

Human Taeniasis in Slovakia (2010–2019): Genetic Analysis of *Taenia saginata* Isolates

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Abstract

Taenia saginata and *Taenia solium*, known as beef and pork tapeworm, are foodborne pathogens of global importance having a substantial impact on human health and economy. The study aimed to summarize the occurrence of human *Taenia* spp. infection in past 10 years in Slovakia based on reports of clinicians and diagnostic laboratories to Public Health Authority of the Slovak Republic. Altogether, 19 human cases were reported in Slovakia in 2010–2019, with the incidence of infection ranging from 0.00 to 0.12/100,000 inhabitants per year. In two patients *T. saginata* infection was confirmed molecularly. Nucleotide sequences of the analyzed *nad1* gene fragments derived from both patients were identical and in a phylogenetic tree clustered together with *T. saginata* Tsa isolate (AM503345), as well as with a homologous sequence of the completely sequenced mitochondrial genome of *T. saginata*. Similarly, *cox1* nucleotide sequences derived from one of the patients and isolates from Asia and/or Europe were identical. A cluster for *cox1* partial gene sequence was placed separately from closely related *Taenia asiatica* and/or *T. solium* isolates in the phylogenetic tree.

Keywords: *Taenia saginata*, *Taenia solium*, epidemiology, genetic characterization, Slovakia

Introduction

TAENIA SAGINATA AND TAENIA SOLIUM are foodborne pathogens of global importance. Humans acquire taeniasis by eating undercooked or raw beef or pork containing cysticerci. Although *T. saginata* persists in many European countries, with both human and animal cases reported, less is known about *T. solium*. Nevertheless, despite industrialization of pig husbandry and improved sanitation, the suspicion of its ongoing transmission persists especially in Eastern and Central Europe (Trevisan *et al.*, 2018).

In Slovakia, only partial information has been published on the epidemiology of human taeniasis. Taeniid eggs were found in 4.9% of 81 children from segregated Roma settlements (Rudohradská *et al.*, 2012) and in 0.8% of 239 children from a village in eastern Slovakia (Štrkolcová *et al.*, 2014).

The study aimed to summarize the occurrence of human *Taenia* spp. infection in the past 10 years in Slovakia.

Moreover, the results of phylogenetic analyses are presented for two cases of *T. saginata* infection.

Materials and Methods

Data on human taeniasis were collected by the Public Health Authority of the Slovak Republic (PHA SR) continuously, since 2010 till the end of 2019. In Slovakia, taeniasis are classified into Group “D” diseases, which must be reported to the PHA SR after a positive laboratory result, usually using the Kato-Katz technique (Kato and Miura, 1954). To make the epidemiological situation comparable with other countries, the “Incidence of infection/100,000 inhabitants per year” was determined by dividing the number of new cases per year by the size of population and then multiplying by 100,000. The population size was determined by the Statistical Office of the Slovak Republic (SOF SR, 2020).

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In April 2017 and May 2018, two cases of *Taenia* spp. infection were diagnosed, and fecal samples were further analyzed by amplification of a 471 bp region of *nad1* and a 396 bp fragment of the *cox1* gene (Bowles *et al.*, 1992; Bowles and McManus, 1993).

The obtained sequences were compared with GenBank entries using Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990). For the alignment of homologous nucleotide sequences, the Clustal W program was used. Evolutionary analysis was performed using the maximum likelihood method and the Tamura–Nei model (Tamura and Nei, 1993). To show the relationships between *T. saginata* nucleotide sequences from the study and homologous *T. saginata* sequences and other *Taenia* spp. with zoonotic potential, phylogenetic trees were constructed using the MEGA X program (Kumar *et al.*, 2018).

Results

Altogether, 19 human cases were reported in Slovakia in 2010–2019, varying between 0 and 7 per year. The average patient age was 31.6 ± 17.9 years, and most were in the 30–40 years age category (Table 1). The incidence of infection was estimated at 0.00–0.12/100,000 inhabitants per year.

Infected patients came from all eight regions of Slovakia. Five cases were recorded in eastern Slovakia, eight in central, and six in western Slovakia. Of the six patients with reported etiological agent of infection, five were infected with *T. saginata* and one with *T. solium*. Six patients reported eating beef, one pork, and three patients unspecified meat products (Table 1). All patients were treated with albendazole and/or praziquantel or niclosamide and were subsequently coprologically negative.

T. saginata infection was molecularly confirmed in two 30-year-old men, one from southwestern (Patient 1), and one

from northwestern Slovakia (Patient 2). Both patients observed the repeated presence of taeniid proglottids in their feces and reported diarrhea, flatulence, and intensive abdominal pain. Patient 1 also suffered from arthralgia, myalgia, and insomnia, and blood analysis showed an increased relative eosinophil count. Patient 2 also suffered from heartburn, skin exanthema, and headache. Both patients mentioned the consumption of steak/steak tartare and underwent antiparasitic treatment.

PCR amplification of *nad1* and *cox1* genes fragments confirmed infection by *T. saginata*. Both *nad1* gene sequences (MH744544 and MH675892) were identical with each other. Using the maximum likelihood method, these sequences clustered together in a phylogenetic tree with, for example, *T. saginata* Tsa isolate (AM503345) as well as with a homologous sequence of completely sequenced mitochondrial genome of *T. saginata* (AY684274). A cluster comprising *T. saginata* sequences was placed separately from closely related *Taenia asiatica* (AP017670) and/or *T. solium* (AB086256) isolates in the tree. Similarly, the *cox1* partial nucleotide sequence (MN337881) obtained was compared with homologous entries from GenBank. Three separate clusters were created in the phylogenetic tree. The *T. saginata* group contained the nucleotide sequence obtained from a human patient in this study and identical *T. saginata* sequences from all over the world. These sequences were distinct from *T. asiatica* and/or *T. solium* from GenBank (Fig. 1).

Discussion

The study confirmed the presence of *Taenia* spp. in humans in Slovakia, with almost annual occurrence of new cases in 2010–2019. During this period, only the intestinal

TABLE 1. HUMAN CASES OF *TAENIA* SPP. INFECTION REPORTED IN SLOVAKIA, 2010–2019

Year	No. of cases	Etiological agent	Gender	Age	Region	Epidemiological anamnesis (meat consumption)
2010	4	<i>Taenia</i> spp.	M	34	Bratislava (WeS)	
		<i>Taenia</i> spp.	F	24	Bratislava (WeS)	
		<i>Taenia</i> spp.	M	54	Žilina (CeS)	
		<i>Taenia solium</i>	F	60	Žilina (CeS)	
2011	3	<i>Taenia</i> spp.	F	34	Banská Bystrica (CeS)	
		<i>Taenia</i> spp.	M	39	Žilina (CeS)	Beef
		<i>Taenia</i> spp.	F	33	Žilina (CeS)	Beef
2012	1	<i>Taenia</i> spp.	M	29	Žilina (CeS)	Meat products
2013	7	<i>Taenia saginata</i>	M	39	Bratislava (WeS)	Meat products
		<i>Taenia</i> spp.	M	28	Košice (EaS)	Pork
		<i>T. saginata</i>	F	3	Košice (EaS)	Beef
		<i>Taenia</i> spp.	F	3	Košice (EaS)	
		<i>Taenia</i> spp.	M	33	Prešov (EaS)	Beef
		<i>Taenia</i> spp.	M	75	Prešov (EaS)	
		<i>T. saginata</i>	M	21	Trenčín (WeS)	
2014	0	—	—	—	—	
2015	1	<i>Taenia</i> spp.	F	23	Žilina (CeS)	
2016	0	—	—	—	—	
2017	1	<i>T. saginata</i> ^a	M	30	Trnava (WeS)	Beef
2018	2	<i>Taenia</i> spp.	M	8	Nitra (WeS)	Meat products
		<i>T. saginata</i> ^a	M	30	Žilina (CeS)	Beef
2019	0	—	—	—	—	

^aPresented case.

CeS, central Slovakia; EaS, eastern Slovakia; F, woman; M, man; WeS, western Slovakia.

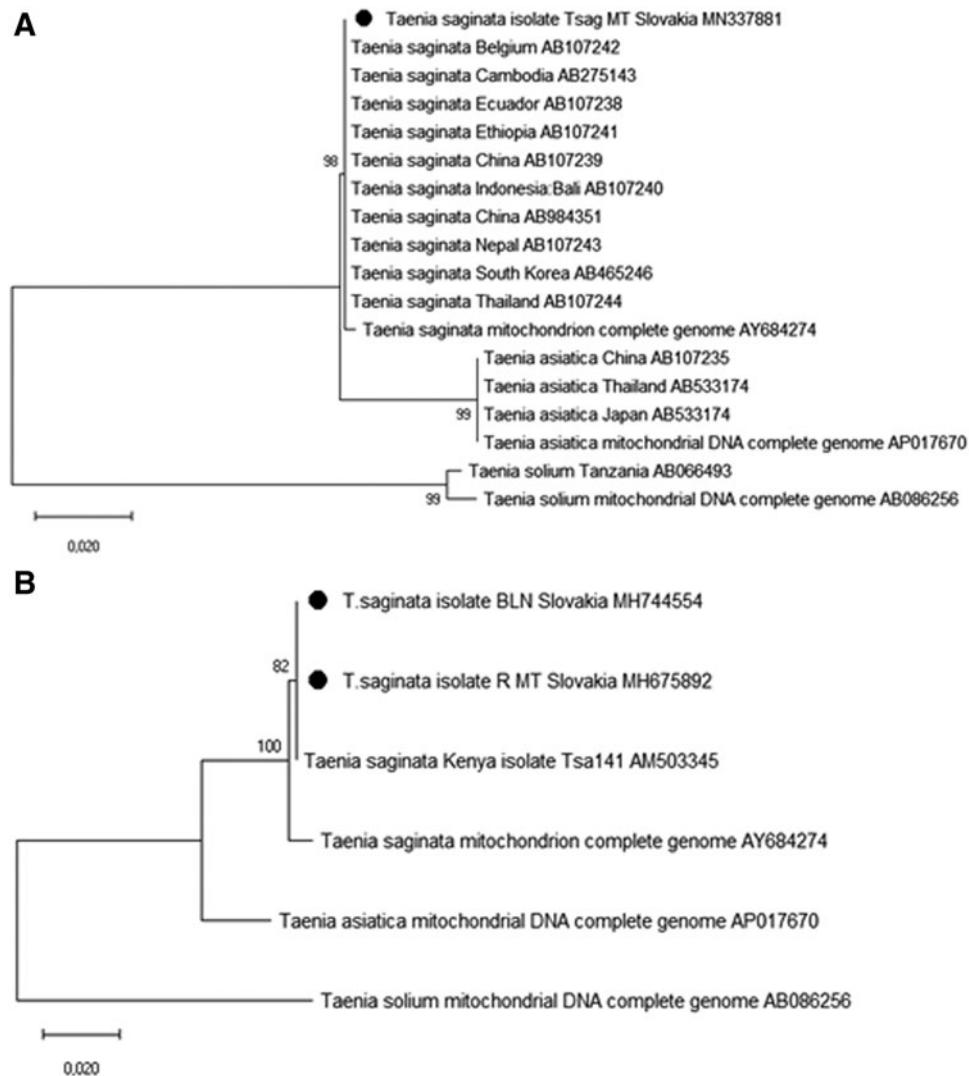


FIG. 1. Evolutionary analysis by maximum likelihood method using the 437 bp long fragment of the cytochrome C oxidase subunit 1 (*cox1*) gene (**A**) and the 488 bp long fragment of NADH dehydrogenase subunit 1 (*nad1*) gene (**B**). The evolutionary history was inferred by using the maximum likelihood method and Tamura–Nei model (Tamura and Nei, 1993). The tree with the highest log-likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. For *cox1* gene, the analysis involved 18 nucleotide sequences and there were a total of 437 positions in the final data set (**A**). For *nad1* gene, six nucleotide sequences were involved and there were a total of 488 positions in the final data set (**B**). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

form of the infection was reported, and based on the consumption of undercooked beef/pork, *Taenia saginata* infection was referred in five patients and *T. solium* in one. In two patients, *T. saginata* was confirmed molecularly.

Although morphological differentiation of *T. solium* and *T. saginata* proglottids is possible, it depends on the quality of the material and the experience of laboratory staff. Therefore, improvement in the differential diagnosis of human taeniasis is essential, and molecular analyses of stool samples or proglottids may be the best way to distinguish the species (Trevisan *et al.*, 2018). Moreover, this could be a relevant tool in suspected patients with negative coprology, where the sensitivity for detecting taeniid eggs is low (Széll *et al.*, 2014).

Genetic information on *T. saginata* is rare; thus, research on intraspecific variation and population genetics is necessary to gather data on the relationship between human and animal isolates from different countries. In this study, a *cox1* nucleotide sequence from a Slovak patient and isolates from Asia and/or Europe were identical. However, one polymorphic site was detected at position 23, corresponding to the 7654th nucleotide on the 13.67 kbp-long mitochondrial genome of *T. saginata* (AY684274). Within the *nad1* gene fragment, four polymorphisms were identified when compared with a fragment of the homologous gene of the mitochondrial genome of *T. saginata* (AY68424).

To conclude, the monitoring of *T. saginata* and *T. solium* is necessary, especially with regard to potential trans-border

transmission of infected meat and the spread of the infection by travelers and refugees entering the EU from endemic countries. Molecular characterization of both species can increase knowledge on the spread of these tapeworms across countries and continents.

Ethical Statement

The study was in accordance with the 1975 Declaration of Helsinki, as revised in 2013. The patients agreed with all examination and analyses and signed informed content.

Disclosure Statement

No competing financial interests exist.

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References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.
- Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Mol Biochem Parasitol* 1992;54:165–173.
- Bowles J, McManus DP. NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *Int J Parasitol* 1993;23:969–972.
- Kato K, Miura M. Comparative examinations. *Jpn J Parasitology* 1954;3:35.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
- Rudohradská P, Halánová M, Ravaszová P, Goldová M, Valenčáková A, Halán M, Papajová I, Pohorencová A, Valko J, Čisláková L, Juriš P. Prevalence of intestinal parasites in children from minority group with low hygienic standards in Slovakia. *Helminthologia* 2012;49:63–66.
- SOF SR, 2020. Demography and Social Statistics. Population and migration. Available at: https://slovak.statistics.sk/wps/portal/ext/home!/ut/p/z1/04_Sj9CPykssy0xPLMnMz0vMAfljo8ziA809LZycDB0NLPyCXA08QxwD3IO8TAwNTEz1wwkpiAJKG-AAjgZA_VFgJc7ujh4m5_j4GBhY-7qYGN04eoUGWgcbGBo7GUAV4zCjIjTDIdFRUBADse0bP/dz/d5/L2dBISevZ0FBIS9nQSEh, accessed May 18, 2020.
- Štrkolcová G, Goldová M, Halánová M, Mandelík R, Valenčáková A, Mojžišová J, Počátková O. Giardiasis, cryptosporidiosis and intestinal helminthoses in selected groups of children. In *Book of Abstracts. V4 Parasitological Meeting. Parasites in the Heart of Europe*. Institute of Parasitology SAS, Košice, Stará Lesná, May 25–30, 2014, p. 42.
- Széli Z, Sréter-Lancz Z, Sréter T. Evaluation of faecal flotation methods followed by species-specific PCR for detection of *Echinococcus multilocularis* in the definitive hosts. *Acta Parasitol* 2014;59:331–336.
- Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512–526.
- Trevisan C, Sotiraki S, Laranjo-González M, Dermauw V, Wang Z, Kärssin A, Cvetkovikj A, Winkler AS, Abraham A, Bobić B, Lassen B, Cretu CM, Vasile C, Arvanitis D, Deksne G, Boro I, Kucsera I, Karamon J, Štefanovska J, Koudela B, Pavlova MJ, Varady M, Pavlak M, Šarkūnas M, Kaminski M, Djurković-Djaković O, Jokelainen P, Stojčević JD, Schmidt V, Dakić Z, Gabriél S, Dorny P, Omeragić J, Alagić D, Devleeschauwer B. Epidemiology of taeniosis/cysticercosis in Europe, a systematic review: Eastern Europe. *Parasit Vectors* 2018;11:569.

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