam, China and Nepal. Seroprevalence studies indicate high rates of exposure to the parasite in several countries (Vietnam, Korea, China and Indonesia) with rates ranging from 0.02-12.6%. In Bali, Indonesia, the prevalence of taeniasis and cysticercosis by immunoblot analysis were 0.72% and 1.65%, respectively (Sutisna *et al*, 1999). However in Irian Jaya, the seroprevalence was much higher at 47.9% (Wandra *et al*, 2003). On the basis of serologic results, 12 (70.6%) of 17, 20 (62.5%) of 32 and 12 (25.5%) of 47 of epileptic seizures, subcutaneous nodules and healthy groups, respectively, were infected with the larval stage of *T. solium*. This study reported the first community-based seroprevalence study of cysticercosis in a rural community in Malaysia. A rate of 2.2% reported in this study was higher than that reported in Bali, Indonesia.

Garcia and Bruckner (1993) noted that the peak incidence of cysticercosis is between 30-50 years of age. Willingham *et al* (2003), who carried out a hospital-based survey over the past 15 years in Vietnam, found a higher prevalence of cysticercosis in an older age group. Similar finding was also noted by Wandra *et al* (2003) in Irian Jaya, Indonesia. Among the epileptic seizures group, the prevalence increased with age, with rates of 40, 71.4 and 100% among patients 18-24, 30-44 and ≥45 years old, respectively. However, there was no significant difference in antibody titers among the age groups in this study.

Garcia and Bruckner (1993) noted that neurocyticercosis usually affects more males than females. Although 2 out of the 3 positive cases in this study were males, the difference

was not significant. There was a similar higher proportion of seropositive male but no significant difference in gender predisposition was reported elsewhere (Carrique-Mas *et al,* 2001; Wandra *et al,* 2003; Willingham *et al*, 2003).

Several methods are being used to detect antibodies for the diagnosis of cysticercosis. In this study, we used a commercially available ELISA test and we detected a seroprevalence of 2.2% among the rural population in Ranau, Sabah. ELISA has been shown to be lacking in specificity (Hira *et al*, 2003) and false positive results due to cross-reacting antibodies could not be ruled out in this study. However, the fact that there have been clinical cases of cysticercosis diagnosed by ELISA and supported by Magnetic Resonance Imaging (MRI) findings, among local Malaysians who had no travel history, suggest that the potential of cysticercosis in rural communities in Malaysia can not be ignored. There are several ethnic minorities in Malaysia who are known to be in close contact with domesticated wild boars or pigs, not only in Sabah, but also in Sarawak and Peninsular Malaysia. As most of these minority groups live in rural areas where personal and environmental hygiene practices may be inadequate, they are at risk of being infected with this parasite.

As discussed earlier, interpretation of ELISA results may be difficult in view of potential cross-reacting antibodies, especially in the tropics where polyparasitism is common. Other complementary methods, such as ultrasonography and magnetic imaging should be employed where available to aid in the diagnosis, especially when tissue biopsy is almost impossible. New approaches have been described, such as multiplex polymerase chain reaction (Yamasaki *et al*, 2004).

Long storage of the serum sample used could have also affected our detection rate. Thus, the rational for using the mean ±3SD optical density as the cut-off point in this study. Based on these findings, further study is proposed, especially among ethnic minorities such as the aborigines and other ethnic minorities of Peninsular Malaysia, to determine the extent of the problem in Malaysia.

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