

## Research Article

# Seroprevalence of Cysticercosis in Children and Young Adults Living in a Helminth Endemic Community in Leyte, the Philippines

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Cysticercosis is a significant public health problem in countries where pigs are raised for consumption and remains an important cause of neurological disease worldwide. The Philippines is considered an endemic area for cysticercosis because cases in both humans and pigs have been reported; however, epidemiologic information stays limited. We conducted a pilot survey of the seroprevalence of human cysticercosis in a village in Leyte, the Philippines, by measuring antibody specific for *Taenia solium* cyst-fluid antigen. There were 497 subjects aged 7–30 years in our study and most subjects were infected with one or more helminths. The overall cysticercosis seroprevalence in this population was 24.6% (95% CI: 20.82% ~ 28.58%) with no significant difference based on age, sex, or other helminth coinfection status. Although the sample may not be representative of the whole community, the findings suggest that cysticercosis is a significant, but underrecognized public health concern in the Philippines.

## 1. Introduction

Human cysticercosis is caused by infection with the larvae (cysticerci) of *Taenia solium*, a cestode (tapeworm) transmitted among humans and between humans and pigs. Humans acquire tapeworm infection from eating raw or undercooked pork meat containing *T. solium* cysticerci. When ingested, cysticerci are activated by stomach acid, pass into the duodenum, and develop into adult tapeworms. The tapeworm body consists of many proglottids, each containing approximately 50,000–60,000 eggs [1]. Both humans and pigs can develop cysticercosis if they ingest *T. solium* eggs passed in human stool directly, or from consumption of food or water contaminated with eggs (fecal-oral transmission). Human cysticercosis can also occur by autoinoculation or

reverse peristalsis of eggs in individuals with *T. solium* tapeworms [2]. The clinical presentation of cysticercosis is nonspecific and varies depending on the location, number, and stage of cysts. The most frequently reported locations are skin, skeletal muscle, heart, eye, and most importantly, the central nervous system, causing neurocysticercosis (NCC) [3–7].

Cysticercosis is endemic in Africa, Asia, and Latin America [8]. It is mainly transmitted 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 and smallholder farming. In recent years, a growing number of cysticercosis cases have been reported in more developed countries as a result of increasing migration and tourism

[2, 9–13]. Theoretically, cysticercosis is straightforward to prevent and control; however, it has not been eliminated and remains neglected in both the endemic developing countries [8, 14] and developed countries [15].

At present, epidemiological surveys of cysticercosis have not been conducted in many endemic areas due to the lack of availability and cost of the diagnostic methods [16]. Diagnostic approaches for cysticercosis include subcutaneous nodule biopsy, neuroimaging, and serological tests [8]. Neuroimaging, that is, computerized tomography (CT) and magnetic resonance imaging (MRI), are very useful for diagnosis of cysticercosis, but they are inaccessible in many poor endemic areas of the world [17]. Immunodiagnostic methods for cysticercosis, which detect parasite antigens or host antibodies to parasite antigens, have been developed and improved greatly in recent years and allow identification of endemic communities where prevention and control measures should be implemented [18, 19].

The Philippines is a developing country whose economy relies mainly on agriculture and is considered an endemic area because both human [20] and porcine cysticercosis [21] have been reported. However, information on the epidemiology of this disease is still quite limited in the Philippines. To appropriately target scarce health care resources, detailed, community based studies of the prevalence of cysticercosis in pig farming areas of the Philippines are necessary. Therefore, we conducted a human seroprevalence study of cysticercosis using a commercially available kit which detects antibodies to cysticercal antigens in a village in Leyte where schistosomiasis and geo-helminths are coendemic. Our results indicate that human cysticercosis is an underappreciated infection in this area.

## 2. Materials and Methods

**2.1. Ethics Statement.** The study was approved by Institutional Review Board at Brown University and at the Philippines Research Institute of Tropical Medicine. Written, informed consent was obtained from each adult participant or from the parents of minors.

**2.1.1. Study Design.** This study was conducted in Macanip, a *S. japonicum*-endemic rice-farming village in Leyte, the Philippines. The study area is endemic for both *S. japonicum* and geo-helminths. The current cross-sectional serologic survey of cysticercosis was performed in subjects who were eligible for a longitudinal treatment-reinfection study of schistosomiasis [22, 23]. *S. japonicum* infected individuals were enrolled in the study during October 2002 if they lived primarily in the study village, and were not pregnant or lactating, and provided informed consent. The study sample consisted of 422 *S. japonicum* infected individuals aged 7 to 30 years and individuals aged 7 to 18 years ( $n = 75$ ) who were not infected with *S. japonicum* were recruited as control subjects. The prevalence of infection with *S. japonicum* in this age range was 60.0% in the community. Due to the overall study design, the sample for serologic survey of cysticercosis has a much higher proportion of *S. japonicum*

infection than the age-specific or general prevalence in the community [23]. For each subject, infections of *S. japonicum*, *A. lumbricoides*, *T. tricurria*, and hookworms were determined through Kato-Katz examination of 2 slides prepared from each of 3 stool samples. All participants received treatment with a split dose of 60 mg praziquantel/Kg of body weight after baseline blood collection and physical examination.

**2.1.2. Blood Collection.** Prior to treatment for schistosome infection, blood was collected into Vacutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ) for all subjects. Serum was prepared, aliquoted, and stored at  $-80^{\circ}\text{C}$  until assayed.

**2.1.3. ELISA Assay.** All serum samples were tested by indirect ELISA assay to quantify the level of antibody reacting with *T. solium* cyst-fluid antigen using a commercial diagnostic kit (Shenzhen Combined Biotech Co. Ltd., China). The reported sensitivity and specificity of this assay kit are 92.5%, and 100%, respectively, with no false positives occurring in samples from patients with trichinosis, fascioliasis, paragonimiasis, and nonparasitic encephalopathy.

The ELISA assay was performed following the instructions and using the diluents supplied with the kit. Briefly, serum samples were diluted 1 : 8 in kit diluent and transferred into wells ( $100\ \mu\text{L}/\text{well}$ ) of the plates that are pre-coated with *T. solium* cyst-fluid antigen followed with blocking by bovine serum. Then, the plates were incubated at  $37^{\circ}\text{C}$  for half an hour and washed five times with washing buffer. Horseradish peroxidase (HRP)-labeled goat anti-human IgG (H + L) antibody ( $100\ \mu\text{L}/\text{well}$ ) was added and incubated at  $37^{\circ}\text{C}$  for half an hour followed by washing five times. After adding chromogenic substrate TMB (3,3',5,5'-tetramethylbenzidine) ( $50\ \mu\text{L}/\text{well}$ ), plates were incubated at  $37^{\circ}\text{C}$  for half an hour and the reactions were stopped with 2 M  $\text{H}_2\text{SO}_4$ . OD values were recorded at 450 nm with reference wavelength of 620 nm by the ELISA microplate reader (Clinibio 128C, ASYSHitch GmbH, Austria).

The serum sample of each study subject was assayed in triplicate. All control sera were run on each plate in duplicate. One negative control serum provided by the kit was diluted 1 : 8 while a standard reference serum prepared using pooled sera from ten cysticercosis patients was diluted serially 2-fold from 1 : 8 to 1 : 1024. Based on the ODs of standard reference sera on each ELISA plate, a standardization method I-STOD (improved-optical density standardization) was used to transform the OD value of all serum samples into standardized antibody concentration as arbitrary units for analysis of antibody level in the population [24]. All sera with OD values greater than 2.1 times of mean OD value of the negative control serum were defined as antibody-positive according to the kit instruction.

**2.1.4. Statistical Analysis.** All statistical analysis was performed using STATA 7.0 software. Because the distribution of specific antibody concentration in the study population was right skewed, natural logarithm transformation

TABLE 1: The prevalence of the four helminth infections in the study sample.

Category	Variable	Number of positives	Percent Positive (%)	95% CI* (%)
Helminth infected	<i>S. japonicum</i>	422	84.9	81.5–88.0
	<i>Ascaris</i>	353	71.0	66.8–75.0
	<i>Trichuris</i>	460	92.6	89.9–94.7
	Hookworm	259	52.1	47.6–56.6
Multiple helminth infections	None	2	0.4	0.5–1.5
	One	31	6.2	4.3–8.7
	Two	100	20.1	16.7–23.9
	Three	193	38.8	34.5–43.3
	Four	171	34.4	30.2–38.8

\* confidence interval.

( $\ln(\text{concentration} + 1)$ ) was performed for each concentration value before multivariate regression analysis. Multivariate linear regression analysis was used to detect the influence of sex, age, and coinfection status for four different helminths (*S. japonicum*, *A. lumbricoides*, *T. tricurua*, and hookworms categorized as Yes/No infection), on the antibody level (natural logarithm transformationed, ( $\ln(\text{concentration} + 1)$ ), specific to *T. solium* cyst-fluid antigen. Multivariate logistic regression analysis was performed to evaluate the relationship between sex, age, or different helminth infections (categorized as Yes/No infection) and *T. solium* seropositivity. Comparison of proportions between different groups was performed using chi-squared test.

### 3. Results

**3.1. Description of Study Population.** The study sample consisted of 497 individuals, of which, 310 were males and 187 were females. The mean age of this sample was 14.9 years with a standard deviation of 6.0 years. Most subjects were infected with one or more helminths. The prevalences of the four helminths infections determined by stool examination (*S. japonicum*, *A. lumbricoides*, *T. tricurua*, and hookworms) were all above 50%, and 93.4% of the subjects were infected with multiple helminths (Table 1). No *T. solium* carrier was detected using the coprological examination method.

#### 3.2. Seroprevalence of Cysticercosis in the Study Sample

**3.2.1. Distribution of *Taenia solium* Cyst-Fluid Antigen-Specific Antibody.** The distribution of *T. solium* cyst-fluid antigen-specific IgG (concentration relative to the standard reference serum) in the study population was right skewed ranging from 0 to 3.015 unit (Figure 1). In accordance with the cutoff defined by 2.1 times of mean I-ODST unit of the negative control serum, the cut-off value for positive antibody concentration was 0.045. In multivariate linear regression analyses, specific antibody level was not related with age or sex. No relationship between *T. solium* cyst-fluid antigen-specific antibody level and infection of *S.*

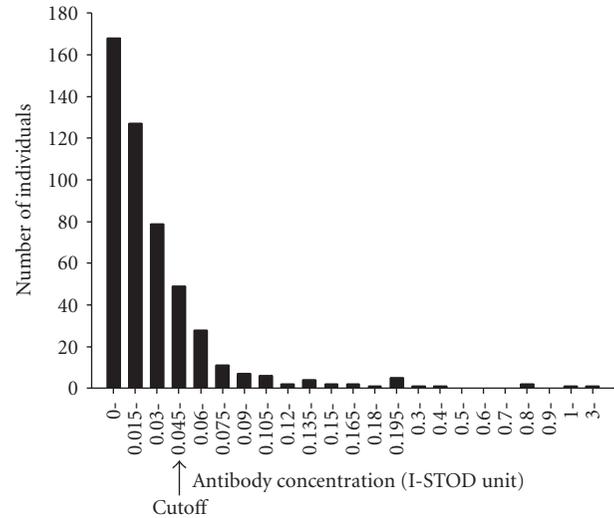


FIGURE 1: The distribution of *Taenia solium* cyst-fluid antigen-specific antibody concentration (I-STOD unit) in the study population. Y-axis: number of individuals; X-axis: antibody concentration by arbitrary I-STOD unit; arrow points to the positive cutoff value for seropositivity.

TABLE 2: Multivariate linear regression analysis between *Taenia solium* cyst-fluid antigen-specific antibody level and potential confounders.

Variable	Coefficient	P	95% CI
Age	-0.0007	.350	-0.0021–0.0007
Sex	-0.0042	.623	-0.0210–0.0126
<i>S. japonicum</i> infection	-0.0096	.402	-0.0321–0.0129
<i>Ascaris</i> infection	0.0058	.529	-0.0122–0.0238
<i>Trichuris</i> infection	-0.0364	.019	-0.0669–0.0060
Hookworm infection	-0.0065	.431	-0.0227–0.0097

*japonicum*, *A. lumbricoides*, or Hookworm was detected. *T. solium* cyst-fluid antigen-specific antibody levels were lower in individuals with *T. tricurua* infection (Trichuriasis) compared to individuals without Trichuriasis ( $P = .019$ ) (Table 2).

**3.2.2. Seroprevalence of Cysticercosis.** The overall seroprevalence of cysticercosis in this study sample was 24.6% (95% CI: 20.8% ~ 28.6%). Seroprevalence in different sex-age groups is shown in Figure 2. Seroprevalence was not related with sex, age, or infection with any of the four helminths species according to results of multivariate logistic regression analysis (Table 3).

### 4. Discussion

Cysticercosis is a long-standing problem in Latin America, a growing problem in Africa and Asia, and an emerging problem in developed countries [25]. A comprehensive estimate of the societal costs of *T. solium* cysticercosis for the Eastern Cape Province (ECP), South Africa, suggested that cysticercosis results in considerable monetary costs to

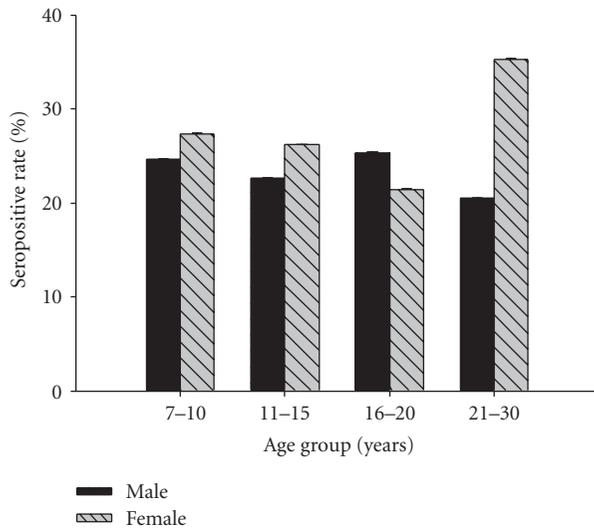


FIGURE 2: Seroprevalence of cysticercosis in different sex-age groups. The numbers of subjects in different groups are as follows. Male: age 7–10 ( $n = 77$ ), age 11–15 ( $n = 97$ ), age 16–20 ( $n = 63$ ), age 21–30 ( $n = 73$ ); female: age 7–10 ( $n = 62$ ), age 11–15 ( $n = 80$ ), age 16–20 ( $n = 28$ ), age 21–30 ( $n = 17$ ). No statistical difference was found for seroprevalence of all groups ( $P = .931$ ).

TABLE 3: Multivariate logistic regression analysis between seroprevalence of cysticercosis and potential confounders.

Variable	Coefficient	$P$	95% CI
Age group	−0.0273	.803	−0.2416–0.1870
Sex	0.1249	.578	−0.3149–0.5646
<i>S. japonicum</i> infection	−0.0103	.972	−0.5941–0.5735
<i>Ascaris</i> infection	−0.1446	.551	−0.6194–0.3302
<i>Trichuris</i> infection	−0.0504	.900	−0.8361–0.7353
Hookworm infection	−0.2831	.199	−0.7149–0.1486

a region that is already economically constrained [26]. A recent study on the disease burden of *T. solium* cysticercosis in Cameroon reported an average annual number of DALYs (Disability Adjusted Life Year) lost due to *T. solium* cysticercosis in West Cameroon amounting for 9.0 per thousand persons which is approximately 4 times higher than the same estimations already available for trypanosomiasis and schistosomiasis in sub-Saharan Africa [27].

In recent years, several epidemiological surveys of cysticercosis using both antigen and antibody detection methods have been carried out in endemic areas in Asia. These studies reported a seroprevalence of cysticercosis ranging from 2% to 48% in different districts in Indonesia, 5%–7% in Bac Ninh province of Vietnam, 0.02%–11.2% in different areas of China [16], and 3.2%–42.6% in Vellore district of south India [28].

In the Philippines, to date, there have been only 15 published cases of neurocysticercosis [20, 29, 30]. The prevalence of cysticercosis in pigs was 0.167% (8,276/4,956,422) based on direct examination of slaughtered pigs between 1970 and 1974 in twenty selected areas in the Philippines [16]. In 2005,

veterinarians at the National Abattoir stated that in Leyte, the Philippines, there were still *T. solium*-infected animals; however, no data were provided to support this claim [31].

To our knowledge, ours is the first community-based study examining the seroprevalence of human cysticercosis in the Philippines. The seroprevalence of cysticercosis in our study sample was 24.6%, although comparable with other surveys in Asia [16], the prevalence was considerably higher than expected, given the absence of documented reports of cysticercosis in this region. Even though antibody detection assays might overestimate current infection prevalence [18, 19], we believe that our data support cysticercosis as a significant public health problem consistent with the poor sanitation, and unpenned animal farming practices in the study site [21, 31]. Additional studies are necessary using other serology tests [2, 18] including antigen detection methods to confirm the high prevalence of cysticercosis in the study area, while neuroimaging (CT scan) of persons testing sero-positive with neurological problems would also be helpful to validate the serological findings.

We did not identify any subjects excreting *Taenia* eggs in stool by Kato-Katz exam. Given the high seroprevalence, this apparent paradox is in fact consistent with the epidemiology of transmission of *T. solium* [19, 32]. For example, 95% of Indian patients with NCC were vegetarians or did not admit to consume pork [16]. The episodic nature of egg excretion [1, 4, 32, 33], coupled with the low sensitivity of microscopic stool examination likely contributed to this discrepancy [4]. Future work should include more sensitive egg detection methods such as a coproantigen-based diagnosis test [33].

Clinically, schistosomiasis japonica has been a main concern in the study area and has been implicated in the neurological complications of the residents [34–37]. In 1950, a study reported a prevalence of cerebral schistosomiasis of 2% among the American soldiers ( $n = 600$ ) who were infected with *S. japonicum* from 1944 to 1945 in Leyte [38], supporting the estimate that cerebral involvement in schistosomiasis occurs in 2% to 5% of infected individuals [39]. The high prevalences of schistosomiasis and neuroschistosomiasis, coupled with a report suggesting that *T. saginata* infections are more common than *T. solium* infections in humans in the Philippines [16, 21], have contributed to the underappreciation of *T. solium* as a potential cause of neurological complications in the study area. The overlapping clinical features of cerebral schistosomiasis japonica [40] and NCC, together with the high seropositive proportion of cysticercosis in our study sample suggest that cysticercosis should remain in the differential diagnosis of seizure disorders in this region.

While our data supports an unexpectedly high estimate of cysticercosis prevalence in the study population, several study limitations merit discussion. First, our study population has a higher prevalence of *S. japonicum* infection compared to the general community. This sampling approach would lead to overestimating the community prevalence of cysticercosis if there are common environmental, genetic, or immunologic factors that predispose to both infections. We believe that our results are generalizable to non-S.

*japonicum* infected populations because we did not detect a significantly different seroprevalence of cysticercosis in the *S. japonicum* infected (24.2%, 102/422) compared to the *S. japonicum* uninfected (26.7%, 20/75) individuals ( $P = .643$ ).

Second, the immunodiagnostic method employed in our study is based on indirect ELISA to detect specific IgG using *T. solium* cyst-fluid antigen and may be susceptible to cross-reactivity to schistosome antibodies. This kit was used in the national epidemiological survey for cysticercosis during 2001–2003 in China [41]. Besides its documented sensitivity (92.5%) and specificity (100%), a later study reported a positive rate of 78% in 70 patients with cysticercosis using this kit [42]. These test characteristics support the validity of the seroprevalence data obtained in our study sample. Currently, there are no commercially available antibody detection kits for serodiagnosis of cysticercosis that have been specifically validated for false-positivity in *S. japonicum* infected individuals. We do not think that false-positive test results due to coinfections with other helminths contributed to our high seroprevalence because the seroprevalence for cysticercosis did not differ in individuals with and without these coinfections (see Tables 2 and 3).

Third, anticysticercal antibody may persist long after the parasite has been eliminated by either immune mechanisms or drug therapy [43]; and antibody may reflect exposure to parasite antigens rather than actual infection [44]. Thus, the detection method applied in the study could contribute to overestimation of the prevalence of active cysticercosis.

Fourth, the age range of our study sample was restricted to 7–30 years, which may have decreased our ability to detect a relationship between cysticercosis seropositivity and host age (Figure 2, Tables 2 and 3) as Fleury et al. observed [45].

Despite the limitations of antibody detection using cyst-fluid antigen, a high community seroprevalence for cysticercosis identifies a “hot spot” where preventive and control measures should be targeted. The high seroprevalence of human cysticercosis in the present study sample suggests that cysticercosis may be a significant, yet unrecognized problem in the Philippines. These pilot data in a selected sample underscore the need for community and school based surveys of cysticercosis as well as studies identifying risk factors for cysticercosis in rural areas of the Philippines. In addition, our results support a reexamination of pigs as a sentinel indicator for human cysticercosis in the same area [46].

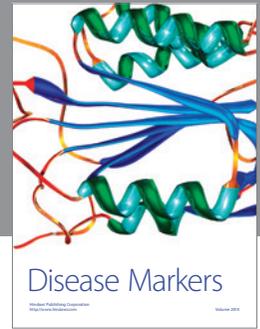
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