

Molecular Characterization of Cysticercosis in Pigs of Maharashtra

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Abstract: By considering the importance of the disease a periodic survey for detection of cysticercosis in pigs was carried out during December 2014 to March 2015 in different parts of Maharashtra.

Prevalence of cysticercosis in pigs: The present study was carried out to determine the prevalence of cysticercosis in pigs slaughtered at Deonar abattoir. Amongst 600 pig carcasses examined, *Cysticercus cellulosae* infestation was recorded in two pigs with a prevalence of 0.33%. The similar non-availability of pig slaughter unit in other areas was a hindrance to the area-wise comparison parameter. In Maharashtra, pigs slaughtered at Deonar abattoir, Mumbai and reported a prevalence of 0.89% and 0.57% of *Cysticercus cellulosae*, respectively. The analysis of data generated by these previous workers has revealed that Mumbai region is endemic of cysticercosis in pigs. However, over the last period of 10-15 years, the incidence has declined gradually. However, the observations in the present study indicate an increment in the prevalence of cysticercosis, i.e., 0.33%.

Out of 600 slaughtered pigs inspected at Deonar abattoir, Mumbai, 02 positive cyst samples were processed for DNA extraction for the detection of *T. solium*. 2 positive cysticerci sample were analyzed by PCR assay which detected gDNA of *Taenia solium* with an amplification of 984 bp when targeted by Cox1 gene which is essential for a positive sample. Thus, the prevalence of cysticercosis in pigs by PCR was recorded to be 0.33%. Employment of PCR assay to detect *T. solium* DNA in muscle lesions for validating the meat inspection results.

Keywords: Cysticercosis, *Cysticercus* cyst, *Taenia solium*.

INTRODUCTION

The recent emergence of infectious diseases, most of which have involved zoonotic parasitic disease agents of public health importance, represents a growing global concern. Parasitic zoonoses of international importance include cysticercosis. These are likely to become increasingly important in the spectrum of emergent and re-emergent diseases for developed and developing countries. Although these infections are not among the leading causes of parasite-induced mortality worldwide, they represent a significant hazard in most developing countries, where they cause considerable economic loss due to condemnation of infected organs and may lead to a major impairment in individual and societal quality of life.

Taeniasis, an obligatory cyclozoonosis is one of the important meat borne parasitic zoonotic disease, caused by adult stages of taeniid tapeworms viz., *Taeniasaginata* and *Taenia solium* in cattle, buffaloes and pigs, respectively. The disease is classified in List B by Office International des Epizooties (OIE) which is neglected zoonoses. Cysticercosis, an underrated zoonotic disease occurs throughout all parts of India, particularly in the north. The prevalence of porcine cysticercosis as judged from slaughterhouse data ranges from 7 to 12%, although the recent study in Uttar Pradesh reported a prevalence of 26% (Prasad *et al.*, 2002). The economic losses due to condemnation of infected meat due to cysticercosis have not been estimated properly but there are several indirect

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evidences available suggesting heavy losses to meat industry (D'Souza and Dhanalakshmi, 2005). The prevalence of taeniasis is reported to be between 0.5% to 2%, although surveys in Uttar Pradesh found 38.0% of people in a pig rearing community had taeniasis (Prasad *et al.*, 2002). Neurocysticercosis is reported as the single most cause of epilepsy in developing world (Prasad *et al.*, 2008).

In case of taeniasis, meat inspection is effective in detecting heavily infected carcasses, however it is not reliable in detecting lightly infected carcasses (Cai *et al.*, 2006). Therefore, there is a need to develop rapid and reliable diagnostic methods to know the exact prevalence of these diseases. In order to overcome limitations in the identification of *Taenia* species based on morphology or enzyme electrophoresis, various molecular approaches have been developed, including the use of DNA probes, Polymerase Chain Reaction (PCR), or PCR coupled to restriction fragment length polymorphism (RFLP) and multiplex-PCR.

Moreover the use of such molecular techniques for clarification of the taxonomic status within cestodes has revealed new insights into the evolution, ecology, and population genetics of these parasites (OIE, 2005). The use of DNA probes is time consuming and relatively insensitive; however, PCR with oligonucleotide primers derived from such species-specific probes is a rapid and sensitive method. Techniques such as PCR-RFLP and multiplex-PCR permit differential diagnosis of *T. solium*. Moreover molecular techniques can be also applied on fresh, frozen or ethanol-preserved parasitic material (OIE, 2005). The application of molecular tools such as PCR has also facilitated the identification of *Taenia* spp. genomic DNA (gDNA) in cysts, suspected lesions or cyst residues from pig and cattle in slaughterhouses in cysticerci of *T. solium* from tissues in humans (Shih *et al.*, 2010). PCR test not only provides an efficient tool for validation of meat inspection results but also can rule out ambiguity in carcass judgment of suspected cases of porcine cysticercosis (Sreedevi *et al.*, 2012).

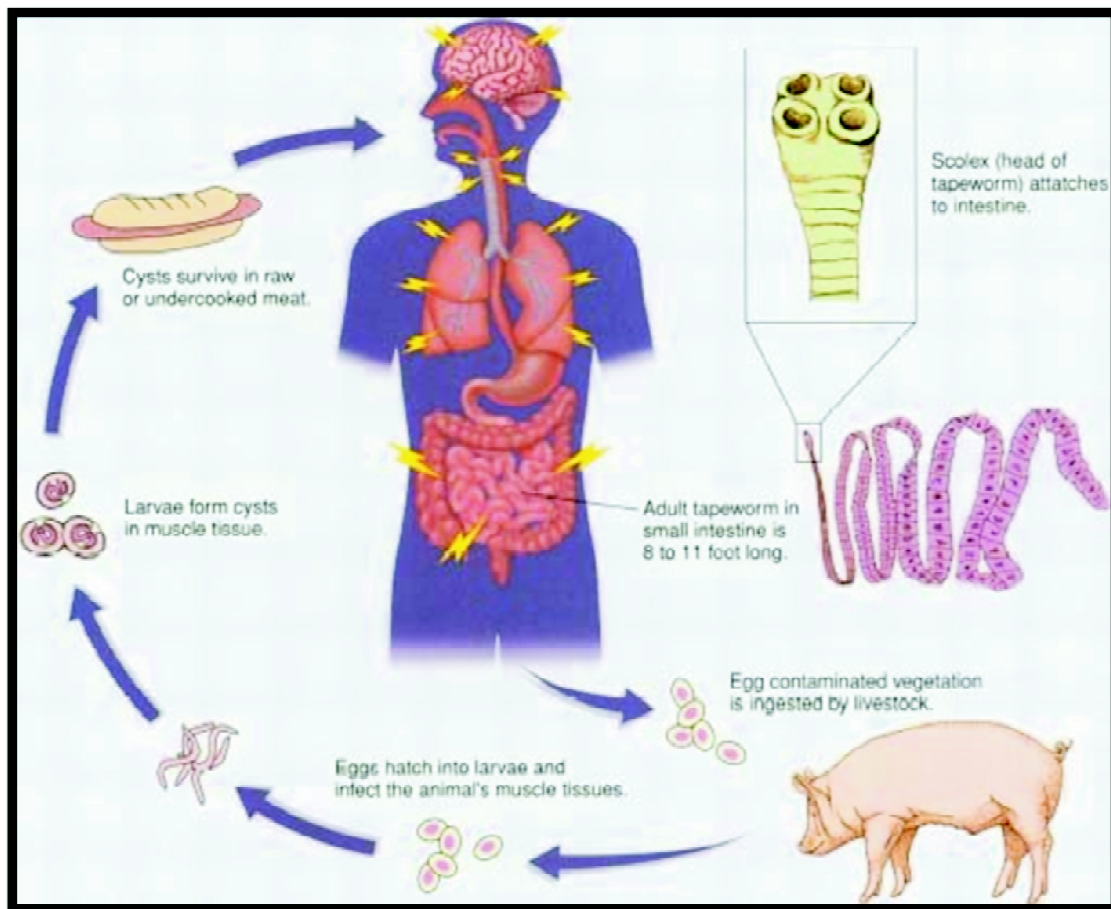


Figure 1 : Life cycle of cysticercosis



Figure 2: Cysticercus cyst sample

In view of above findings, PCR is the most suitable method for specific detection of *Taenia solium* in suspected cases. In this context, the present study is undertaken with the following objectives- To collect the cysticercus cyst samples of slaughtered pigs from different regions of Maharashtra and to study the prevalence of cysticercosis in pig of Maharashtra by Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

The present study was undertaken to determine the prevalence and molecular characterization of cysticercosis in slaughtered pigs of Maharashtra. The survey work was conducted to inspect different slaughtered food animals during the period from December, 2014 to March 2015 to pursue different aspects of the study for identification of parasitic diseases in different areas of Maharashtra including Pune, Nagpur, Srirampur, Sangamner and Deonar abattoir (Mumbai). The animals slaughtered at Deonar abattoir are usually from different districts Maharashtra *viz.*, Dhule, Nashik, Jalgaon, etc.

Postmortem Inspection Procedure

Deep incisions were taken at common predilection areas/sites of the carcasses such as shoulder muscle, thigh muscle, masseter muscle, neck, diaphragm and heart to detect the presence of cysticerci. Muscle samples infested with cysticerci were collected and placed in an insulated box containing ice and

brought to the laboratory, Department of Veterinary Public Health, Bombay Veterinary College for further processing (Table 1). In positive cases, the intensity of infestation was recorded by counting the number of cysts per unit area of muscle sample (7.5 × 2.5 cm). The intensity of infestation was graded into three categories *viz.*, low (1 cyst), moderate (2-3 cysts) and heavy.

Processing of Sample

The muscle tissues were washed with cold (4°C) phosphate buffer saline (PBS) prior to processing. Cysts were first separated from adherent host tissues and then collected in cold PBS. Further, cysts were gently washed with cold PBS three times and were stored separately in absolute ethanol at -20°C until use.

Table 1
Collection of pig cyst samples from different slaughter houses of Maharashtra

Sr. No.	Month	Caracass Unit of Animal	Total No. of Animal Inspected	No. of Positive Sample of Cysticercosis	% Prevalence
1.	Dec. 2014	Pig	100	00	-
2.	Jan. 2015	Pig	180	00	-
3.	Feb. 2015	Pig	200	00	-
4.	Mar. 2015	Pig	120	02	-
Total		600	02	0.33	



Figure 3: DNA sure Tissue mini kit

Estimation of Prevalence (%) of Cysticercosis

The prevalence (%) of cysticercosis was recorded by the following modified formula.

Prevalence (%)

$$= \frac{\text{Number of infected individuals at particular point in time}}{\text{Number of total individuals at particular point in time}} \times 100$$

Molecular Characterization of the Metacestodes

Molecular characterization of *T. Solium*, suspected cysts collected from one individual slaughtered pig was considered as one isolate for respective disease.

Extraction of Genomic DNA for Detection of *T. solium*

Genomic DNA was extracted from *T. solium* cysticerci dissected from muscles of cysticercosis infected pigs and preserved in 70% ethanol at -20°C.

The DNA was isolated using the DNASure Tissue mini kit (Genetix Biotech Asia Pvt. Ltd)

according to the manufacturer's protocol as described in flowchart.

PCR Amplification

Oligonucleotide primers

The following oligonucleotide primers are used in the study. The primer sequence and product size are shown in Table 2.

3.5.2 Reaction mixture for PCR

The reaction mixture was prepared on ice containing box to a final volume of 25 µl as shown in Table 3.

3.5.3 Polymerase chain reaction (PCR) cycling conditions for detection of *T. solium*

The PCR assay for the detection of *T. solium* was standardized as per the method of Sreedevi *et al.* (2012) with slight modifications. A total of 40 PCR cycles were run with the following conditions: one initial denaturation cycle at 94°C for 2 min, followed by 40 repeated cycles with temperatures at 94°C for

Table 2
Primers for diagnosis of *T. solium*

Serial Nos.	Location on DNA	Primer sequence	Expected product size	Reference
Cox 1	<i>T. solium</i>	Forward Reverse	5'-TTGATTCCTTCGATGGCTTTTCTTTTG-3' 5'-CGA-AAA-CAT-ACG-ACA-ACT-GC-3'	984 bp Yamasaki <i>et al.</i> (2004)

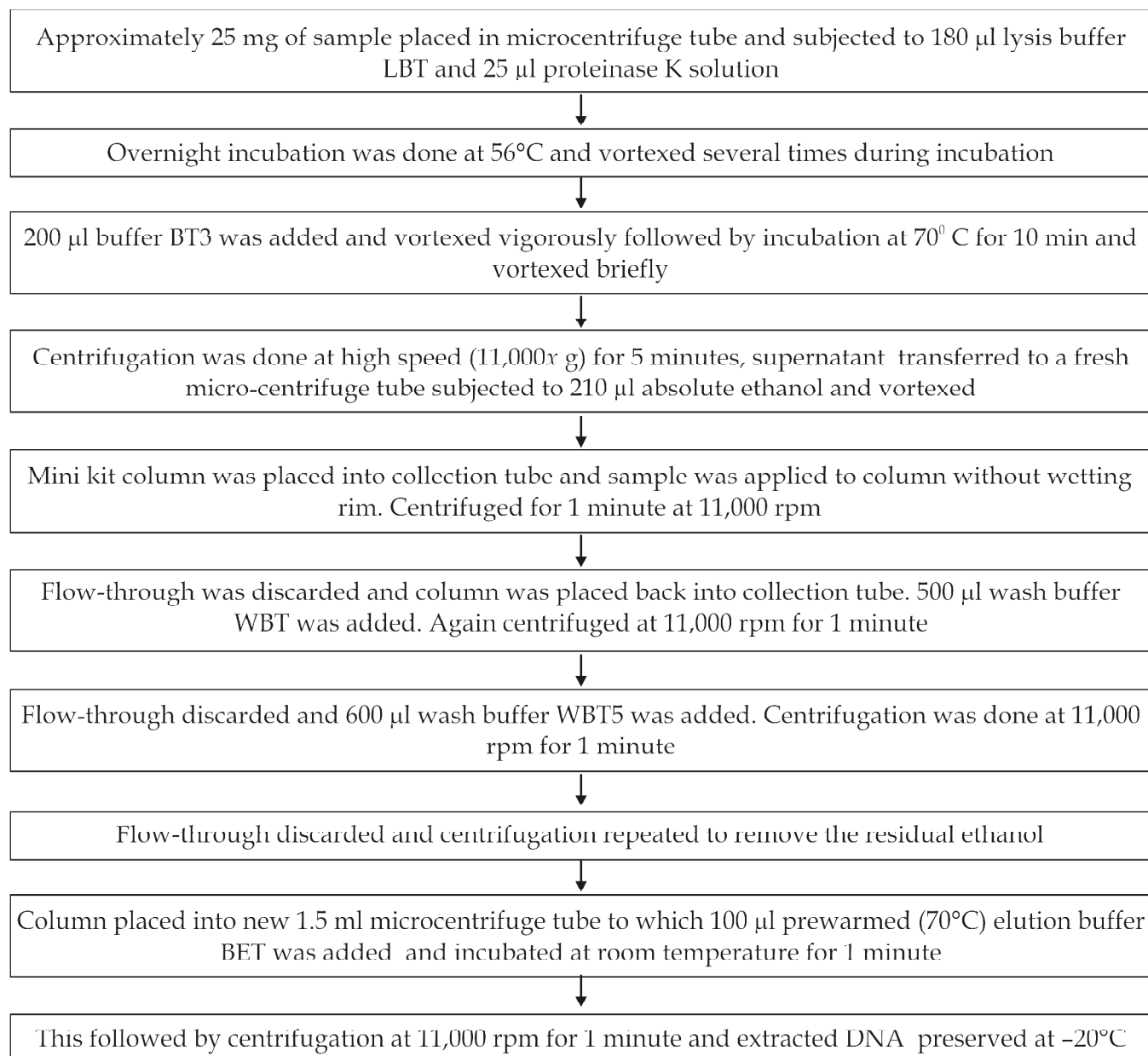


Table 3
Reaction mixtures for PCR

Sr. No.	PCR Reagents	Quantity (ml)	Final concentration
1.	10x Buffer	2.5	2.5x
2.	dNTPS (10 mM)	0.5	5 mM
3.	MgCl ₂ (25 mM)	2.0	50 mM
4.	Cox1- F (12.5 p mol)	1.0	12.5 p mol
5.	Cox 1-R (12.5 p mol)	1.0	12.5 p mol
6.	Taq polymerase (2U)	0.5	1.0 U
7.	Nuclease free water	12.5	-
8.	Template DNA 15 ng	5	75 ng
9.	Total (ml)	25	

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 held at 4°C in thermal cycler.

3.5.4 Electrophoresis of the PCR product

After completion of PCR, amplified products were analysed and confirmed by agarose gel electrophoresis (0.8% for cysticercosis). Any nonspecific reaction or difference in size of band was observed by running the 100bp DNA ladder along with PCR products.

Table 4
Prevalence of cysticercosis in pigs by PCR

Name of slaughter house	Species	No. of carcasses inspected	No. of positive carcasses by PM	Carcasses found positive by PCR	% prevalence by PM	% prevalence by PCR
Deonar abattoir, Mumbai	Pig	600	02	02	0.33	0.33
Total					0.33	0.33

TAE (Tris/acetate/EDTA) solution 50 × was diluted upto 1 × by taking 20 ml TAE in 980 ml distilled water. Placed a clean gel caster on a horizontal smooth surface, sealed its open ends with a paper tape and fixed the appropriate comb into gel caster. Agarose gel was prepared by dissolving agarose in 100 ml 1 × TAE in a glass flask and mixed this carefully in a microwave set at 170°C for one minute and then again repeated until clear solution was formed. 5µl ethidium bromide (concentration of 0.5µg/ml) was also added at the base of the flask and allowed to cool before solidification this gel was poured on a plastic tray fixed on a stand containing a plastic comb for wells formation.

After solidification of the gel, the combs were removed. Gel was shifted into plastic tank containing TAE (1x), comb was removed carefully. Loading sample was prepared by adding 1µl (6x) loading dye. 5µl PCR product mixed and loaded in a gel from 2nd well. In a first well, 6µl DNA ladder (100 bp) was loaded. The electrophoresis apparatus was set at 80 volts, run for approximately 45 minutes. After required time carefully gel was removed and shifted on U.V Transilluminator of gel documentation system (Bio-Rad Gel Doc.) and photographed.

3.5.5 Estimation of prevalence of cysticercosis by PCR

Total of Two positive samples obtained during PM inspection from Deonar Abattoir of Maharashtra were processed for the detection of *T.solium* by PCR and the prevalence was estimated.

RESULTS AND DISCUSSION

By considering the importance of the disease a periodic survey for detection of cysticercosis in pigs was carried out during December 2014 to March 2015 in different parts of Maharashtra.

Gel Electrophoresis Showing All Positive Samples by PCR

Figure : Cox1 gene in positive cysticercus cyst PCR assay

PCR test with Cox1 primers to detect *Taenia solium* from pigs carcasses.

Lane 1 - 100-bp DNA ladder,

Lanes - 2 and 4 - test samples extracted from cysticercosis positive pigs.

Lane 12 - Negative control.

CONCLUSION

Keeping in mind the public health importance of zoonotic metacestodes and economic losses due to condemnation of infected organs/meat, the present research was undertaken to study the prevalence and attempt the molecular characterization of DNA of procured positive samples for detection of etiological agent of cysticercosis in the pigs of Maharashtra. For that, different slaughter houses comprising of pigs (600) were examined during postmortem inspection procedure for the presence of cysticerci of Maharashtra.

Prevalence of Cysticercosis in different areas of Maharashtra

In Maharashtra, only Deonar abattoir is having a scientifically designed pig slaughter unit. Hence, a total of 600 slaughtered pigs were inspected, out of which 02 pigs showed inspected samples. Thus the prevalence of cysticercosis noted was 0.33% in pig population. More number of cyst were found in thigh muscle followed by shoulder muscles, neck muscles and masseter muscles. Specifically for the detection of *T. solium*, total 600 slaughtered pigs were inspected at Deonar abattoir, Mumbai for the presence of *Cysticercus cellulosae* cysts. Out of those 2 positive cysticerci samples collected from Deonar

abattoir were processed for the genomic DNA isolation as well as analyzed by PCR assay which was found positive.

- It is recommended that the postmortem inspection is useful for the diagnosis of cysticercosis in slaughtered pigs
- In cysticercosis, the common site of predilection and more number of cysts were observed in thigh muscle followed by thigh, shoulder, neck, and masseter muscles.
- In case of porcine cysticercosis, the prevalence recorded from pigs slaughtered at Deonar abattoir, Mumbai showed 0.33% prevalence based on postmortem findings and PCR was detected positive as well.
- Because of public health significance of disease, hygienic measure during processing of meat and people awareness about the disease and safe disposal of infected viscera/ meat is required for effective prevention and control of disease.
- Owing to economic and public health significance of cysticercosis, it is necessary to conduct periodic surveys after every two to three years to monitor occurrence of human as well as porcine cysticercosis as revealed by number of reports of neurocysticercosis in human beings from different part of India.

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