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Short communication

Comparison of cerebral and non-cerebral coenurosis by genetic markers of glycolytic enzyme (enolase) and mitochondrial sequences in sheep and goats

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ABSTRACT

Coenurosis is a debilitating disease caused by the larval stage of *Taenia multiceps*. The larval stage of *T. multiceps* appears in two cerebral and non-cerebral forms known as *Coenurus cerebralis* and *Coenurus gaigeri*,respectively. This leads to the hypothesis that there are genetic intraspecific variabilities within this species. Molecular analysis in the present study showed that *C. cerebralis* and *C. gaigeri* were 100% genetically identical based on exonic region of enolase (*ENO*) gene and both mitochondrial (*cox1* and *nad1*) markers. In addition, an intronic mutation at *ENO* gene (position: 1171) was detected in the Iranian *C. cerebralis*. The phylogenetic analyses based on the *ENO*, *cox1*, *nad1* sequence data also suggest that *T. gaigeri* may not be distinct from *T. multiceps* and there is only one single valid species within *T. multiceps*.
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1. Introduction

Coenurosis is mainly a disease of sheep, goats and cattle which is characterized by locomotor disturbances. The causal agent of this disorder is *Coenurus cerebralis*, the larval stage of the dog tapeworm *Taenia multiceps*; it develops in the brain or spinal cord and produces pathognomonic lesions (Akbari et al., 2015). Some documents have reported occurrence of coenurosis in tissues other than brain or spinal cord; such coenuri has been referred as *Taenia gaigeri* in goats and has mainly been reported from the Asian countries (Oryan et al., 1994, 2014).

The location of the cyst(s) outside the central nervous system (CNS) suggests that they may be a different strain or genetic variant of *T. multiceps* or may reflect a different host response to the parasite in goats (Rostami et al., 2013). The taxonomy of tapeworms belonging to the family Taeniidae has been controversial because of the paucity of adult phenotypic characters and the great plasticity of the larval form in different intermediate hosts (Nakao et al., 2010). However, molecular examination of coenuri structures helps in clarifying the taxonomic status and phylogenetic position of the parasite.

Therefore, the present study was carried out to elucidate the molecular evaluation of the two larval forms of *C. cerebralis* and *Coenurus gaigeri*. In addition to the known mitochondrial markers, we used a glycolytic enzyme, enolase (*ENO*) gene for the first time, as a molecular marker, for characterization and differentiation of the larval stages of *T. multiceps*.

2. Materials and methods

2.1. Collection of the cyst isolates

Between March 2013 and September 2014, a total of 25 isolates from the larval stage of *T. multiceps* were obtained from the naturally infected sheep and goats (5 cerebral and 20 non-cerebral cysts) in Fars Province, Iran. The protoscolices were removed from the cysts, washed three times with saline and stored at −20 °C before extraction of the genomic DNA.

2.2. Extraction and amplification of the genomic DNA by PCR

Genomic DNA from 25 samples including the coenuri cysts obtained from brain and muscles was extracted, using

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a commercially available kit (Qiagen DNAeasy; Qiagen, Valencia, California) according to the manufacture's protocol. Two specific primers of enolase gene (*ENO*, 1533 bp) (forward: 5′-ATCAGATGTTTGCGTGAGTTT-3′ and reverse: 5′-GGTGATTACAAAGGATTGCGGAAGT-3′) were designed, using the software Primer Premier 5.0 based on *ENO* gene of *T. multiceps*(accession number JQ627651). The specificity of these primers was checked on all sequences available from the GenBank database, using the Basic Local Alignment Search Tool (BLAST) program (www.ncbi.nlm.nih.gov/BLAST/). The primers were synthesized by CinnaGen Company (Tehran, Iran). In additional the nucleotide sequences of mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*, 444 bp) and NADH dehydrogenase 1 (*nad1*, 462 pb) genes were amplified, using the previously described methods (Zhang et al., 2007; Lavikainen et al., 2008; Oryan et al., 2010).

2.3. DNA sequencing and analysis

The products were directly sequenced, using a capillary DNA analyzer (ABI 3730; Applied Biosystems, Foster City, California) after sequencing reactions with a Big Dye Terminator v. 3.1Cycle Sequencing Kit (Applied Biosystems). Further comparison of the continuous sequences was made with the previously available taeniid sequences of the mitochondrial DNA (*cox1* and *nad1*) and mRNA of (*ENO*) in NCBI (National Center for Biotechnology Information), using CLC Main Workbench 5 software (CLC Bio, Aarhus, Denmark). Creation of multiple-sequence alignment and construction of a phylogenetic tree were determined, using Clustal W program, Neighbor-Joining (NJ) method in the MEGA 4 soft ware (Tamura et al., 2007). In addition, the G + C content of the *ENO* was calculated in different taxa, using Oligo Calculator#XPS##x00AE;.The *ENO* mRNA (1080 pb), and the predicted amino acid sequences were aligned and compared, using the CLC Main Workbench 5 software.

3. Results

All the three fragments (*ENO*, *cox1* and *nad1*) were successfully amplified from the cerebral and non-cerebral isolates by PCR method. No amplification product was detectable in the negative controls. The assembling of DNA sequence in both directions, using forward and reverse primers, yielded a fragment containing 1533, 396 and 462 consensus nucleotides for *ENO*, *cox1* and *nad1* regions, respectively. The GenBank accession numbers were provided for *ENO* (KP982890, KP982891), *cox1* (KT253933, KT253934) and *nad1* (KT253935 and KT253936) for the cysts obtained from the brain (naturally infected sheep: strain B) and muscle sources (naturally infected goats: strain M), respectively. All samples examined in our study were 100% identical to each other at *ENO*, *cox1* and *nad1*. However, intra-specific variations were detected between the Iranian *T. multiceps* strains and other strains existing in the GenBank database based on *cox1* (0–3.03%) and *nad1* (0–2.85%). The *T. multiceps cox1* sequences of JX507239 and HM143886 showed the lowest (0%) and highest (3.03%) genetic diversity to our isolates, respectively. Both *T. multiceps* strains were originated from China. Also, the lowest (0%) and highest (2.85%) genetic diversity to our *nad1* sequences belonged to the Iranian (HM101470) and UK (AJ239104) isolates.

The G + C contents of the *ENO* gene sequences of *T. multiceps* isolates were 52%. Compared to the previously reported *T. multiceps*(JQ627651), the *ENO* sequences alignment showed that the cerebral strain (B) in the present study showed a point mutation (T to C transition) at position 1171. Interestingly, this position showed sequence heterogeneity (Y: both C and T) in the non-cerebral strain (M). This polymorphic region in *ENO* was intronic, so no amino acid change in this region was observed. In addition, there was a noticeable interspecific variation in *ENO* sequences of different *Taenia* sp. in which 26 amino acid substitutions were found among different *Taenia* species.

Comparison of the extracted *ENO* mRNA sequence of our *T. multiceps* strains to the published mRNA sequences for other *Taenia*species showed that the most genetic homology (100%) belonged to the previously reported sequence for *T. multiceps* (JQ627651) and followed by 88%, 97% and 98% to *T. asiatica* (LM127470), *Taenia solium* (KF658454) and *Taenia pisiformis* (KF040090), respectively. In addition, the phylogenetic trees based on *ENO*, *cox1* and *nad1* showed that both cerebral (B) and non-cerebral (M) forms of larval stages were grouped into the *T. multiceps*clade.

The result of the phylogenetic analysis of enolase is shown in Fig. 1. Based on *ENO* phylogenetic tree, the closest taxa to our *T. multiceps*strains were *T. asiatica* (LM127470, EF420377) and *T. solium*(KF658454). Comparison of the amino acid sequence of *ENO* showed that our strains had 100% identity with that previously reported for *T. multiceps*(JQ627651) in GenBank. However, *T. pisiformis* showed the greatest amino acid diversity (88.41%) to our *T. multiceps* isolates. Unexpectedly, *Echinococcus granulosus*(GU080332) was more closely related to the *Taenia* clade in comparison to the *T. pisiformis* (KF040090) based on the *ENO* sequence analysis.

4. Discussion

Circumscription of *Taenia* species based on morphology has remained problematic. Approximately 42 valid species and three

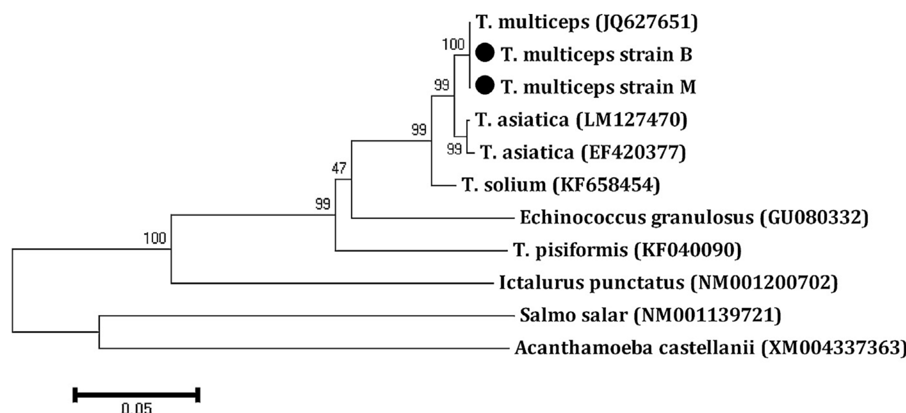

Fig. 1. Phylogenetic tree based on *ENO* gene sequence data, constructed according to the Neighbor-Joining (NJ) algorithms, showing the position of *T. multiceps*(strain B and M), compared to other related species in the GenBank. Numbers above the branches indicate bootstrap values (%) from 1000 replicates.

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