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Relative seroprevalence of cysticercus antigens and antibodies and antibodies to *Taenia* ova in a population sample in south India suggests immunity against neurocysticercosis

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ABSTRACT

We evaluated the exposure of a community in Vellore district of south India to *Taenia solium* infection and its relationship to the prevalence of neurocysticercosis (NCC) causing active epilepsy. Seroprevalence of *Taenia* cysticercus antigens and antibodies were determined in 1064 randomly chosen asymptomatic individuals, antibodies to *T. solium* ova in 197 selected sera, and prevalence of taeniasis by a coproantigen test in 729 stool samples. The prevalence of NCC causing active epilepsy in Vellore district was determined in a population of 50 617. Coproantigens were detected in 0.8% (6 samples), *Taenia* cysticercus antigens in 4.5% (48 sera) and cysticercus IgG antibodies in 15.9% (169 sera) of the population. Cysticercus antibodies were directed against relatively low molecular weight cyst glycoprotein antigens in 14.9% (158 sera) of the population. IgG antibodies to *Taenia* ova were found in 81 (41.1%) of the selected samples. Prevalence of NCC causing active epilepsy was 1.3 per 1000 population. These results show high exposure of the population to the parasite and a relatively high prevalence of active infections (4.5% antigen positives) but a low prevalence of NCC causing active epilepsy (0.13%). These findings may indicate that the population is protected against developing neurocysticercosis. IgG antibodies directed against *Taenia* ova and low molecular weight cyst antigens may contribute to protection.

1. Introduction

The helminth cestode *Taenia solium* causes two forms of infections in humans: taeniasis and cysticercosis. Taeniasis is an infection of the intestine with the adult tapeworm and is acquired by ingestion of pork containing viable *T. solium* larvae. The adult tapeworm possesses 700–1000 proglottids of which the mature distal gravid proglottids detach from the worm either singly or in groups of 2–5 segments and pass into the faeces. A proglottid contains approximately 40 000 eggs and it is estimated that an adult worm can release up to 300 000 eggs into the environment every day.[1] Patients with taeniasis are, therefore, the source of *Taenia* ova in the environment. Poor sanitary facilities, open-air defecation, free-ranging pigs that feed on human faeces, and pork consumption are factors that sustain taeniasis. In these conditions high levels of *Taenia* eggs

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are maintained in the environment over long periods, even though a very small percentage of the population is infected with a tapeworm.[2]

Cysticercosis is an infection that is caused by *T. solium* metacestode larvae. Humans get cysticercosis by ingesting *Taenia* eggs from the environment. The eggs hatch in the intestine to release oncospheres, which actively migrate through the intestinal wall and are transported by the blood stream to different organs. Clinically important *T. solium* infections are those of the brain (neurocysticercosis [NCC]) and eye.[2,3]

In southern India, NCC was found to be the cause of active epilepsy (AE) in at least 0.13% of the population between 2 and 60 years old.[4] NCC accounted for one-third of all causes of AE in this population. In the population free of seizures, 16% were seropositive for cysticercus antibodies to infection specific *T. solium* cyst antigens.[5] This indicated a high exposure of the population to *Taenia* eggs. It also suggested that most people who were infected with *T. solium* ova did not develop NCC. In settings of chronic exposure to high levels of *Taenia* eggs, Fleury et al.[6] suggest that a sizable proportion of the population acquires protective immunity to the infection. In an endemic rural Mexican community, they found that only 9.1% of the population had NCC as determined by a positive CT scan although 44% of the population were seropositive for cysticercus antibodies. They attributed the low infection rate to protection by acquired immunity.

In this study, we determined the prevalence of taeniasis and seroprevalence of *Taenia* cyst antigens and of antibodies to ova and cysts of an asymptomatic population sample from south India. We also determined the antigens against which cysticercus antibodies were raised. Based on our findings we speculate on the relationship between exposure to the parasite and development of NCC amongst members of the community.

2. Materials and methods

2.1. Population sample

This study was carried out in Vellore district (population 3.5 million) in the south Indian state of Tamilnadu (population 62 million). Free-range pig rearing, consumption of pork from the non-regulated market and poor sanitation are prevalent in the district. A population of 50 617 from Vellore district was screened for AE and all detected cases were investigated for NCC.[4] A representative sample of the population, 1600 people without history of seizures, was randomly selected for study of *Taenia* serology. Twenty-five percent of this population were from urban Vellore and 75% from an adjacent rural population in Kaniyambadi Block.[4] The urban and rural populations of Vellore district intermingle freely due to employment and commerce. Populations residing in pig-rearing areas or who were involved in pig rearing were noted.

Blood from 1064 consenting individuals, between the ages of 2 and 60 years, was collected between March 2004 and February 2005, and the serum stored at −20 °C until assay.[5] Early morning stool samples were collected from 729 individuals and processed for assay of coproantigens.

2.2. Taenia serology – assay of antigens

Circulating *T. solium* metacestode antigens were assayed in all sera by an ELISA using monoclonal antibodies to excretory secretory products of *T. saginata* metacestodes.[7] The ELISA was 94% sensitive for cysticercosis with no cross reactivity with sera of other parasitic infections.[8]

2.3. Taenia serology – assay of antibodies to cyst and ova

Cysticercus IgG antibodies against lentil lectin-specific *T. solium* cyst glycoproteins were detected on immunoblots.[9] Sera were considered positive for cysticercus IgG antibodies on reaction to one or more of seven *T. solium* cyst glycoproteins of molecular weights 50, 38–42, 24, 21, 18, 14 and 13 kDa.[10] These antigens are considered to be infection specific and their antibodies diagnostic for cysticercosis.

IgG antibodies to *T. solium* ova were determined in 197 sera on immune blots. Briefly, *T. solium* ova were obtained by thawing frozen gravid segments and aspirating the released fluid. The fluid was spun at 503 g for 5 min and the presence of *Taenia* eggs in the supernatant was confirmed by microscopy. The supernatant fluid, containing 1.125×10^8 whole and broken ova/ml, was adjusted to 1 mM phenylmethylsulfonyl fluoride in PBS, homogenized in a homogenizer with seven up and down strokes/min for 3 min, spun at 12 000 g for 10 min and the supernatant used as enriched extract of *Taenia* ova antigens.

Taenia ova antigens, 8 μg protein/mm gel, were subjected to SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) (4–20%, 1 mm thickness) and electrotransferred to polyvinylidene fluoride membranes. The blots were probed with sera (1:100 diluted), followed by rabbit anti-human IgG-HRP and developed as given for the immune blots in the determination of cysticercus antibodies. Sera were considered positive for antibodies to *Taenia* ova antigens on appearance of any immune bands irrespective of the molecular weight of the antigen. Sera from 25 healthy laboratory staff, obtained with consent, served as negative controls for antibodies to *Taenia* ova antigens.

2.4. Taeniasis – assay of coproantigens

Stool samples were received in the laboratory within 6 h of collection and kept at 4 °C until processed. Samples were extracted with two volumes of 0.15 M PBS pH 7.2 + 0.3% Tween 20 + 5% formalin and extracts stored at 4 °C and assayed for coproantigens within 24 h. Coproantigens were assayed by a capture ELISA using rabbit antibodies to *T. solium* somatic antigens by the method of Allan et al.[11] A sample was considered positive for coproantigens when the absorption of the sample was 1.6-fold greater than the mean absorbance at 490 nm + 3 SD of six negative controls assayed on the same microtitre plate.

Table 1
Seroprevalence of *Taenia* cyst antigens and antibodies of the study population living in Vellore district, India

	Population									
	All						Pig rearing areas		Non-pig rearing areas	
	Total		Urban		Rural		Rural		Rural	
Number	1064		167		897		569		324	
	n	%	*n*	%	*n*	%		%	*n*	%
Antigen positive	48	4.51	2	1.19[a,c]	46	5.12[a]	38	6.7[b,c]	8	2.5[b]
Antibody positive	169	15.9	10	6.0[d,e]	159	17.7[d]	103	18.1	55	17.0[e]

[a] $P = 0.023$ comparing antigen positivity between urban and rural populations.
[b] $P = 0.006$ comparing antigen positivity between rural populations residing in pig rearing and non-pig rearing areas.
[c] $P = 0.003$ comparing antigen positivity between the rural population residing in pig rearing areas and urban population residing in non-pig rearing areas.
[d] $P < 0.001$ comparing antibody positivity between urban and rural populations.
[e] $P < 0.001$ comparing antibody positivity between urban and rural populations living in non-pig rearing areas.
Information on area of residence was not available for 4 individuals of the rural population.

Saline smears of all stool samples were subject to microscopic examination for the presence of parasites.

2.5. Clinical significance of the tests

The clinical significance of each of the assays is as follows:

- Cysticercus antigens in serum (using ELISA): presence of these antigens denotes active infection by the cysticercus (cysticercosis).
- Cysticercus IgG antibodies to one or more of seven infection specific cyst glycoprotein antigens in serum (using immune blots): presence of these antibodies denotes exposure to the cysticercus antigens. This occurs when *Taenia* ova are ingested and evolve into the larval form (cysticercus) in the tissues of the body such as the subcutaneous tissues, muscles, CNS or eyes. Presence of these antibodies does not always denote active or ongoing infection as the antibodies could be persisting from an infection that has resolved.
- Antibodies to *Taenia* ova in serum: presence of these antibodies denotes the entry of the *Taenia* ova into the blood stream of the host but does not indicate establishment of disease by the larval form (cysticercus) either current or remote.
- Coproantigen in stools: This test detects the presence of adult (*Taenia*) antigens in the stools. Presence of coproantigen indicates the presence of an adult *Taenia* worm in the gut (taeniasis) of the person.

2.6. Statistical analysis

Fisher's exact test was applied to determine differences between populations. Differences were considered significant at $P < 0.05$.

3. Results

The population who consented to provide blood for the study were 84.3% (897/1064) rural and 15.7% (167/1064) urban (Table 1).[5] Two-thirds of the rural population (63.4%, 569/897) resided in regions where pig rearing was prevalent (Table 1). The urban population did not rear pigs.

3.1. Seroprevalence of Taenia cyst antigens

Taenia cyst antigens were detected in 4.5% (48/1064) of the total population, with significantly higher levels in the rural compared to the urban population ($P = 0.023$) (Table 1). Cyst antigens were present in a significantly higher number of the rural population residing where pig rearing was prevalent (6.7%, 38/569) compared with those who lived where pig rearing did not occur (2.5%, 8/324) ($P = 0.006$) or the urban population who did not rear pigs (1.2%, 2/167) ($P = 0.003$) (Table 1).

Only five people positive for cyst antigens were also positive for cysticercus antibodies. In light infections antigens are more sensitively detected than antibodies,[12] which may be one reason that antibodies were not detected in the majority of infected people in an asymptomatic population.

3.2. Seroprevalence of antibodies to Taenia solium cyst antigens

IgG antibodies to infection specific cyst antigens were detected in 15.9% (169/1064) of the total population with significantly higher levels in the rural compared to the urban population ($P < 0.001$) (Table 1).[5] Cysticercus antibody levels did not differ between rural populations who lived in pig rearing or non-pig rearing areas (Table 1).

Cysticercus antibody levels were significantly higher in the rural compared to the urban population who lived in non-pig rearing areas (Table 1).

Stratified for age seroprevalence of cysticercus antibodies in the population was 11.9% (26/219), 17.4% (113/648) and 14.8% (28/189) in the age groups 6–15, 16–45 and 46–60 years respectively.[5]

Antibodies to only the 13 or 14 or 18 kDa antigens or a combination of the 13, 14 and 18 kDa cyst glycoproteins with the other cyst antigens were present in 14.9% (158/1064) of the total population (Figure 1). Antibodies to only the high molecular weight cyst glycoproteins, 50 kDa or 38 kDa or 24 kDa or 21 kDa, were noted in 1.0% (11/1064) of the population (Figure 1).

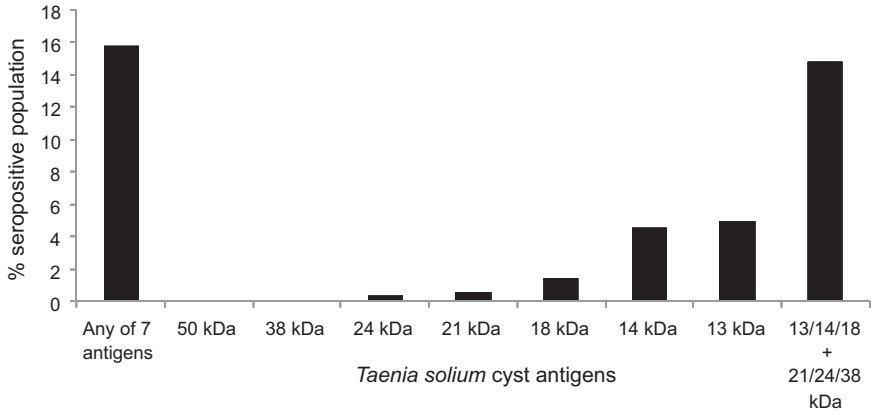

Figure 1. Seroprevalence of IgG antibodies to *Taenia solium* infection specific cyst antigens determined on immune blots in the study population of Vellore district, India.

Among all sera, five from the rural population were seropositive for both *Taenia* antigens and antibodies. Antibodies to 13 and/or 14 and/or 18 kDa cyst antigens were detected in all five sera.

3.3. Antibodies to Taenia ova antigens

Antibodies to *Taenia* ova antigens were analyzed in 197 sera of which 136 (69.0%) were negative and 61 (31.0%) were positive for antibodies to *T. solium* cyst glycoproteins. Antibodies to the enriched *T. solium* ova antigen extract were detected in 81/197 (41.1%) of the sera and were directed against proteins of 22–52 kDa. None of the 25 negative control sera exhibited antibodies to the ova antigens. IgG antibodies to *T. solium* ova were detected in 50/136 (36.8%) sera negative and in 31/61 (50.8%) sera positive for antibodies to *T. solium* cyst glycoproteins. Representative immune blots of sera positive and negative for antibodies to *Taenia* ova antigens are shown in Figure 2.

Sera of all individuals with intestinal parasites, determined by stool microscopy, were among the 197 sera tested

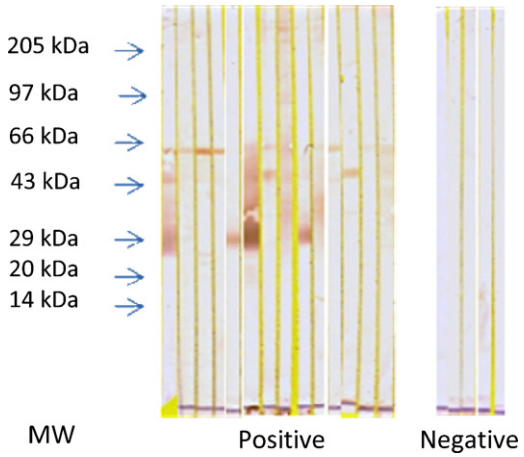

Figure 2. Representative immune blots of sera positive and negative for antibodies to *Taenia* ova in the study population of Vellore district, India. Serum IgG antibodies to *Taenia* ova antigens were directed against proteins of 22–52 kDa. Negative control sera did not exhibit antibodies to the ova antigens.

for antibodies to *Taenia* ova antigens. Antibodies to *Taenia* ova antigens were detected in 13 of 45 people with intestinal parasites (*Giardia*, hookworm and *Ascaris*) but not in those with *Hymenolepis nana* infection. If antibodies to *Taenia* ova antigens detected in persons with these non-cestode intestinal parasitic infections are considered cross-reactive and non-specific to *Taenia*, *Taenia* ova antibodies would be present in 34.5% (68/197) and not 41.1% of the community. This remains a high prevalence.

3.4. Coproantigen assay and stool microscopy

All stool samples were from the rural population, 62% (452/729) (from pig rearing areas and 38% (277/729) from non-pig rearing areas. Coproantigens, determined by the coproantigen ELISA, were detected in 0.8% (6/729 samples) of the rural population, with a prevalence of 1.1% (5 samples) in the population from pig rearing areas and 0.36% (1 sample) in the population from non-pig rearing areas. Of the six coproantigen positive people none were positive for cysticercus antibodies and one was positive for cyst antigens.

Intestinal parasites from stool microscopic examination were detected in 6.1% (45/729 samples) of the population, with *Giardia* in 2.7% (20 samples), hookworm in 1.9% (14 samples) and *Hymenolepis nana* in 1.1% (8 samples) of the population. *Ascaris*, *Trichuris* and *Strongyloides* were seen in 0.5% (4 stool samples each).

Coproantigen positive individuals were informed of the test results and advised taenicid treatment. All positive individuals agreed to be treated. They were admitted to hospital, administered 2 grams niclosamide with a laxative, and stools collected for 24 h. Neither scolex nor segments were recovered in any purge. Stool samples collected from each individual within two weeks of treatment were negative for coproantigens.

4. Discussion

NCC is the most common parasitic infection of the CNS and a common cause of recent onset seizures in many countries of South America, sub-Saharan Africa and Asia.

[2] In India, NCC is the cause of a third to half of AE,[13-15] which indicates the magnitude of the disease in the country. The results of our study support this significant exposure of the population in India to *Taenia* infections but also suggest chronic exposure to infectious *Taenia* forms may protect the population against NCC.

In our study a prevalence of 0.8% taeniasis, in a region with poor sanitary facilities and open-air defecation, suggested widespread exposure of the population to *Taenia* ova. Antibodies to *Taenia* ova antigens in 41% of the population are an indication of the pervasive spread of infectious *Taenia* forms in the environment. Coproantigen data indicated that populations residing in pig rearing areas were more than 3-fold exposed to *Taenia* ova than those living in non-pig rearing areas. High exposure to *Taenia* ova was also reflected in the high level of active infection in the population, 4.5% as estimated by the Ag-ELISA. The infection was higher in areas with higher prevalence of taeniasis.

Widespread environmental contamination and exposure to *Taenia* oncospheres were also evident in the high percentage (16%) of the population seropositive for antibodies to infection specific cyst antigens. Cysticercus antibody levels however, did not change as the prevalence of taeniasis changed. The high prevalence of cysticercus antibodies (>11%) across ages (6–60 years) supports constant and chronic exposure of the population to infectious *Taenia* forms.

Although 22.8% of the rural population and only 7.2% of the urban population manifested markers of *Taenia* infection (antigen and antibodies), the prevalence of NCC in both populations determined at the time of the serological studies was similar, 1.28 and 1.02 per 1000 people respectively.[4] Development of disease (NCC) did not appear related to magnitude of exposure. Similar findings have been made by Fleury et al.[16] who showed that the proportion of severe cases of NCC were comparable between populations of high or low exposure to *Taenia*.

Our studies showed that while over 41% of the population were exposed to *Taenia* ova and 20% exhibited markers of *Taenia* cyst infection (i.e. presence of cyst antigen or antibodies to infection specific cyst antigens) only 0.13% developed NCC with AE. The findings are similar in other regions endemic for *T. solium* infections. In Ecuador, Rodriguez-Hidalgo et al.[17] found that despite high exposure to the parasite active infection in the population was low. This was presumed to reflect endemic stability. In Peru and Colombia, it was found that up to 50% of cysticercus seropositive individuals did not develop infection or had cysticercosis that resolved spontaneously.[18] *Taenia* epidemiology portrays high exposure but low infection, which is taken to indicate the low efficiency of *Taenia* ova to establish cysticercus infection. Low infectivity of *Taenia* ova has been demonstrated in pigs, where only about 1% of ingested eggs lead to an infection.[19]

From the observations in our study, we subscribe to the suggestion of Fleury et al.[6,16] that high exposure to *Taenia* but low levels of infection may also indicate acquired immunity that protects the population against NCC. In animals, acquired, protective immunity to *Taenia* infections has been demonstrated through ingestion of eggs and by immunization. Pigs, sheep and mice immunized with oncosphere proteins were protected against infection on re-challenge with *Taenia* ova.[20–23] This protective immunity was IgG antibody and complement mediated and was most effective against the infecting oncospheres in the early stages of disease, before immune evasion mechanisms of the parasite were activated and infection established.[24] The immunity wanes with time after exposure to oncospheres, but is maintained when exposure is continuous, as in tapeworm carriers.[25]

Molinari et al.[26] have demonstrated in vitro that the viability and mobility of *T. solium* oncospheres treated with sera of NCC patients plus complement are significantly reduced, suggesting cysticercus antibodies may protect their hosts against re-infection. We suggest that constant exposure to low levels of *Taenia* ova induce and maintain IgG anti-oncosphere antibodies in the population of Vellore district. This acquired immunity may protect the population against NCC by killing the infecting larvae soon after infection and preventing the establishment of *T. solium* cyst infections.

Cross-immunity between *Taenia* species may also protect the population against infection.[27] Sheep exposed to, or immunized with, *T. hydatigena* eggs resisted infection with *T. ovis* eggs and more effectively than animals immunized with *T. ovis* eggs to prevent infection with *T. hydatigena*.[27] Cross-immunity of *Taenia* species that infect humans with other *Taenia* species in the environment may similarly moderate infections and protect the population.

Taenia species exhibit stage specific, host protective antigens. In addition to protective oncospheric antigens, metacestode antigens have been described as protective immunogens in animals. Mice immunized with metacestode antigens and challenged with *T. taeniaeformis* eggs, developed cysts that exhibited early degeneration.[28] Pigs immunized with metacestode antigens showed significant decrease in cysticercosis a year later and on reinfection they exhibited metacestodes in varying stages of degeneration compared to pigs that had not been immunized.[29] It is possible that this situation exists in humans as well as there is similarity of Taeniid immunity across species. We suggest that since a significant proportion (16%) of the population of Vellore district were healthy but seropositive for infection specific cyst antibodies, they may reflect an immunized population capable of clearing larva and cysts in early stages of development. This immunity would contribute to preventing infection.

Successful vaccines against cysticercosis in pigs and sheep have used oncosphere glycoproteins of molecular weight 52, 47, 18 and 16 kDa as immunogens.[30] The low molecular weight 18 kDa oncosphere glycoprotein, in particular, is considered an efficient protective immunogen.[31] In our study, antibodies to cyst glycoprotein antigens of low molecular weights, 13, 14, 18 kDa, predominated in a population that was healthy and free of NCC but living in a *Taenia* endemic region. In pigs these antibodies appear early in infection with *T. solium*, while antibodies to higher molecular weight cyst antigens 42 and 50 kDa appear late in infection.[32] In the population under study, the detection of the antibodies to low molecular weight cyst antigens reflected ingestion of *Taenia* ova that did not lead to disease. The absence of antibodies to 42 and 50 kDa glycoprotein

cyst antigens in the majority of the seropositive population may also indicate absence of infection. Antibodies to *Taenia* ova are considered the most effective protective antibodies against establishment of infection. The absence of disease in the absence of antibodies to ova may support a role for antibodies to low molecular weight cyst glycoproteins in protecting the population against NCC.

In summary, these studies show widespread exposure of the population of Vellore district to *T. solium,* who through constant exposure to low levels of infective *Taenia* ova may acquire protective, antibody-mediated immunity to NCC. Antibodies to oncosphere proteins and low molecular weight cyst glycoproteins may form the armamentarium of this protective immunity.

Authors' contributions: TJ, VR, PD, JM and AO contributed to the design of the study; TJ, PB and VP performed laboratory studies; MVR carried out community studies. All authors analyzed the data, drafted the manuscript, and read and approved the final version. VR, PD and AO are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: The study was approved by the Institutional Review Board of the Christian Medical College, Vellore, India.

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