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Case Report

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Taeniasis, a neglected tropical disease, from Sistan and Baluchestan, Iran

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ABSTRACT

The World Health Organization (WHO) reports that human taeniasis is a neglected tropical disease. It has a worldwide distribution, even in developed countries. Three species of Taenia (*Tania saginata, Taenia solium,* and *Taenia asiatica*) can infect humans. The definitive hosts are humans, while intermediate hosts are cattle or pigs. Consuming raw or undercooked beef can lead to *Taenia saginata* taeniasis, while the primary source of infection for *T. asiatica* and *T. solium* is raw or undercooked pork. *Taenia saginata* taeniasis is the most prevalent in Islamic countries such as Iran, in which pork consumption is very low. It has been reported that human taeniasis has a prevalence between 0.0028% to 3% in Iran. Little is known about the molecular characterization of *T. saginata* in Iran. In this study, *T. saginata* was diagnosed based on its morphological and molecular characteristics. This is the first report on the molecular definition of *Taenia saginata* from Sistan and Baluchestan, Iran.

Keywords

Taenia, Taenia saginata, Taeniasis, neglected tropical disease

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Abbreviations

WHO: World Health Organization

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Case Description

According to the World Health Organization (WHO), human taeniasis is a neglected tropical disease. The disease is globally distributed, even in developed countries [1]. *Taenia saginata* is the most prevalent species of tapeworm worldwide [1].

The epidemiology of taeniasis is associated with cultural practices, such as consuming undercooked meat or eating organs of intermediate hosts infected with viable metacestodes[1].

Humans can be affected by three species of Taenia: (*Taenia solium, Taenia asiatica,* and *Tania saginata*). The consumption of raw or undercooked beef can lead to *Tania saginata* taeniasis in humans, while the primary source of infection for *T. asiatica* and *T. solium* is raw or undercooked pork. The only definitive hosts are humans, while intermediate hosts are cattle or pigs[1].

Although *T. saginata* cannot use humans as intermediate hosts, *T. solium* can cause neurocysticercosis in humans. Human taeniasis caused by T. saginata has been reported in different parts of Iran, including Mazandaran, Guilan, Golestan, Tehran, Alborz, Khorasan-e-Razavi, Ardabil, Esfahan, Fars, and Ilam provinces in the form of case reports or original studies[2-5].

According to previous research, human taeniasis has a prevalence between 0.0028% and 3% in Iran [6, 7].

This study employed morphological and molecular techniques to identify *Taenia spp*. in a human specimen from Sistan-and-Baluchestan, Zabol.

A man aged 46 visited the doctor with complaints of chronic abdominal pain, flatulence, and dyschezia. He reported passing *Taenia proglottids* in his feces. He worked as a government employee and sometimes slaughtered a calf for personal use. However, he stated that he had never eaten raw beef. It is possible that he ingested contaminated material while handling the carcass and touching his mouth. The patient was treated with praziquantel and, after three months, showed no symptoms, with no evidence of eggs/proglottids in the stool sample.

Morphological examination

The proglottids were sent to the parasitology laboratory for morphological analysis. To do this, the specimens were pressed between two microscope slides and stained with the acid carmine method [8]. Under the microscope, morphological characteristics such as the number of uterine branches were carefully examined[9].

Molecular Analysis

Cytochrome c oxidase subunit I (coxI genes) of mitochondrial DNA has been used to study genetic structures in taeniid cestodes. The coxI gene shows genetic diversity among taeniid cestodes, including *E.granulosus, T.taeniaeformis,* and *T.saginata* [10].

The DNA was extracted using the DNA Blood & Tissue kit (MBST, Tehran, Iran) according to the manufacturer's protocol.

The DNA sample was then subjected to PCR amplification of the mitochondrial cytochrome c oxidase (cox1) genes using specific primers : (cox1 F=5`-CATGGAATAATAATGATTTTC-3`) and (cox1 R =5`-ACAGTACACACAATTTTAAC-3`) as mentioned by Anantaphruti [11]. The total reaction volume for PCR was 25 µl, which contained 12.5 µl of 2X Master mix, 1 µl of each primer (10µM), 2 µl of template DNA (approximately 100 ng), and 8.5 µl dH2O.

The PCR procedure consisted of 38 cycles with the following steps: initial denaturation for 5 minutes at 94°C, followed by denaturation for 30 seconds at 94°C, annealing for 45 seconds at 50°C, and extension for 45 seconds at 72°C. Finally, there was a step of final extension for 10 minutes. Each PCR reaction included distilled water as a negative control and Taenia DNA as a positive control. After the procedure, the PCR products were electrophoresed on a 1.5% agarose gel in 0.5× Tris–borate–EDTA buffer and stained with CyberSafe. The PCR product was purified and sequenced using the Sanger method (Pishgam).

A phylogenetic tree was created using the CoxI gene and a representative selection of available sequences from GenBank. The maximum-likelihood method in MEGA was used for this, along with the Tamura-Nei model of nucleotide substitution and 1,000 bootstrap replications. The mitochondrial coxI gene sequence of Echinococcus multilocularis (NC000928) was used as an outgroup for phylogenetic analysis.

Morphological characteristics, such as the number of uterine branches, were examined microscopically. The specimen was identified as T. saginata, because it had over 16 uterine branches (Fig 1). The PCR analysis revealed a PCR with a length above 1200 bp (Fig.1). The amplicon was sequenced and then registered under the accession number OR889487 in GenBank. Upon sequence analysis, it was found that the amplicon had 100% identity to *T. saginata* strains registered in GenBank under the following accession numbers: MT074050 (Cambodia), MW750280 (South Korea), AB533173, AB465239,

AB465235 (Thailand).

The nucleotide sequence obtained in this study exhibited 99.9% similarity to the *T.saginata* sequence data registered under accession numbers: MN452862 (China) and AB107245 (Thailand) and 99.8% identity to the *T. saginata* sequence data registered under accession numbers: MK644930-MK644934 (South Korea), AB984351, AB533172 (China), AB465244(Japan), AB107244 (Thailand), AB107242 (Belgium), AB107240 (Indonesia: Bali), AB107238 (Ecuador). Despite the difference in the nucleotide sequence, the translated amino acid sequence had 100% similarity with the abovementioned records.

Based on phylogenic analysis, the CoxI sequence retrieved from this study clustered with the *T. saginata* cox I gene, distinct from *T. solium* and *T. sciatica* (Fig2).

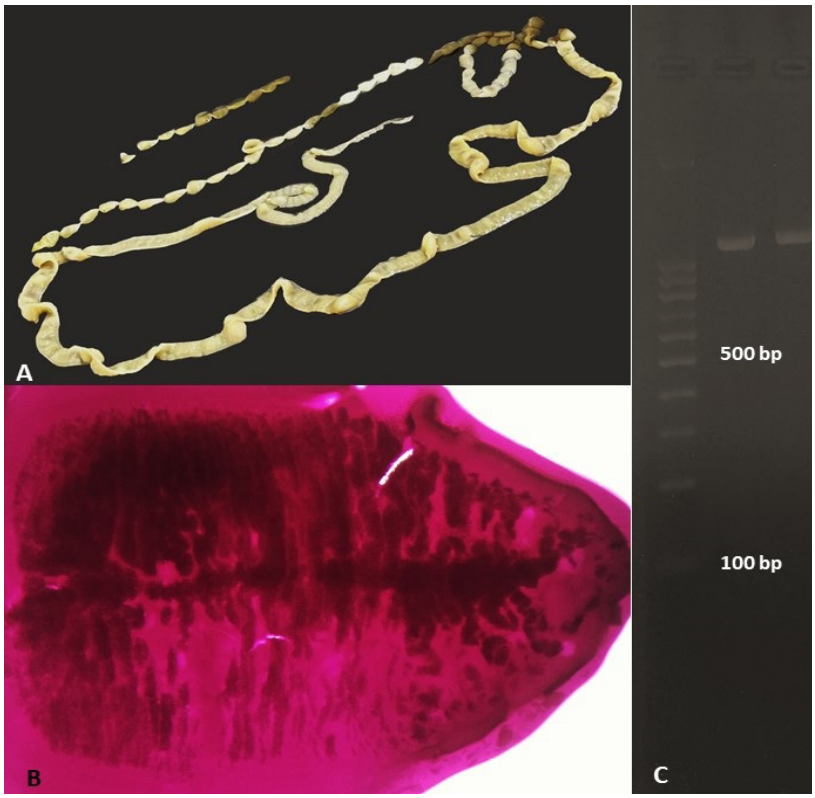

Figure 1.
A) Unstained Taenia B) Carmine acid stained proglottid of Taenia. C: Gel electrophoresis of 924 bp targeting the cox1 fragment of Taenia saginata using PCR.

The diagnosis was *T. saginata* despite the similarity of the lateral branches of the uterus in *T. asiatica* and *T. saginata*. This is because T. asiatica infection is primarily caused by consuming pork and boar meat or internal organs, which are not consumed in Muslim countries like Iran, where this study was conducted[12].

Humans with *T.saginata* taeniasis may be asymptomatic or experience symptoms such as digestive issues, itching of the anus, bloating, abdominal pain and discomfort, mild diarrhea, and weight loss.

In developing countries, human taeniasis is a significant public health problem that is usually diagnosed by observing eggs or gravid proglottids in the stool.

Taenia eggs cannot be used to distinguish between Taenia species. However, stained gravid proglottids can be used to differentiate between *T. saginata* and *T. solium* taeniasis. However, since pork consumption is very low in Iran, it can be assumed that most cases reported based on Taenia eggs are *T. saginata*[12].

Identifying Taenia species by morphological characteristics, such as the number of uterine branches in gravid proglottids, is challenging and requires considerable experience [13, 14]. In a fresh sample of gravid proglottid, proper staining should reveal a clear and sharp uterine structure.

Southeast Asian countries have reported *T. asiatica*, which has a similar morphology to *T.saginata.* However, pork consumption is very low in Islamic countries; its presence is also unlikely.

The final host, intermediate host, and environment influence human taeniasis transmission and spread. The spread of taeniasis in humans caused by *T. saginata* is linked to certain factors, such as consuming raw or undercooked beef, the health status of people, and proper management of municipal wastewater.

Various full articles and case reports [2] have documented cases of human taeniasis caused by *T. saginata* in Iran. Additionally, there have been reports of appendicitis caused by this parasite [5]. However, the genotype of *T. saginata* detected in Iranian patients is poorly understood.

Despite the morphological similarity between *T.saginata* and *T.asiatica*, the nucleotide sequence of the two showed a significant difference in comparison. Anantaphruti et al. conducted a study in Thailand that sequenced a partial region of the cox1 gene (924 bp) and identified 14 haplotypes among *T. saginata* isolates. The most common haplotype was A, followed by B. The study concluded that the sequence obtained from *T. saginata* in this research is similar to haplotype A[11].

Managing *T.saginata* human taeniasis requires a comprehensive approach that includes better health education, improved sanitation, enhanced beef inspection, accurate diagnosis, effective treatment, and

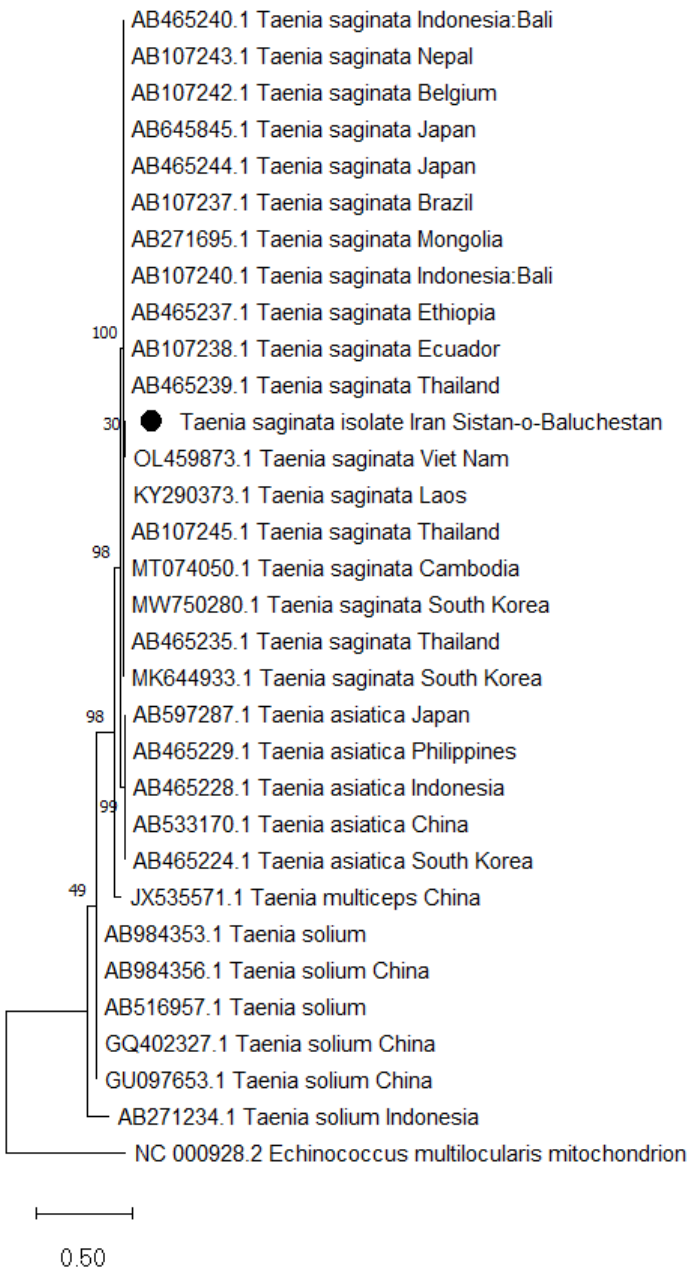

Figure 2.
The phylogenetic tree was drawn based on cox1 sequences of Taenia sequence in this study and retrieved representative sequences from GenBank. The phylogenic tree was inferred using the Maximum Likelihood method and the Tamura-Nei model. Evolutionary analyses were conducted in MEGA11.

close monitoring of taeniasis cases. It is important to remember that greater consumption of illegally slaughtered beef could result in a rise in human taeniasis cases.

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Conflicts of Interest

We have no conflicts of interest related to this work.

Authors' Contributions

M. K. S. collected the sample, E.E. supervised the laboratory tests and wrote the original draft, M.A.D., and J.K. performed laboratory tests, and S.N. edited the manuscript.

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