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SHORT COMMUNICATIONS

Prevalence of porcine cysticercosis in Vellore, South India

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Background: Porcine cysticercosis is acquired by pigs through consumption of human faeces containing *Taenia solium* ova and indicates the presence of active transmission of the parasite between pigs and humans.

Methods: The prevalence of porcine cysticercosis was assessed by an antigen ELISA and enzyme linked immunoelectrotransfer blot (EITB) for antibodies in rural and urban areas of southern India.

Results: Of the 112 porcine blood samples, 13 (11.6%) were positive for cysticercal antigens and the free-range pigs were 3.6 times more likely to be infected than the slaughtered pigs and 67 (59.8 %) tested positive for serum antibodies indicating high exposure to *T. solium* eggs.

Conclusion: The high prevalence of porcine cysticercosis recorded in the study areas mandates public health measures, which includes meat inspection.

Keywords: *Taenia solium*, Cysticercosis, Pig, ELISA, EITB, India

Introduction

Porcine cysticercosis, a parasitic zoonosis caused by the metacestode larval stage of *Taenia solium*, is acquired by pigs through consumption of human faeces, feed or drinking water containing *T. solium* ova.[1] *Taenia solium* cysticerci are commonly found in the muscles, eye or brain. While tongue palpation and meat inspection are the preferred diagnostic tools in massive infections among pigs, serological tests are useful in the diagnosis of mild or latent infections among the pig population.[2,3] Human consumption of infected pork causes taeniasis (adult tapeworm infestation) and a tapeworm carrier is the source of infective eggs which causes human and porcine cysticercosis. The presence of porcine cysticercosis in an area indicates the presence of active transmission of the parasite between pigs and humans and pigs are normally targeted in order to control the problem.[4] There are limited studies from India reporting the prevalence of porcine cysticercosis.[5–10] This study aimed at estimating the prevalence of porcine cysticercosis among reared free-ranging pigs and pigs that were slaughtered for human consumption in Vellore city, southern India.

Materials and methods

The study was carried out between January and December 2007 in Vellore city of Tamilnadu, India. Pig rearing families in the village and urban areas of Vellore were identified and asked for permission to collect blood from their pigs. We identified 12 pig rearing families spread across seven villages and two urban wards in Vellore by conducting field visits. Of the 12 families four (three rural and one urban) consented for blood collection from the pigs they owned. The head of the pig rearing family chose which animal was sampled; 8–10 ml of blood from live pigs was collected from either the ear lobe veins or superficial veins in the legs by qualified personnel.

There are only six slaughter points in and around Vellore city and all the owners were approached. Around 25 ml of blood per pig was collected at the time of slaughter on a weekly basis from each of the slaughter points. The pigs killed at these slaughter points are primarily sourced from the neighbouring areas predominantly from rural, independent semi-commercial pig rearing families. These pigs are often reared in similar environmental conditions as the free ranging pigs except that they are heavier and older than the free ranging pigs.

A total of 112 blood samples were collected: 25 from live pigs reared in two areas (one village and one urban slum) and 87 from the six slaughter points.

Serum separated from the blood was stored at -70 °C until assay. Circulating *T. solium* metacestode antigens were assayed using a modified ELISA with monoclonal antibodies to excretory/secretory products of *T. saginata* metacestodes. Briefly, 100 μl sera, deproteinized with 5% trichloroacetic acid (TCA) and neutralized with 0.156M carbonate/bicarbonate buffer pH 10, were added to microtitre plate wells coated with

capture antibody (B158C11A10; 5 μg/ml) for one hour at 25 °C, followed by incubation for one hour with 100 μl detecting biotinylated antibody (B60H8A4; 1.25 μg/ml) and for one hour with streptavidin peroxidise. Bound peroxidase was developed with o-phenylenediamine / H_2O_2, reactions stopped with 50 μl H_2SO_4 (4N) and plates read at 490nm/655 nm.

A sample was considered positive for *T. solium* cysticercus antigens when the absorbance at 492nm was above the mean +3SD of six negative control sera run on the same microtitre plate.[3,11,12]

Detection of cysticercal antibodies in serum was by an enzyme linked immunoelectrotransfer blot (EITB) using lentil lectin specific *T. solium* cyst glycoproteins as antigens.[13] Antigens for the EITB were isolated from cysts obtained from local naturally infected pigs and purified by lentil lectin–Sepharose chromatography by a purification protocol similar to that followed at the CDC, USA. [13] A sample was considered positive for cysticercal antibodies following the criteria of Tsang et al.,[14] which was determined by reaction to one or more *T. solium* glycoproteins of molecular weights 50, 38–42, 24, 21, 18, 14 and 13 kDa.

Statistical analysis

The overall proportions of seropositivity to cysticercal antigens and antibodies were calculated and compared between the free-ranging and slaughtered pigs. Statistical associations between the status of the pig (free-ranging or slaughtered) and the outcomes were determined using χ^2 tests for association and OR with 95% CI.

Results and Discussion

Positivity for circulating cysticercal antigens

Prevalence of porcine cysticercal antigens was 24% and 8% among live/free ranging pigs and slaughtered pigs, respectively, with a combined prevalence of 11.6%. The free-ranging pigs were more likely to have circulating cysticercal antigens when compared to the slaughtered pigs with an OR of 3.61 (CI 1.08 to 11.97; Table 1).

Table 1. Prevalence of porcine cysticercal antigens and antibodies in Vellore, south India

Biomarker	Reared free ranging pigs $n = 25$ (%)	Slaughtered pigs $n = 87$ (%)	Overall $n = 112$ (%)
Serum antigen result by ELISA			
Positive	06 (24)[a]	07 (8)	13 (11.6)
Negative	19 (76)	80 (92)	99 (88.4)
Serum antibody result by EITB			
Positive (reaction on 1 or more bands)	13 (52)	54 (62)	67 (59.8)
Negative	12 (48)	33 (38)	45 (40.2)

[a] $p < 0.05$ (χ^2 test), OR = 3.61 (95% CI 1.08 to 11.97)

The prevalence of porcine cysticercal antigens was 15.4% and 6.4% among male and female pigs; no statistically significant associations were detected between the sexes either among free ranging or slaughtered pigs.

Positivity for cysticercal antibodies

The overall seroprevalence of *T. solium* cysticercus antibodies was 59.8% (67/112) and was higher in the slaughtered pigs (62.1%) compared to the free-range pigs (52.0%) although this finding was not statistically significant ($p > 0.05$). The prevalence was lower among male pigs (55.4%) when compared to female pigs (66.0%). Of the 67 serum samples tested positive for *T. solium* cysticercus antibodies by EITB assay, 83% (56/67) showed reaction on 1 to 3 bands.

Correlation between cysticercal antigens and antibodies

Among the pigs without active cysticercal infection, 63% (62/99) demonstrated evidence of exposure or past or present infection in terms of testing positive on one or more bands of the EITB assay and this figure was 38% (5/13) among pigs which were having active cysticercal infection as presented in Table 2. The antigen and antibody results were negatively correlated with a Pearson's correlation coefficient of -0.09 and not statistically significant with a p value of 0.347.

Human cysticercosis is a major public health problem in India. The study area is endemic for human cysticercosis with the burden of human neurocysticercosis being 1.09/1000, contributing to 33% of active epilepsy in the community and 15.9% of the general community having cysticercal antibodies in their serum.[15]

Estimating the true disease burden among pigs is very difficult as the gold standard is a thorough examination of a dissected pig carcass for cysts in the skeletal muscles, liver, heart or brain. Although several tests exist to detect the presence of cysts in pigs, the efficacy and interpretations of results are often difficult to compare. A study done in Zambia has shown that a Bayesian approach using a combination of lingual examination, serological testing and expert opinion provides a better estimate of the disease.[3] Our study is limited by the lack of data on the detection of the cysts by lingual examination of the pigs or the carcasses.

In a study done in three states in southern India, disease detection among pigs was better with serum testing for antigens

Table 2. Correlation between porcine cysticercal antigens and antibodies

	Serum antigen result by ELISA	
Serum antibody result by EITB	Negative (%)	Positive (%)
Negative	37 (37)	8 (62)
Positive on 1 to 3 bands	53 (54)	3 (23)
Positive on more than 3 bands	9 (9)	2 (15)
Total	99	13

Pearson's R = -0.09, significance = 0.347

using ELISA when compared to classical meat inspection and immunoelectrophoresis methods.[5] The prevalence of porcine cysticercal antigens in our study was higher (11.6%) than the seroprevalence rates (6.2 to 6.5%) estimated in three states of southern India,[5] and a reported prevalence of 6.3% on lingual examination of pig carcasses from northern India.[10]

Estimates using a Bayesian framework provide a sensitivity of 35.8% and a specificity of 91.7% for the Ab-ELISA using crude metacestode antigen of *T. crassiceps*.[13] In our study, we have used lentil lectin specific *T. solium* cyst glycoproteins as antigens and we expect the test to have higher sensitivity and specificity. The Ag-ELISA for cysticercosis (used in this study) has been shown to cross react with *T. hydatigena*. The presence of *T. hydatigena* in pigs varies very much according to geographical regions. In Africa, *T. hydatigena* is very common in sheep and goats but has a relatively low prevalence in pigs. In Vietnam and Laos, *T. hydatigena* infections may reach a prevalence of >50% in pigs. Information on the magnitude of *T. hydatigena* infections among animals in India is very limited with one study from northern India reporting a prevalence of *T. hydatigena* among the canine population to be 41%. The prevalence of *T. hydatigena* cysticercosis was 37%, 27% and 8.3% among the sheep, goat and porcine populations, respectively, with a higher prevalence among rural pigs and during the rainy season.[16] Thus in the absence of necropsy data and lack of information on *T. hydatigena* infection in pigs in Vellore district coupled with a high number of EITB antibody positives which does not cross react with *T. hydatigena*, it can be assumed that the Ag-ELISA positive results will in most cases be due to *T. solium* infections. The prevalence of cysticercal antibodies (nearly 60%) in our study is higher than that reported from Peru (43%), a region which is highly endemic for cysticercosis.[17] This suggests that most of the pigs in our study area are exposed to human faeces infected with *T. solium* ova.

Authors' contributions: VR, JM, AO designed the study and drafted the manuscript. TJ carried out all laboratory work. MVR carried out the community studies, contributed in analysing and drafting the manuscript. PD, AO and JV helped in the analyses and drafting of the manuscript. All authors analyzed the data, read and approved the final manuscript. AO, VR and JM are guarantors.

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Competing interests: None declared.

Ethical approval: This study has been approved by the Institutional Review Board of the Christian Medical College, Vellore, India.

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