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Short communication

Genetic similarity between *Taenia solium* cysticerci collected from the two distant endemic areas in North and North East India

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ABSTRACT

Taenia solium taeniasis/cysticercosis is a major public health problem in developing countries. This study reports genotypic analysis of *T. solium* cysticerci collected from two different endemic areas of North (Chandigarh) and North East India (Dibrugarh) by the sequencing of mitochondrial *cytochrome c oxidase* subunit 1 (*cox1*) gene. The variation in *cox1* sequences of samples collected from these two different geographical regions located at a distance of 2585 km was minimal. Alignment of the nucleotide sequences with different species of *Taenia* showed the similarity with Asian genotype of *T. solium*. Among 50 isolates, 6 variant nucleotide positions (0.37% of total length) were detected. These results suggest that population in these geographical areas are homogenous.

1. Introduction

Taenia solium, a neglected zoonotic parasite is endemic in underdeveloped countries where pig raising and pork consumption are not restricted (Dorny et al., 2009). *T. solium* infection is a cause of serious concern due to increase in morbidity and considerable economic loss through wastage of infected organs. Taeniasis is a disease of human intestinal tract caused by the adult tapeworm. The infection is acquired by the ingestion of undercooked pork contaminated with cysticerci. After ingestion, cysticerci migrate to the intestine, where it develops into an adult tapeworm and release proglottids filled with eggs which are passed in the stools. The ingestion of eggs by the intermediate host (pigs and human) results in the development of cysts in the soft tissues which progress to cysticercosis. Intestinal taeniasis leads to mild clinical manifestations and may remain asymptomatic. Human cysticercosis is caused when man becomes an accidental intermediate host either by ingestion of *T. solium* eggs or by autoinfection. The larval stage often localizes in subcutaneous tissue, skeletal muscles, central nervous system (CNS) and eye. Neurocysticercosis (NCC), the most severe form of cysticercosis results from the presence of cysticerci in the central nervous system. It is a major cause of adult acquired epilepsy and other neurological morbidity in many areas of the world (Nash and Garcia, 2011). It is estimated that approximately 50 million people are infected with taeniasis/cysticercosis and 50,000 deaths occur from cysticercosis annually, worldwide (CDC, 1993; Hoberg, 2002; Eddi et al., 2003). In India, taeniasis has a prevalence of 2–38% and 8.7–50% patients presenting with recent onset of seizure had NCC (Rajshekhar, 2004). In a seroprevalence study in and around Chandigarh, anti-cysticercus antibodies were found in 17.3% subjects with highest prevalence (24%) reported from slum areas, however only 8% of seropositive had history of epilepsy, suggestive of neurocysticercosis (Khurana et al., 2006). A hospital based clinico-serological study from Dibrugarh, Assam showed the seroprevalence of 78.43% in patients with ring enhancing lesion in Computed Tomography (CT) scan of brain suggestive of neurocysticercosis in this area (Kotokey et al., 2006). The prevalence of porcine cysticercosis varies from 7–26% in north and south India (Rajshekhar, 2004).

Knowledge of the genetic structure of cestode parasites can be applied to the epidemiology and control of these parasites, because genetic variants may differ in their infectivity and pathogenicity (Thompson, 1995). Mitochondrial DNA analysis of *T. solium* worldwide revealed that *T. solium* could be divided into two genotypes, Asian and American genotypes (Nakao et al., 2002). Clinical manifestations in human cysticercosis appear to be well correlated with the genotypes of *T. solium* (Campbell et al., 2006). In Asian countries, majority of the patients with NCC present with a single enhancement brain lesion and a very few patients have massive

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infections with multiple cysts (Singhi, 2011). In Latin America, the most common presentation of NCC is multiple cysts without signs of inflammation. Subcutaneous cysticercosis (SCC) is rare in Latin America (Cruz et al., 1994) but very common in Asia (Garcia et al., 2003). A possible reason for the clinical difference in different geographical areas might correlate with the variation in genotypes of *T. solium*, although other factors such as nutritional status and ethnic difference of patients cannot be ignored.

Molecular techniques based on Polymerase chain reaction (PCR) have been used for the differential diagnosis of species and strains and to gain knowledge of genetic diversity in the parasitic population (Brouwer et al., 2001; Eom et al., 2002; Kral'ova, 1996; Siles-Lucas et al., 1993). Mitochondrial sequences are widely used in molecular biology as genetic markers for ecological, phylognetic and evolutionary studies. Genetic variations provide the genetic material for natural selection leading to allele fixation within population and speciation. Within *cox1*, differential nucleotides are dispersed over the entire length and served as diagnostic marker for human taenid cestodes i.e. *T. solium*, *Taenia saginata* and *Taenia asiatica* or differentiation of 2 genotypes of *T. solium* i.e. Asian genotype and American/African genotype (Yamasaki et al., 2004). Martinez-Hernandez et al., 2009 have analyzed the different mitochondrial gene (*cox1*, *cyt b* and *nad*) and nuclear genome (5.8S+ITS1+18S, LMWA1 and LMWA2) sequences and it was found that most of polymorphic sites were present in *cox1* gene. So in the present study, genetic analysis of isolates of *T. solium* cysticerci from 2 different endemic regions of India i.e. Chandigarh located in North India and Dibrugarh in North East India which are 2585 km apart was carried by comparing sequences of mitochondrial gene: *cytochrome c oxidase* subunit 1 (*cox1*).

2. Materials and methods

T. solium cysticerci were collected from 50 freshly slaughtered and heavily infected pigs from slaughter houses located in Chandigarh ($n = 25$) and Dibrugarh ($n = 25$) respectively. The pigs were raised in widely different geographical areas in North and North East India and thus were considered from different pig breeds. The carcass containing cysts were transported to the department of Parasitology, PostGraduate Institute of Medical Education and Research (PGIMER), Chandigarh and Regional Medical Research Center, Dibrugarh, respectively for collection of cysticerci. The cysticerci were separated, washed with distilled water and examined under the microscope for morphological confirmation. These were fixed in 95% ethanol and stored at −20 °C till further use. Cyst from each animal was considered as an isolate. The cysts collected at Dibrugarh were transported to Chandigarh under refrigerated condition for further processing.

2.1. Molecular analysis

DNA extraction: The cyst samples were washed thrice in PBS to remove ethanol and genomic DNA was extracted from each sample by QIAamp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

For molecular identification, PCR amplification of *cox1* gene was performed by using the primers and PCR conditions as described previously (Nakao et al., 2002) with minor changes. Briefly, PCR was carried out in a 50 µl reaction mixture containing 2 µl DNA, 0.2 mM premixed solution of dNTP, 10 pmol of each primer, 1x PCR buffer, and 1 U of TaqDNA polymerase (Bangalore Genei). Amplification program included an initial denaturation step of 95 °C for 5 min and 30 cycles each of denaturation (95 °C for 30 s), annealing (56 °C for 30 s), extension (72 °C for 90 s) and final extension of 72 °C for 10 min. After agarose gel electrophoresis (1%), PCR products were purified and sequenced.

2.2. Phylogenetic analysis

Previously published sequences of *Taenia* species retrieved from the National Center for Biotechnology (http://www.ncbi.nlm.nih.gov) were used as the reference sequences (Fig. 1). Nucleotide sequence analysis was performed with BLAST sequence algorithms and sequences were aligned using Clustal W (Thompson et al., 1994). *T. saginata* and *T. asiatica* sequences were used as outgroup. The genetic distance was calculated by using Kimura two-parameter distance estimates and samples were clustered using the Neighbour joining algorithm using Mega 4 software. Bootstrap analysis was performed using 1000 replicates.

3. Results

T. solium cysticerci were identified by the partial sequencing of *cox1* gene. The size of amplified PCR product was 1800 bp. For analysis nucleotide sequences of 1620 bp from all these isolates were aligned with reference sequences of other taenid cestodes i.e. *T. saginata*, *T. asiatica* and Asian and American/African genotype of *T. solium* (Fig. 1). In this study, single nucleotide polymorphism was found and no addition or deletions were detected. Among 50 North and North East Indian isolates, 4 haplotypes were found. These haplotypes were differed at 6 variant nucleotide positions (0.37% of total length).

Among 25 North Indian isolates, three haplotypes (Accession number KC709806, $n = 20$; KC709808, $n = 2$ and KC709809, $n = 3$) were found and these showed polymorphism at nucleotide positions 52 (G–A), 291 (A–G) and 524 (C–T). The predominant haplotype reported in this study with accession number KC709806 showed 100% homology with *cox1* gene sequence of *T. solium* cysticercus from India, Nepal, Japan, Madagascar (AB066489, AB491986, AB516957, AB781355, respectively), 99.9% homology with Madagascar (AB781357), 99.8% homology with China, Thiland, Korea, Nepal, Madagascar (AB066485/GQ402327, AB066487, DQ089663, AB491985, AB781359, respectively), 99.5% homology with Indonesia: Irian Jaya (AB066488), 99% homology with Tanzania (AB066493) and 98.8% homology with Brazil, Cameroon and Mexico and Madagascar (AB066492, FN995665, FN995660 and AB781361, respectively). Isolates in this haplotype showed 99.8% homology to North East Indian isolates (KC709811).

The nucleotide sequence of all 25 North East Indian isolates (Accession number KC709811) showed 100% homology with each other and differed from North Indian isolates at nucleotide positions 366(C–T), 1041 (A–G) and 1164 (C–T). Interestingly, these isolates like American and African genotype has 'T' at position 1164 and showed 99.8% homology with *cox1* gene sequence of *T. solium* cysticercus from India (India, Nepal, Japan, Madagascar (AB066489, AB491986, AB516957, AB781355, respectively), 99.7% homology with Madagascar (AB781357), 99.6% homology with China, Thiland, Korea, Nepal, Madagascar (AB066485/GQ402327, AB066487, DQ089663, AB491985, AB781359, respectively), 99.3% homology with Indonesia:Irian Jaya (AB066488), 99% homology with Tanzania (AB066493), 98.8% homology with Madagascar (AB781361) 98.9% homology with Mexico (FN995660) and 98.7% homology with Brazil, Cameroon (AB066492, FN995665), respectively.

Further in dendrogram analysis, all the isolates were clustered with Asian genotype of *T. solium* (Fig. 1). All the North Indian isolates were clustered in one clade and all the North East Indian isolates clustered together and represented the same clone.