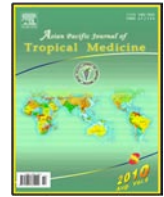


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## Document heading

## Seroprevalence of cysticercosis in North Indian population

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## ABSTRACT

**Objective:** To estimate the seroprevalence, morbidity of *Taenia solium* (*T. solium*) cysticercosis and its relationship to socio-economic, sanitary parameters. **Methods:** Using multistage stratified random sampling, 2 500 subjects from urban ( $n=1\ 250$ ) and rural population ( $n=1\ 250$ ) of Lucknow, India were registered. Blood, stool samples, socioeconomic and demographic data were collected. Serum enzyme-linked-immunosorbent-assay (ELISA) was used to detect anticysticercus IgG and IgM antibodies. Microscopic examination of stool samples after processing by concentration method was done to observe taeniasis and other intestinal parasites. CT scan of seropositive cases presenting with seizures was done for confirmation of neurocysticercosis. **Results:** The overall, urban and rural seroprevalence of *T. solium* cysticercosis was 3.48%, 4.64% and 2.32%, respectively. The risk factors significantly associated with the disease were pig rearing in both study populations, unsanitary waste disposal in urban, vegetarian diet and open defecation in rural population. One case of intestinal taeniasis was observed. Twenty-six of 30 cases undergoing CT scan were diagnosed as neurocysticercosis. **Conclusions:** Seroprevalence of cysticercosis is high in the study community. Prevalence of cysticercosis is related to roaming pigs and behavioral and environmental practices in local community. Health education and identification of tapeworm carriers seems promising control strategy.

## 1. Introduction

Cysticercosis is a frequent infection in developing countries where pigs are raised within the human habitat[1]. Cysticercus is the larval tissue stage of the pork tapeworm *Taenia solium* (*T. solium*). When humans consume uncooked measily pork, the larva attaches to the gut wall and grows into the intestinal tapeworm. The life cycle is maintained when pigs eat food contaminated with human feces containing tapeworm eggs and develop porcine cysticercosis, a process facilitated by husbandry practices in villages where pigs are free roaming scavengers and human fecal contamination is widespread. Human cysticercosis is defined as presence of *T. solium* larvae in human tissues and may occur due to fecal contamination

of water, food or through self-infection. Although cysticercosis may localize throughout the body, most clinical manifestations are related to their presence in the central nervous system called neurocysticercosis. The cysts can invade parenchyma, the subarachnoid spaces, and the ventricular system, causing seizures, hydrocephalus, and other neurologic dysfunction.

Currently, the best clinical procedures for diagnosing neurocysticercosis are neuroimaging studies including CT scan and MRI. However, these are impractical for population based screening for cysticercosis. Immunoserologic assays, such as enzyme-linked immunoelectrotransfer blot assay (EITB) or enzyme-linked immunosorbent assay (ELISA), detect antibodies against *T. solium* cysticerci. Consequently, they are useful in identifying a population at risk of contact with the parasite[2].

Cysticercosis is potentially eradicable through surveillance and human interventions for which community data of prevalence and risk factors of cysticercosis is crucial. Community based data is not available for India, although a large number of hospital-based studies have reported a high

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incidence of neurocysticercosis in patients of epilepsy<sup>[3]</sup>. In the current study we have estimated the community prevalence of cysticercosis in urban and rural Lucknow, North India using ELISA for detection of anticysticercus IgG and IgM antibodies. CT scan, stool examination were used as additional techniques to find the proportion of neurocysticercosis cases in the seropositive cases and rule out any cross reactivity of ELISA with intestinal taeniasis or other parasites.

## 2. Material & methods

### 2.1. Study design and participants

In urban of Alambagh and rural of Mati, cross-sectional study design was undertaken to study the rate of exposure to cysticercosis in the population in relation to sanitary and socioeconomic parameters and its morbidity. A multistage stratified random sampling approach was adopted. Door to door survey was done (from January 6 to May 4), and households were contacted. A total of 2 500 subjects from 1340 families were randomly registered. Institutional Ethics Committee approved research work and all the norms of Helsinki code were followed during the conduct of the study<sup>[4]</sup>.

### 2.2. Sample size calculation

Sample size calculation was done based on a suspected cysticercosis prevalence of 4% with a probable error of 20% on either side.

$N = 4 \times p \times q / L^2$ , where  $p$  = assumed prevalence,  $q = 1 - p$ ,  $L$  = allowable error (% of  $p$ ). In our case  $L = 20\%$  of  $4 = 0.8$ , and  $N = 4 \times 4 \times 96 / 0.82 = 2 400$ .

### 2.3. Assessment

After prior consent of individuals in case of adults and parents in case of minor, the indexed cases were subjected to the following measures:

#### 2.3.1. Questionnaire

A pre-designed questionnaire was used to retrieve information regarding socio-demographic status, source of drinking water, food habits, sanitation, main occupation of each participating household. Information on personal hygiene, dietary habits, consumption of pork, defecation practices, occupational hazards, and symptoms relevant to cysticercosis were recorded.

#### 2.3.2. Clinical assessment

Study group was submitted to detailed clinical evaluation with emphasis on history of epilepsy, ocular disturbances, and evidence of raised intracranial tension, subcutaneous or muscular nodules.

#### 2.3.3. Stool examination

Stool examination was undertaken in the study to rule

out any cross reaction in ELISA, due to intestinal taeniasis, or other parasites commonly known to cross react. Stool samples collected from the population were processed by concentration method. Initially macroscopic examination was performed for visible worms or *Taenia* segments and then formalin was added to preserve the samples for further analysis by wet mount examination.

### 2.3.4. Serology

Serum collected from the study population was evaluated for the presence of anticysticercus antibodies by an in-house experimental ELISA with anti human IgG and IgM antibodies as detailed by Husain *et al*<sup>[5]</sup>. ELISA used has a diagnostic sensitivity and specificity of 93.5% and 84.2%<sup>[5]</sup> while it is reported to be 100.0% sensitive and 71.9% specific in follow up detection of neurocysticercosis<sup>[6]</sup>. Pooled control positive and negative sera were run in each batch. Samples were run in triplicate and mean optical density (OD) was tested. The ODs were converted to titers for easy comparison in ELISA Units (EU) as per our standardized laboratory practice using the formula:

$$\text{Titer in EU} = \frac{\text{Sample OD} - \text{Negative control (NC) OD} \times 100}{\text{Positive control OD} - \text{NC OD}}$$

Titer of more than 50 EU was considered positive. Variation in intra-sample OD of <10% was acceptable. In case of discrepancy in readings the test for the sample was repeated. Twenty five percent of the samples were run twice to check to reproducibility of the test, which was found to be 99.6%.

### 2.3.5. CT scan

To find the proportion of morbidity due to neurocysticercosis, CT scan of seropositive cases presenting with seizures was done. All 38 seropositive cases presenting with epilepsy were called for the CT scan. However, 5 refused to give consent and 3 cases were lost to follow-up. Hence, CT scan was done in 30 cases (15 urban and 15 rural).

## 2.4. Statistical analysis

Differences in distribution of categorical variables were analyzed using  $\chi^2$  test. All significance in tests were two-tailed, and a  $P$  value of <0.05 was considered significant.

## 3. Results

### 3.1. Population characteristics

Twenty five hundred study subjects from rural community of Mati and urban community of Alambagh (1 250 from Mati and 1 250 from Alambagh) were randomly selected. Mean age of subjects was (24.79±16.43) years old ranging from 1.5 years to 85 years. The study group comprised of 1 142 males and 1 358 females from 1 340 families. Of the total registered subjects 51.20% (640/1 250) of urban and 50.90% (636/1 250) of rural population were vegetarian.

Samples from urban of Alambagh had a mean age of (23.54 ±14.96) years old with 690 females and 560 males, from 718 families. Out of these 914 were Hindus, 88 Muslims, 154

belonged to Scheduled Castes (SC), Scheduled Tribes (ST) and Backward Class (OBC), and 94 were Punjabi Sikhs. Pork eaters comprised 1.24% of the subjects. Water supply was derived in majority of subjects from tap ( $n=153$ ), hand-pumps ( $n=61$ ) and tube-well ( $n=36$ ). Three hundred and ten of 1250 subjects indulged in sanitary disposal of sewage while 940 had indiscriminate ways of disposal. Heaps of domestic waste were seen in parks or open spaces being scattered by the animals at the end of colonies or clusters of houses. Occasionally local municipal authorities removed this waste.

Samples from rural in Mati had mean age of  $(26.18 \pm 17.83)$  years old comprising 668 females and 582 males from 622 families. The group included 988 Hindus, 52 Muslims, 166 SC, ST, OBC and 4 Punjabi Sikhs. Pork eaters comprised 4.24% (53/1250) of the subjects. An indiscriminate mode of sewage disposal was evident in 1233 subjects while only 17 practiced a sanitary mode. The waste was dumped outside every house or backyard giving free access to animals. However dry waste was burnt in some houses. Drinking water was consumed from hand-pumps only.

Sanitation measures were not followed at both places. Washing hands properly before food and after defecation was not strictly followed. Washing of raw vegetables or fruits twice before consumption was generally considered sufficient. Washing leafy vegetables with alkaline potassium permanganate solution to eliminate *Taenia* eggs was not known to any of the subjects included in the study.

### 3.2. ELISA

Anticysticercus IgG and IgM antibodies were detected using two sets of ELISA in serum of all cases registered in the study. Presence of antibodies of either IgG or IgM type was taken as positive. Overall 87 cases of 2500 tested positive in the population. Mean titer of positive samples in IgG was  $(114.38 \pm 56.56)$  EU (87/2500), and was  $(108.96 \pm 53.16)$  EU (86/2500) in IgM; whereas mean titer of negative cases in IgG was  $(16.59 \pm 5.87)$  EU (2413/2500) and  $(15.20 \pm 4.52)$  EU (2414/2500) in IgM. Fifty-two cases had both ELISA's positive. It was interesting to note that the relatives of the cases ( $n=46$ ) with a positive ELISA had elevated titers though not above the cut-off level denoting possible prior exposure. Of the 1340 families studied, multiple cases of cysticercosis ( $n=25$ ) were diagnosed in 8 families.

Urban population had 58 cases of IgG and 52 cases of IgM ELISA positive. Mean IgG titer of positive cases was  $(120.50 \pm 48.20)$  EU and of negative cases was  $(14.59 \pm 8.40)$  EU.

Mean IgM titer of positive cases was  $(103.20 \pm 52.10)$  EU and of negative cases was  $(13.62 \pm 6.8)$  EU. Rural population had 29 cases of IgG and 34 cases of IgM ELISA positive. Mean IgG titer of positive cases was  $(108.26 \pm 34.67)$  EU and of IgM was  $(114.72 \pm 34.6)$  EU. While IgG titer of negative cases was  $(18.59 \pm 6.98)$  EU and of IgM was  $(16.78 \pm 8.43)$  EU.

### 3.3. Seroprevalence of cysticercosis

Overall seroprevalence of cysticercosis was found to be 3.48% in the population. Seroprevalence in urban population was 4.64% and was 2.32% in rural population. No predilection for any sex was observed. While 52.80% were males out of 87 IgG seropositive cases, 45.00% were males in 86 cases who tested positive in IgM ELISA.

The risk factors for the disease were evaluated in terms

of a questionnaire and direct observation at site. Table 1 depicted the incidence of risk factors in the seropositive cases in the urban and rural populations. Pig rearing was significantly associated to the infection in both populations ( $P < 0.001$ ). Indiscriminate waste disposal was found to be significantly associated in the urban population ( $P < 0.05$ ) while in the rural population such association was found with vegetarian diet ( $P < 0.05$ ) and non availability of toilets ( $P < 0.001$ ).

### 3.4. Symptoms

Among 87 cases (87/2500) with a positive serology, 38 subjects had a history of transient ill defined seizures lasting for mean duration of 90 seconds, 77.33% had generalized seizures while 22.67% had focal seizures. Subcutaneous and/or intramuscular nodules were observed in 6.80% (6/87) cases. Headache was observed in 34.40% (30/87) cases, blurring of vision in 13.79% (12/87) cases and stomachache in 5.74% (5/87) cases. Thirty-nine seropositive subjects were asymptomatic. CT scan showed 21 cases with single and 5 with multiple ring enhancing lesions in the brain parenchyma, 4 cases had a normal CT scan.

**Table 1**

Association of risk factors with cysticercosis in infected individuals.

Risk factors value	Urban ( $n=1250$ )			Rural ( $n=1250$ )		
	$n$ (%)	$\chi^2$	$P$	$n$ (%)	$\chi^2$	$P$
Sex		0.65	0.42		1.74	0.29
Female	35 (60.3)			19 (65.5)		
Male	23 (39.7)			10 (34.5)		
Age group		0.15	0.70		1.86	0.17
<18 yrs	32 (55.2)			14 (83.3)		
>18 yrs	26 (44.8)			15 (51.7)		
Diet		0.70	0.40		4.81	0.03
Vegetarian	31 (53.4)			16 (55.2)		
Non-vegetarian	27 (46.6)			13 (44.8)		
Water source		0.06	0.80			NA
Tap water	53 (91.3)			0 (0.0)		
Hand pump	5 (8.7)			29 (100.0)		
Pig rearing		152.2	<0.001		58.19	<0.001
Present	6 (10.3)			17 (58.6)		
Absent	52 (89.7)			12 (41.4)		
Toilet		0.77	0.38		25.59	<0.001
Available	38 (65.5)			2 (6.8)		
Not available	20 (34.5)			27 (93.2)		
Waste disposal		4.24	0.04		0.96	0.33
Sanitary	21 (36.2)			1 (3.4)		
Indiscriminate	37 (63.8)			28 (96.6)		

In the urban seropositive 37.93% (22/58) individuals had seizures and 6.89% (4/58) had subcutaneous or muscular nodules. Headache was observed in 31.03% (18/58) cases, blurring of vision in 13.79% (8/58) and stomachache in 6.8% (4/58) cases. Twenty-six cases were asymptomatic. Out of 15 cases with CT scan, 10 had single cyst, 2 had multiple cysts

and 3 had normal scan.

In the rural seropositive 55.17% (16/29) individuals had seizures and 6.89% (2/29) had subcutaneous or muscular nodule. Headache was observed in 41.37% (128/29) cases, blurring of vision in 13.79% (4/29) and stomachache in 44.82% (13/29) cases. Thirteen cases were asymptomatic. CT scan of 15 cases showed 11 with single cysts, 3 with multiple cysts and 1 had a normal scan.

### 3.5. Stool examination

Population screened showed *Entamoeba coli* in 33 cases, *Entamoeba histolytica* (*E. histolytica*) in 27 cases and *Ascaris lumbricoides* in 6 cases in their stool examination. In the urban population ( $n=36$ ) *Entamoeba coli* was found in 23 cases, *E. histolytica* in 11 cases, *Ascaris lumbricoides* in 2 cases. In the rural population ( $n=30$ ) *Escherichia coli* was found in 10 cases, *E. histolytica* in 16 cases, *Ascaris lumbricoides* in 4 cases. Only one case of intestinal taeniasis was observed. This case did not test positive in IgG or IgM cysticercus by ELISA.

## 4. Discussion

A baseline epidemiological data for prevalence of cysticercosis in a population can be critical for adopting strategies for its control and eradication, as prevention seems to be most effective in this disease. The prevalence data reported from India is largely based on hospital based studies. No community based human cysticercosis prevalence data is available in India<sup>[3]</sup>. Radiological procedures are the diagnostic gold standard but are impractical for population based studies. Immunoassays like ELISA and EITB, which have been used in serum for diagnosis and sero-epidemiological surveys and are the method of choice for screening for exposure to cysticercosis. An attempt has been, made in this study to estimate the seroprevalence of cysticercosis in general population of North India with a valid sample size, exactly representing the existing population, using ELISA as a “survey tool”. The instant study also compares rural and urban population in the same geographic location and identifies community, behavioral and environmental practices which need modification to prevent continuous transmission of the disease. An overall high cysticercosis seroprevalence of 3.48% was observed in general population in terms of presence of anti *T. solium* IgG and/or IgM antibodies detected by ELISA. In urban population the seroprevalence was 4.64% and 2.32% in the rural population. Both IgG and IgM ELISA were done and positivity in both or either test was taken as positive. The difference in the seroprevalence rates between the urban and rural groups was statistically significant.

In an only study reporting prevalence of cysticercosis from community, further to the referred review by Rajshekhkar *et al* in 2003<sup>[3]</sup>, Khurana S *et al* have compared prevalence data in urban, rural and slum areas of Chandigarh, India<sup>[7]</sup>. They have reported an overall prevalence of 17.3% with 24.0% from slum, 20.0% from rural and 8% in urban population. However, much cannot be concluded from the study as it was conducted in a sample of 600 subjects, does not represent general population and hence the results are not comparable to the instant study. It ends up reflecting an

overall high prevalence of cysticercosis in the area. Crowded living conditions, indiscriminate sewage disposal, poor food hygiene and sanitary conditions in urban areas may account for the difference in the urban and rural seroprevalence. Similar observations are reported by Garcia *et al* in Peru where high seropositivity rates were found in persons with multiple predisposing factors, suggesting that these apparent risk factors and behaviors acted cumulatively<sup>[8]</sup>. High titers, though below the cut-off limit were observed in family members of cases with positive titers particularly in urban population. These observations were concurrent to findings of Goodman *et al* who reported three times more likelihood of a positive EITB is for cysticercosis in family members of patients of neurocysticercosis, than the general population<sup>[9]</sup>. Such different levels of exposure within families is explainable in light of studies conducted by Garcia *et al*<sup>[10]</sup> and Meza-Lucas A *et al*<sup>[11]</sup> who also report high antibody titers in the family members of the patients. Current study confirms these findings and reinforces that immunoassays may not always reflect active infection but a sub clinical infection or a past infection may also give positive result due to transient antibody response. Immunity to exposure to same risk factors as the infected individual for a prolonged duration, contact with the infected person itself or self cured cysticercosis can be a reason for high titers in the family of seropositive individuals. Apart from the elevated antibody titers in the family members, 25 cases were diagnosed of cysticercosis in 8 families. The familial aggregation may be suggestive of presence of a tapeworm carrier in the family, besides predominance of other risk factors.

CT scans were done in 30 cases presenting with epilepsy and having a positive serology. Neurocysticercosis was present in 26 cases that showed presence of ring lesions in CT scan. Four of the seropositive cases did not show lesions. The cases not showing lesions could have been cases of abortive, healed lesions; other systemic cysticercosis or the CNS lesions may have been missed in CT scan. However, this proportion of cases of confirmed neurocysticercosis supports the fact that only one-third of *T. solium* seropositive individuals harbor cysts within the brain<sup>[12]</sup>. Contrary to the three-tier “iceberg” analogy of human taeniasis-cysticercosis<sup>[10]</sup> in the current study only two distinct tips were observed. Most noticeable tip was of 26 cases with confirmed neurocysticercosis and second comprised of 39 seropositive asymptomatic individuals additive to 4 cases without lesions in CT scan.

In absence of comprehensive Indian study of risk factors comparisons are drawn from epidemiological studies conducted worldwide which reveal that contact between pigs and human feces, lack of inspection of pork, consumption of undercooked pork, consumption of unclean vegetables and water contaminated with infected human feces, living with a tapeworm carrier, poor personal hygiene are potential risk factors<sup>[13]</sup>. Sanchez *et al* have found lack of potable water, lack of sanitary toilet, earthen floor, lack of education, lack of knowledge about the parasite besides other reported factors<sup>[14]</sup>. Likewise, we have observed a general poor personal as well as community hygiene with lack of awareness of disease in our survey, contributing to seropositivity defying any geographic limitations. High socioeconomic status does not seem to protect against exposure, as demonstrated by seropositivity. Age and gender have also been associated with seropositivity in some studies<sup>[15]</sup> while we did not observe any such predilection

similar to Xu *et al*[16]. In the current study pig rearing was found to be significantly associated with seropositivity in both urban (52/58) and rural (17/29) population.

Association of vegetarian diet (16/29) with the infection was observed in the rural area. Out of 13 non-vegetarians in the rural infected group, 9 were pork eaters. Non availability of toilets in rural area was observed to be promoting the rate of infection, similarly creating conducive conditions for completion of the parasite's life cycle. This confirms the point particularly in Asian perspective where the domestic pigs have free access to human feces, human feces being used as manure, local customs promoting consumption of raw pork. Unsanitary sewage disposal (37/58) was also found associated significantly to seropositivity but only in urban population. The finding can be rationalized considering sewage disposal in open drains, which finally contaminates drinking water and caters a large proportion of population. Sewage disposal seems to propagate the infection laterally as well, as these drains and openings were accessible to pigs which were let loose for diet by the owners and roamed around the human habitation. Problem is aggravated by the fact that the eggs of *T. solium* are infectious immediately when passed in the stool and do not require the activation period in the soil as in contrast to most helminth parasites. Thus, continuity of infection goes unchecked naturally also. Higher seropositivity in urban area with high consumption rate of tap-water supply (53/58) was not surprising after the observation.

It is significant to note here that both the areas under study are serviced by primary health centers responsible for providing treatment and prevention measures under the government run health schemes. Specifically in terms of helminthoses these centers dispense antihelminthics (albendazole) regularly for treatment and prevention of intestinal parasites. However, presence of seropositivity within a community like in the current study implies that the parasite cycle exists and needs interventional measures depending on the burden of disease in the population. Apparently this strategy is not effective in preventing transmission of the disease and a larger targeted program is required. However, this can be a reason for observed low taeniasis in the study population. The findings of our study rebut the existence of taenia cysticercosis complex and hints that the infection is more caused due to closeness with carrier and environmental factors than autoinfection.

To conclude, our study is a significant addition to prevalence data of cysticercosis in North India with a valid sample size and sampling method. We recommend proper sewage disposal, penning of pigs far from human habitation, high level of cooking hygiene practices, for effective control of cysticercosis. Active finding and treatment of probable tapeworm carriers especially the personnel related to cooking should be accompanied by health education and control swine cysticercosis. We also suggest presumptive treatment of the suspected cases and their families with antihelminthic treatment. The study clearly brings home the point that the community is at risk as a whole and individual hygienic practices do not decrease risk of disease.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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