

Original Research Article

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Detection of Porcine Cysticercosis in Nagaland of North East, India

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ABSTRACT

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A total of 360 pig carcasses were examined through meat inspection in different market places of Dimapur and Kohima district of Nagaland, India in the month of March, 2019. Out of which, 6 (1.67%) were found positive for porcine cysticercosis with visible cysts. The serum samples were collected from 300 pigs and out of which 10 (3.00%) serum samples were found positive for *Cysticercus cellulosae* antibody. Polymerase chain reaction (PCR) assay was performed to confirm *Cysticercus cellulosae* and to validate the results of meat inspection. Oligonucleotide primers targeting against the large subunit rRNA gene (TBR primers) of *Taenia solium* were used in this study. On reactivity in PCR test, the TBR primers yielded products of 286bp in cysticercosis positive cases.

Introduction

Cysticercus cellulosae, the metacestode stage of *Taenia solium* is an underrated and a neglected zoonotic disease involving pig and man (Willingham *et al.*, 2008). Porcine cysticercosis plays a crucial role in the transmission and maintenance of human taeniosis and cysticercosis (Pinto *et al.*, 2000). In Indian context, meat consumption is greatly influenced by culture, traditions, customs and taboos especially in the rural societies. In north eastern states of India pork is considered as a traditional food item (Anonymous. 2012) and has much higher pork consumption than the other parts of the country. The demand for

pork was increasing along with prices in both Assam and Nagaland according to the traders. Thus, accurate inspection of pork is very important.

Although the pork consumption is highest in North East India but the scientific procedure of diagnosis and meat inspection is still lacking. While meat inspection is the preferred diagnostic tool to detect heavily infected carcasses but it is not reliable in detecting lightly infected carcasses (Cai *et al.*, 2006). So detection of antibodies by ELISA (Ab-ELISA) can be used as an alternative tool for the diagnosis of porcine cysticercosis (Deckers *et al.*, 2008).

Moreover, meat inspection requires expertise of the meat inspector; otherwise cysticerci may be confused with *T. hydatigena* cysticerci (Kedra *et al.*, 2001), hydatid cysts (Deplazes *et al.*, 2005) and left over of muscle fasciae (Geysen *et al.*, 2007). To overcome such type of difficulties application of ELISA and molecular tools like Polymerase chain reaction (PCR) can be effective in diagnosis and validation of meat inspection results.

So considering the public health significance of the disease, the purpose of this study was to evaluate the performance of ELISA for ante-mortem diagnosis in pigs and estimating the prevalence of porcine cysticercosis in slaughtered pig through meat inspection and PCR for validation of results.

Materials and Methods

Study area

The study was carried out between January 2019 to February 2019 in Dimapur and Kohima district of Nagaland, India. Three blocks from each district were selected for collection of sera sample and for carcass examination.

Sample collection

Collection of blood samples

A total of 300 blood samples were collected from the anterior vena cava of pigs by using BDV accutainer@needles (gauge 19) and BDV acutainer@plain tubes (10 ml). The blood samples were kept standing in an ice box at +4°C to ensure no haemolysis occurred while in the field.

At the laboratory, blood was centrifuged to separate serum from blood clot. Serum was harvested into barcoded 2 ml vials that were stored at -20°C until processing.

Carcass Inspection

A total of 360 pig carcasses were examined as per the standard method [10] for the presence of *Cysticercus cellulosae* from different market places of Dimapur and Kohima district. Majority of the pigs were brought from Uttar Pradesh, India to Dimapur and distributed to the rest of the districts of Nagaland on weekly basis (Fig.1). Fifty gram (g) of tissue each from brain, tongue, liver and skeletal muscles of infected pig carcasses were brought to the laboratory in ice-box

Serological tests

ELISA was performed as per the manufacturer's protocol of RIDASCREEN® *Taenia solium* IgG kit (RBiopharm AG, Germany). The absorbance was read at 450 nm with an ELISA reader (Lab systems Multiskan Plus, Thermo Fisher Scientific, USA). Samples having percent positivity value 0.50 or above (%P ≥ 0.05) were categorized as positive and below 0.50 as negative.

Extraction of DNA from cysts

Extraction of DNA from cysts/suspected lesion was made possible using a commercially available QIAamp tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions (Kolesarova *et al.*, 2012). Briefly, 200 µl of cyst/lesion homogenate, 20 µl of proteinase K stock solution, and 200 µl of lysing buffer were pipetted into 1.5 ml Eppendorf tube. The mixture was incubated at 37 °C for 1 h and then at 70 °C for 30 min before the addition of 200 µl of absolute alcohol and mixing by vortexing. The mixture was then transferred to the QIAamp spin column placed in a clean 2 ml collection tube and centrifuged at 8000 RPM in MiniSpin centrifuge (Eppendorf, Wesseling-Berzdorf, Germany) for 1 min at

room temperature. The QIAamp spin column was washed twice with 500 µl of the washing buffers by spinning for 1 min. The QIAamp spin column was placed in a clean 1.5 ml eppendorf tube and the DNA was eluted with 100 µl of elution buffer preheated at 70 °C. Maximum DNA yield was obtained by spinning at 12,000 rpm for 1 min at room temperature. From the suspended nucleic acid 5 µl was used in the PCR amplification. The extracted DNA was quantified using spectrophotometer at 260 nm wave length

Oligonucleotide primers

The oligonucleotide primers specific to *T. solium* were adopted from already published sequences. The primers TBR3 (5'-GGC TTG TTT GAATGG TTT GAC G-3) and TBR-6 (5'-GCT ACTACA CCT AAA TTC TAA CC-3) against large subunit rRNA gene (Jardim *et al.*, 2006).

PCR amplification and detection of PCR product

The PCR reaction was performed in a thermal cycler (Eppendorf, Hamburg, Germany) in 20 µL volume containing 2µl DNA sample (100 ng/ µl), 1 µl (10 pmol) of each forward and reverse primer, 10 µL of DreamTaq Green PCR Master Mix (2X) (Thermo Scientific™, USA) and 7 µL of nuclease free water (Thermo Scientific™, USA). A total of 40 PCR cycles were run with the following conditions: one initial denaturation cycle at 94°C for 3 min, followed by 40 repeated cycles with temperatures at 94°C for 30 s (denaturation), 59°C for 30 s (annealing, specific for primers) and 72°C for 1 min. After the final cycle, the preparations were kept at 72°C for 5 min for final elongation, and the PCR products were stored at 4°C in thermal cycler for further use (Sreedevi *et al.*, 2012). Five microliters of PCR amplicons was analyzed on ethidium bromide stained 2%

agarose gel (In Genius Gel documentation system, Syngene, UK). The sizes and quantities of PCR products were verified by comparison with a 100 bp plus quantitative ladder (Thermo Scientific™, USA)

Results and Discussion

Overall sero-prevalence of porcine cysticercosis in Dimapur and Kohimadistrict was recorded as 3.00 % (Table 2). Higher sero-prevalence of porcine cysticercosis was recorded in Dimapur (3.33 %) compared to Kohima district (2.67%) of Nagaland. These findings were in close conformity of Barua *et al.*, (2018) where they recorded sero-prevalence of porcine cysticercosis in 4 states of North East India as 2.72 %.

This might be because, in our study area, the vast majority of pigs are confined in pens, even among smallholders, compared to more extensive (i.e., free roaming and scavenging) pig husbandry practices which may predominate in the other study settings.

All the 360 pigs inspected at slaughter were from different market places of Dimapur and Kohima district of Nagaland, India (Fig. 2 & Fig. 3). The overall prevalence of porcine cysticercosis based on post-mortem inspection was 1.67% (Table 1). The prevalence was higher in Dimapur (2.22 %) compared to Kohima district (1.67%) of Nagaland.

Previously, Sarma (1977) recorded 6.64 % positive cysticercosis from greater Guwahati of Assam. Plain (1991) recorded highest infection 11.90 % infection from North Eastern region of Assam. Borkataki *et al.*, (2012) conducted study in three districts of Assam for a period of one year and found 93 pigs (9.50%) positive for cysticercosis out of 978 pigs. Although the previous workers recorded higher prevalence of porcine cysticercosis, Barua *et al.*, (2018) recorded

prevalence as 0.92 %in 4 states of North East India. This might be due to importing of scavenging pigs from other parts of India as reported by butchers.

Table.1 Prevalence of porcine cysticercosis pigs of Dimapur and Kohima district of Nagaland

District	Study area	Animals inspected(No.)	Cyst positive pig (%)
Dimapur	Medziphema	60	1 (1.67%)
	Khuhboto	60	2 (3.33%)
	Nihokhu	60	1 (1.67%)
Total		180	4 (2.22%)
Kohima	Sechu-Zubza	60	1 (1.67%)
	Kohima	60	0 (0.00%)
	Zakhama	60	1 (1.67%)
Total		180	2(1.11%)
Overall Total		360	6 (1.67%)

Table.2 Sero-Prevalence of porcine cysticercosis in Dimapur and Kohima district of Nagaland

District	Study area	No. of Pig Serum	Sero Positive (%)
Dimapur	Medziphema	50	2 (4%)
	Khuhboto	50	2 (4%)
	Nihokhu	50	1 (2%)
Total		150	5 (3.33%)
Kohima	Sechu-Zubza	50	2 (4%)
	Kohima	50	1 (2%)
	Zakhama	50	1 (2%)
Total		150	4 (2.67%)
Overall Total		300	9 (3%)

Fig.1 Unloading of pigs in Dimapurbrought from Uttar Pradesh, India



Fig.2 Cysts of *Cysticercus cellulosae* in skeletal muscle of pig carcass

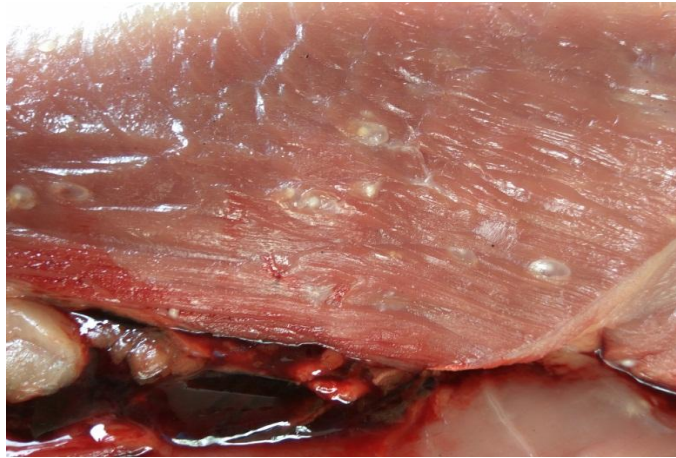
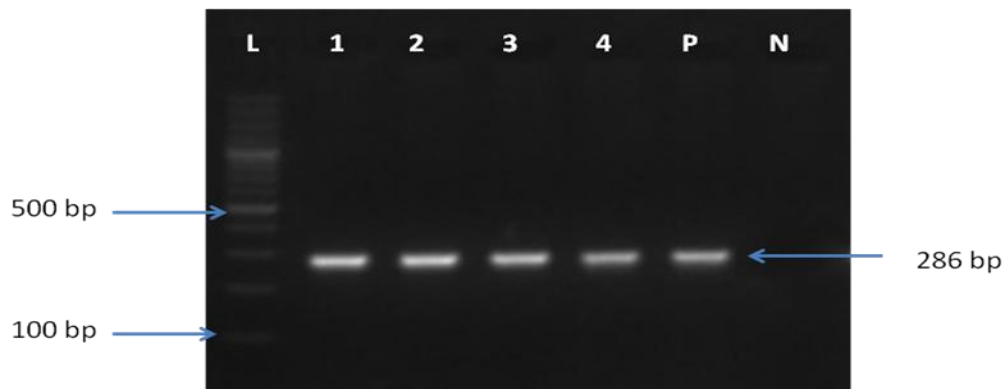


Fig.3 Cysts of *Cysticercus cellulosae* collected from tissue samples



Fig.4 PCR assay with TBR primers to detect *Cysticercus cellulosae* from pigcarcasses. Lane L: 100-bp DNA ladder; Lane 1 to 4: DNA samples extracted from cysticercosis positive pigs. Lane P: Positive control and Lane N: Negative control



Identification of *T. Solium* cysticerci from the infected pig carcasses and suspected carcasses was based on amplification of large subunit rRNA gene (TBR) gene with a product size of 286 bp. All the positive cases show positive amplification of TRB gene (Fig. 4). Dalmasso *et al.*, (2004) extracted DNA from degenerated and calcified cysts/lesions and found positive amplification for TRB gene. Lino Junior (2004) also reported the importance of PCR test with TBR primers as a reliable method for detection of cysticerci in tissues from human autopsies that are in advanced evaluative stages.

From the above study it was found that porcine cysticercosis is still a major public health concern. However there is less information regarding the prevalence of this disease has been recorded from North Eastern states of India. Therefore, an extensive study regarding the prevalence, transmission, risk factors and prevention of this neglected zoonotic disease is utmost concern.

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