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Cysticercosisis caused by metacestodes of Taenia solium, a two-host zoonotic cestodeof the order cyclophyllidea. It is primarily an infection of pigs that acts asan intermediate host of T.

solium and is an under-rated zoonotic diseaseclassified in list B by the OIE. Humans acquire cysticercosis throughfaeco-oral contamination with T. solium eggs from tapeworm carriers(Garcia and Del Bruto, 2000).

Cysticerci (larval cysts) can develop in any organof the body, but those develop in the CNS (brain and spinal cord) produce themost serious form of infection known as neurocysticercosis (NCC). It is an emerging public health andagricultural problem in South-East Asia, particularly in lesser developed areaswhere pigs are raised for consumption under traditional husbandry practices. Thecurrent state of knowledge concerning T. soliuminfections confirms the emergence of cysticercosis as a serious threat toeconomic productivity and human health in many areas of South-East Asia region(Rajshekhar et al, 2003; Ito et al, 2004).

Thelodgment and development of cysts in muscles and various visceral organs of pigproduces a series of inflammatory pathology. However, the animal usually remainsasymptomatic during the course of this disease (Singh et al., 2013). In some cases trembling, twitching, mouth and earparalysis, ataxia, dribbling, excessive salivation, eye blinking and tearing, walking in circles in infected swine were observed (Prasad et al., 2006; Trevisan et al., 2017).

There have been various researches in the field of cysticercosisregarding prevalence, serological and molecular diagnosis and epidemiology (D’Souza

etal., 1998; Boa et al., 2006; Sreedevi et al., 2011; Khaing et al., 2015). Itis sometimes difficult to ascertain the exact etiology merely on postmorteminspection. The metacestodes of Taenia solium can be misdiagnosed withmilk spots, Sarcocystis, T. hydatigena cysticerci or piece of fat and left over of muscle fasciae(Sreedevi, 2013).

Molecular techniques have been developed which are highlyspecific and sensitive in species-specific tapeworm detection anddifferentiation of collected parasite materials like proglottids and eggs in faeces(Yamasaki et al., 2004a; Nunes et al., 2005; Mayta et al.

, 2008), cysts (Yamasaki etal., 2004), affected muscle (Sreedeviet al., 2011), serum (Ramahefarisoaet al., 2010), histopathologicalsections (Yamasaki et al.

, 2004b; Shih et al., 2010) and cerebrospinalfluid (Yera et al., 2011). Thepresent study was conducted in pigs slaughtered for human consumption to knowthe prevalence of porcine cysticercosis, histopathology of affected organs andmolecular identification of cysts obtained from affected pigs in Nagpur andMumbai region of Maharashtra state of India. Materials and MethodsCollection of Taenia solium cysts A total of 1000 pigs comprising of 843 from Mumbai and 157 fromNagpur region of Maharashtra, India were examined after slaughter for presenceof metacestodesof Taenia solium (Cysticercus cellulosae).

Gross lesions werenoted and affected muscles from thigh, shoulder region, tongue, liver, diaphragm and brain were collected aseptically in sterilized polythene bags andtransported to the laboratory for parasitological,

histopathological andmolecular identification. Parasitological processing of cysts The cysts were dissected outaseptically from the muscles without any adherent host tissue. After washingthrice with cold Phosphate Buffer Solution (pH- 7. 2-7. 4), 2-3 numbers of cystsfrom each lot were pressed between two clean, grease free glass slides andexamined under microscope for morphological evaluation of scolex.

Histopathological processing Samples of muscle tissues and organs(tongue, liver, diaphragm and brain) with metacestodes were fixed in 10%formalin. After fixation, the tissues were processed, embedded in paraffin wax, sectioned at 4-5 ? m thickness and stained with Haematoxylin and Eosin. Molecular CharacterizationDNA isolation from cyst WholeDNA was extracted from the cysts collected from Nagpur and Mumbai region (designatedas N1, M1 and M2) using Qiagen DNeasy Bloodand Tissue Kit (Cat. Nos. 69504 and 69506) as per the manufacturer’s protocol.

Polymerase Chain ReactionOligonucleotide PrimersFormolecular identification of the cysts, two different PCR protocols(conventional and multiplex PCR) were employed. The conventional PCR was targeted against the large subunit rRNA gene (LSUrRNA) of Taenia solium using the primersTBR-3 (5?-GGCTTGTTTGAATGGTTTGACG-3?; positions 34–55) as forward and TBR-6 (5?-GCTACTACACCTAAATTCTAACC-3?; positions 319–297) as reverse (Jardim etal., 2006; Sreedevi et al., 2012). The multiplex PCR was targeted against cytochromec oxidase subunit 1 gene (cox1)of taeniid cestodes using different forward primers: 5?-TTGATTCCTTCGATGGCTTTTCTTTTG-3?, specific for T. saginata (positions 322-348); 5?-ACGGTTGGATTAGATGTTAAGACTA-3?, specific for T. asiatica

(positions 880-904) and 5?-TTGTTATAAATTTTTGATTACTAAC-3?, specific for T.

solium Asian genotype (positions 165-189). Reverseprimer 5?-GACATAACATAATGAAAATG-3? (positions 1148-1129), used in this study wascommon to all species (Yamasaki et al., 2004a). The oligonucleotide primers were synthesized at Eurofins Genomics IndiaPvt. Ltd.

(Bengaluru, India). Cycling conditionsAmplificationreactions were carried out in a volume of 25 ? l in 0. 2 ml PCR tubes in athermal cycler (Applied Biosystems, USA). For conventional PCR, 3 ? l of DNA templateand 10 pM of each TBR primer (forward and reverse) were added to the 2X Master Mix (Promega). Conventional PCR protocols consisted of one initial denaturationcycle at 94? C for 2 minutes followed by 40 cycles of denaturation (30 s at94°C), annealing (30 s at 59°C), and extension (60 s at 72°C), plus one cycleof 5 min at 72°C. Forthe multiplex PCR, 2 µl of DNA template and 10 pM of each forward and reverseCox I primers were added to the 2XMaster Mix (Promega). Standard multiplex PCR protocolsconsisted of one initial denaturation cycle at 94? C for 3 minutes followed by 35cycles of denaturation (30 s at 94°C), annealing (30 s at 60°C), and extension(90 s at 72°C), plus one cycle of final extension at 72°C for 5 min. Subsequently, PCR-amplified products were electrophoresed on 1. 5 % agarose gels.