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Original Article

Taenia asiatica: Mitochondrial signatures based analysis of an emerging public health threat in India

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ABSTRACT

Background: *Taenia asiatica* is a zoonotic tapeworm, commonly known as Asian *Taenia*. It is an emerging sister species of *T. saginata* with pigs as intermediate hosts. The present study aimed at genetic characterization and population structure analysis of *T. asiatica* metacestodes in slaughtered pigs in Haryana, north India.
Methods: In total, the vital organs of 253 slaughtered pigs were screened for the presence of *T. asiatica* metacestodes. The molecular identification and phylogenetics were performed targeting the mitochondrial NADH dehydrogenase subunit 1 (*nad1*) and cytochrome C oxidase subunit 1 (*cox1*) genes. The median-joining haplotype network and population structure analyses were performed with the sequences generated herein and GenBank-archived *T. asiatica* sequences for both mitochondrial signatures.
Results: Out of 253 pigs screened, the liver of only one animal showed the presence of *T. asiatica* metacestodes. The sequences generated herein exhibited 99.60 % and 98.85 % similarity to the GenBank-archived sequences of *T. asiatica* corresponding to the *nad1* and *cox1* genes, respectively. Overall, 2 and 6 haplotypes for the overall data set with low nucleotide (0.00399 ± 0.00237 and 0.00095 ± 0.00042) and low haplotype (0.400 ± 0.237 and 0.131 ± 0.054) diversities were recorded for the *nad1* and *cox1* genes, respectively. The negative values recorded for the neutrality indices exhibited deviations from neutrality and hence, propounded recent population expansion or purifying selection or selective sweep.
Conclusions: The findings of the present study are of significant medical importance considering an emerging global public health threat of the neglected tapeworm *T. asiatica*.

1. Introduction

The tapeworm species *Taenia solium*, *T. saginata* and *T. asiatica* are the etiological agents for human taeniosis [1,2]. *Taenia asiatica* was mistaken to be *T. saginata* for more than 200 years, and it was described as a different species by Eom and Rim in 1993 [3]. For the first few years, the parasite remained the subject of taxonomic debate for the morphological similarities of its adult stage to *T. saginata* [4]. However, its larval stage is viscerotropic in comparison to the musculotropic nature of the larvae of *T. saginata* and *T. solium* [5]. Now, *T. asiatica* is an established species and can be differentiated from the other two human *Taenia* based on morphological characteristics such as mature and gravid proglottids and scolex in adults, and bladder surface in the larval stage [3].

To date, *T. asiatica* has been reported from different Asian countries, including Korea, Taiwan, the Philippines, China, Thailand, Indonesia, Vietnam, Japan, Lao PDR, Nepal, and India, in definitive as well as intermediate hosts [2,3,6–15], thus, commonly known as Asian *Taenia* [16]. However, the morphological similarities between *T. asiatica* and *T. saginata* render identification confusions in sympatric populations [8]. The identification based on molecular tools involving mitochondrial markers revealed *T. asiatica* to be a sister species of *T. saginata* [5]. However, accurate identification of a parasite is extremely important to understand its infection biology, pathogenesis and eventually, for its control [17].

To understand the genetic relatedness and phylogeographical distribution of a pathogen, mitochondrial signatures/genetic markers are usually targeted due to their maternal inheritance, absence of recombination, and conserved structure with high evolution and mutation rates [18]. The phylogenetic relationship among different *Taenia* sp. (*T. asiatica, T. saginata,* and *T. solium*) has been assessed primarily with

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cox1 and *nad1* mitochondrial markers [5]. Both genetic markers have also been employed in the present study for the molecular characterization and phylogeographical analysis of the generated *T. asiatica* isolate along with the GenBank-archived isolates from different hosts. Furthermore, neutrality test analysis can contribute important information about the changes (evolutionary patterns, spatio-temporal dynamics, and genetic exchange) occurring in populations, which could prove instrumental in the implementation of effective control strategies. Keeping all the facts in mind, the present study molecularly characterized the metacestodes of *T. asiatica* in pig intermediate hosts and performed phylogenetic, median-joining haplotype network, and neutrality tests analyses of retrieved isolates with respect to the GenBank-archived Asian *Taenia* isolates.

2. Methods

2.1. Study area and sample collection

In the present cross-sectional study, the vital organs of 253 slaughtered pigs (intended for human consumption) were screened for the presence of metacestodes of *T. asiatica* at Chandigarh and Hisar in north India (Supplementary Fig. 1). The liver exhibiting the presence of metacestodes of *T. asiatica* was collected (Supplementary Fig. 2), cleaned thoroughly, and transported at 4 °C to the laboratory of the department of Veterinary Parasitology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India.

2.2. Genomic DNA extraction and PCR amplification

The genomic DNA of the metacestode was extracted with the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) as per the manufacturer's protocol. The DNA obtained was further subjected to amplification by targeting the cytochrome C oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes using the primers and protocols of Yamasaki et al. [19] and Bowles and McManus [20], respectively, with minor modifications (Supplementary Table 1). The amplicons were electrophoresed through a 1.25 % agarose gel along with a 100 bp marker (DNAmark™ 100 bp, G-Biosciences, USA) and then visualized under a gel documentation system (GelDoc Go Gel Imaging system, Bio-Rad, USA).

2.3. Sequencing and phylogenetic analysis

The amplified products (n = 1 for *cox1* and *nad1* each) were subjected to custom Sanger sequencing bi-directionally using the amplification primers (Barcode BioSciences, Bangalore, India). Analyzed sequences were aligned, joined, and edited to generate the consensus sequences using the BioEdit sequence alignment editor [21]. The NCBI nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm was used to search for similar or related sequences in GenBank. Finally, the consensus sequences of the identified *T. asiatica* species were deposited in GenBank under the accession numbers LC819657 (*cox1*) and LC819656 (*nad1*). The phylogenetic analysis of the *cox1* and *nad1* isolates generated herein, along with the GenBank-archived *cox1* (n = 74) and *nad1* (n = 4) *T. asiatica* sequences (Supplementary Tables 2 and 3, respectively) was performed by using Molecular Evolutionary Genetic Analysis (MEGA) 11.0.10 software [22]. For inferring the evolutionary history, the best substitution model was found to be the Tamura-Nei model for both genetic markers. Bootstrap analyses were conducted using 1000 replicates and the distance scale was estimated at 0.2. Other GenBank-archived human *Taenia* species; *T. solium* [MN973965 (*cox1*) and AY395068 (*nad1*)] and *T. saginata* [JN986713 (*cox1*) and OL422142 (*nad1*)] sequences were also included for the phylogenetic analysis. *Ascaris suum* [KT282029 (*cox1*) and MK160503 (*nad1*)] sequences were used as outgroups to root the phylogenetic trees for both genetic signatures.

2.4. Median-joining haplotype network and population structure analyses

The relationships between *T. asiatica* haplotypes for the mitochondrial *cox1* and *nad1* genes based on the country of origin were assessed by performing the median joining haplotype network analysis with PopArt software (http://popart.otago.ac.nz) [23]. The GenBank-archived *T. asiatica* sequences corresponding to mitochondrial *cox1* and *nad1* genes with 100 % coverage to LC819657 (*cox1* gene) and LC819656 (*nad1* gene) sequences were retrieved and analyzed. In total, 73 and 4 sequences corresponding to the *cox1* and *nad1* genes, respectively, including the sequences generated herein (n = 1 each) were involved in the median-joining haplotype network analyses (Tables 1 and 2 for *cox1* and *nad1* genes, respectively). Furthermore, the misidentified *cox1* sequences (n = 2; GU074019 and AF429314) were not included in the haplotype network analysis.

Population genetic differences were determined based on population diversity indices [number of variable sites, total number of mutations, number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π)] for the overall dataset by using DnaSPv6 software [24]. DnaSPv6 software was also used to calculate the neutrality indices (Fu and Li's F, Fu and Li's D and Tajima's *D*) to test the hypothesis of selective neutrality [24].

3. Results

Out of 253 slaughtered pigs screened, only one (0.39 %) revealed the presence of *T. asiatica* metacestodes in its liver. The amplification of the genomic DNA targeting the mitochondrial *cox1* and *nad1* genes of *T. asiatica* yielded the fragments of ~270 bp and ~500 bp, respectively.

Table 1
Various haplotypes of *Taenia asiatica* identified based on the *cox1* gene.

Haplotype	Number of sequences	Accession numbers (Country)
Hap_1	68	AP017670 (Japan), AF445798 (South Korea), NC_004826 (South Korea), AB533170 (China), AB533174 (Thailand), AB533175 (Thailand), KJ187960 (India), KJ187963 (India), KJ187964 (India), AB465211 (China), AB465212 (China), AB465213 (China), AB465214 (Indonesia), AB465215 (Indonesia), AB465216 (Indonesia), AB465217 (Thailand), AB465218 (Thailand), AB465219 (Thailand), AB465220 (Thailand), AB465221 (Thailand), AB465222 (Thailand), AB465223 (Thailand), AB465224 (South Korea), AB465225 (South Korea), AB465226 (China), AB465227 (China), AB465228 (Indonesia), AB465229 (Philippines), AB465230 (Taiwan), JQ517298 (Thailand), JQ517299 (Thailand), JQ517300 (Thailand), JQ517301 (Thailand), JQ517302 (Thailand), JQ517303 (Thailand), JQ517304 (Thailand), JQ517305 (Thailand), JQ517306 (Thailand), JQ517307 (Thailand), JQ517308 (Thailand), JQ517309 (Thailand), LC175223 (Japan), AB574399 (Japan), AB574474 (Japan), AB597275 (Japan), AB597278 (Japan), AB597281 (Japan), AB597284 (Japan), LC558225 (Japan), LC558226 (Japan), LC558227 (Japan), LC558228 (Japan), LC558229 (Japan), LC558230 (Japan), LC558231 (Japan), LC558232 (Japan), LC558233 (Japan), AB066494 (Taiwan), AB107234 (Taiwan), AB107235 (China), AB107236 (Indonesia), LC405943 (Japan), AB608742 (Japan), AB608739 (Japan), AB608736 (Japan), AB588922 (Japan), AB597287 (Japan), LC819657 (India)
Hap_2	1	KJ187961 (India)
Hap_3	1	KJ187962 (India)
Hap_4	1	GU074020 (China)
Hap_5	1	MN448470 (China)
Hap_6	1	MN448469 (China)

Table 2
Various haplotypes of *Taenia asiatica* identified based on the *nad1* gene.

Haplotype	Number of sequences	Accession numbers (Country)
Hap_1	4	AF445798 (South Korea), NC_004826 (South Korea), AP017670 (Japan), LC819656 (India)
Hap_2	1	AJ239108 (Taiwan)

The BLASTn analysis of the sequences generated herein exhibited 98.46–99.58 % and 98.94–99.60 % similarity to the GenBank-archived *T. asiatica* sequences corresponding to the *cox1* and *nad1* genes, respectively. The phylogenetic tree for the *cox1* gene of *T. asiatica* revealed the presence of two genotypes, A and B (Fig. 1). The present study isolate, along with GenBank-archived sequences corresponding to the definitive (human) and intermediate (pig) hosts, constituted the genotype A. However, the sequences retrieved from the Tibetan foxes, human, and pigs resulted in the formation of genotype B (Fig. 1). In addition, two sequences (GU074019 and AF429314) sorted themselves along with the outgroup, indicating that they were misidentified sequences (Fig. 1). Furthermore, due to the limited number of sequences available in GenBank for the *nad1* gene of *T. asiatica*, no such separation of sequences was observed (Fig. 2).

The median-joining haplotype network for the *cox1* gene exhibited the presence of a star-shaped configuration with a central predominant haplotype, Hap_1 (Fig. 3). It constituted the sequences from Japan, South Korea, China, Thailand, Indonesia, the Philippines, Taiwan, and India. Other haplotypes, Hap_2 to Hap_6, were found arranged around the central predominant haplotype, exhibiting 1–2 mutational steps. On the contrary, no such finding was observed for the *nad1* gene (Fig. 4).

The summary of the results for the neutrality tests and population diversity indices for both the mitochondrial signatures of *T. asiatica* are given in Table 3. A lower number of haplotypes (n = 6 and 2) with low nucleotide (0.00095 ± 0.00042 and 0.00399 ± 0.00237) and haplotype (0.131 ± 0.054 and 0.400 ± 0.237) diversities were observed for the *cox1* and *nad1* genes, respectively, for the overall datasets. Significant negative values were recorded for Tajima's *D* (−2.002, $p < 0.05$), Fu and Li's F (−3.497, $p < 0.02$) and Fu and Li's D (−3.440, $p < 0.02$) for the overall dataset corresponding to the *cox1* gene of *T. asiatica*.

4. Discussion

Human taeniosis/cysticercosis is a major public health concern in the developing countries due to their endemicity in Asia, Africa, and Latin America [25]. The present study attempted to molecularly identify the metacestode stage of *Taenia asiatica* in pig intermediate hosts based on two mitochondrial genetic signatures, *cox1* and *nad1*. The study also addressed the analysis of population structure and median-joining haplotype networks of *T. asiatica* for both genes. To the best of our knowledge, the sole previous molecular study targeting the occurrence of *T. asiatica* had documented its presence only in one state, Uttar Pradesh of India [15]. However, the infection rate recorded (0.39 %) in pig intermediate hosts in the present study was much lower than that of the previous study (25 %). The finding could be associated with the different geographical locations in both the studies, as pigs undergo limited anthropogenic mobility through trade. Furthermore, the 'Clean India mission', under which the Government of India had provided financial assistance to the economically weaker sections to build toilets in order to prevent open defecation, might have interrupted the transmission cycle of Asian *Taenia* in India [17]. However, a comprehensive molecular study involving different genetic signatures on the parasitic stages of *T. asiatica* in the intermediate and definitive hosts from different regions of India is warranted to further understand the actual epidemiology of the parasite.

In resource-limited settings or economically weaker communities in India, the ideal conditions prevail for the infection biology of porcine

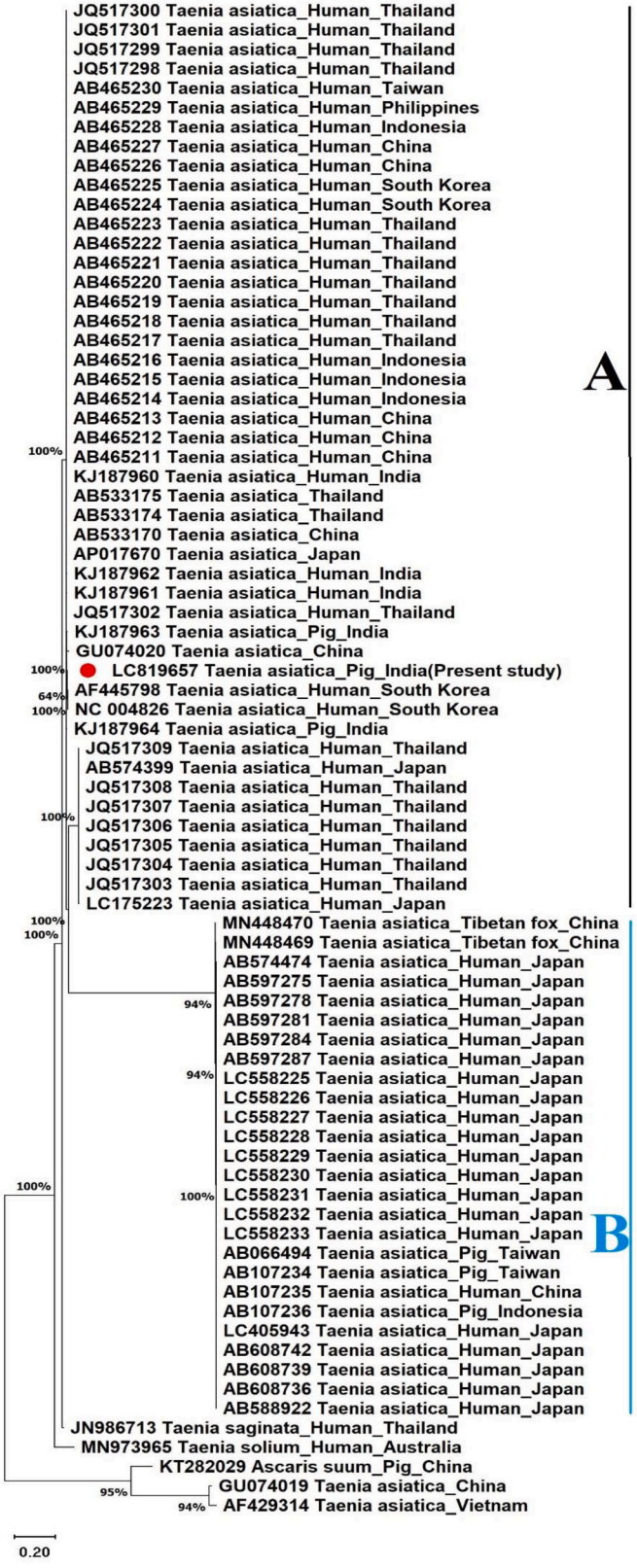

Fig. 1. Maximum likelihood tree inferred for *Taenia asiatica* from partial *cox1* gene sequence. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Bootstrap values are indicated on each node. The bar represents 0.20 substitutions per site. *Ascaris suum* (KT282029) was used as an outgroup species to root the tree.

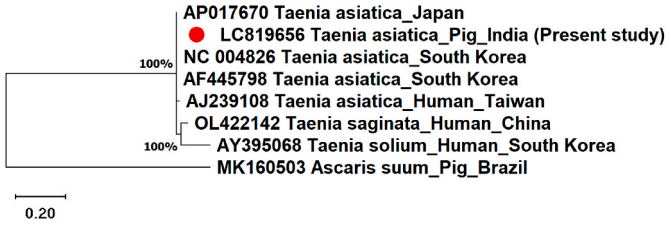

Fig. 2. Maximum likelihood tree inferred for *Taenia asiatica* from partial *nad1* gene sequence. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Bootstrap values are indicated on each node. The bar represents 0.20 substitutions per site. *Ascaris suum* (MK160503) was used as an out-group species to root the tree.

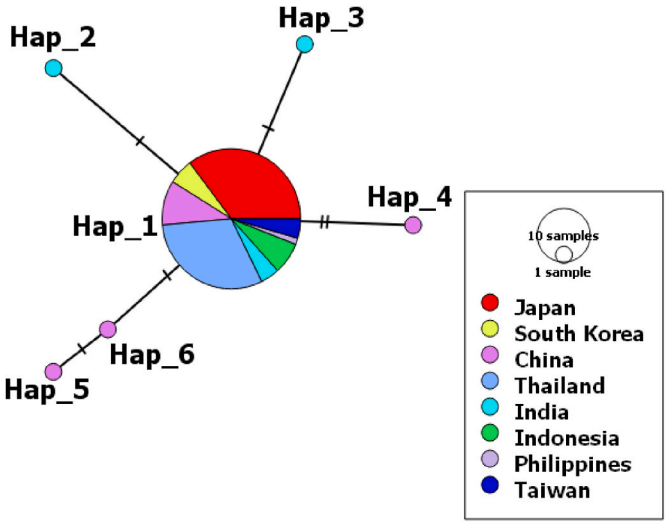

Fig. 3. The median-joining haplotype network of *Taenia asiatica* from different countries based on the partial mitochondrial *cox1* gene sequence. Each circle depicts a unique haplotype and the circle size is relative to haplotype frequency. Nucleotide differences/haplotype substitutions/mutations are denoted by the hatch marks/bars across the lines connecting the haplotypes with each bar representing a single nucleotide variation. A colour code to the country of origin is given.

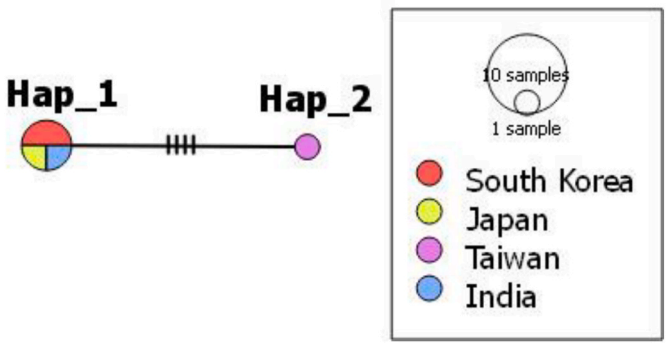

Fig. 4. The median-joining haplotype network of *Taenia asiatica* from different countries based on the partial mitochondrial *nad1* gene sequence. Each circle depicts a unique haplotype and the circle size is relative to haplotype frequency. Nucleotide differences/haplotype substitutions/mutations are denoted by the hatch marks/bars across the lines connecting the haplotypes with each bar representing a single nucleotide variation. A colour code to the country of origin is given.

Table 3
Genetic diversity and neutrality indices for *Taenia asiatica* isolates based on *cox1* and *nad1* genes.

Indices	*cox1* (~270 bp)	*nad1* (~500 bp)
Number of isolates/sequences	74	5
Number of mutations	6	4
Number of variable sites	6	4
Number of haplotypes	6	2
Haplotype diversity (Hd) ± SD	0.131 ± 0.054	0.400 ± 0.237
Nucleotide diversity (π) ± SD	0.00095 ± 0.00042	0.00399 ± 0.00237
Tajima's *D*	−2.002*	-1.093^{NS}
Fu and Li's D	−3.440**	-1.093^{NS}
Fu and Li's F	−3.497**	-1.113^{NS}

Note: Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.02$, ^{NS}-not significant ($p > 0.10$).

cysticercosis (due to *T. asiatica* or *T. solium*) [17]. Nevertheless, it is extremely difficult to adjudge the etiology of porcine cysticercosis at the postmortem as misdiagnosis could result due to milk spots, sarcocystosis, pieces of fat and left over muscle fascia [17]. Hence, researchers should opt for molecular characterization of the parasitic metacestodes to avoid misidentification [17].

Based on the geographical origin, nucleotide substitutions, and pathogenesis, two genotypes have been reported for *T. solium* [17]. However, no such observations have been reported in the case of *T. asiatica.* Nonetheless, in the present study, two different genotypes, A and B had been observed for the *cox1* gene of *T. asiatica*. Intriguingly, these genotypes did not exhibit any geographical delimitations/origin. Moreover, in the genotype B along with humans and pigs, Tibetan fox isolates were also arranged, which is an unusual introduction to the established life cycle. It was worth noticing that the median-joining haplotype network did not exhibit any genotypic distinction, and the sequences corresponding to both genotypes constituted the predominant haplotype, Hap_1. Whereas the Tibetan fox isolates constituting the Hap_5 and Hap_6 haplotypes differed from the predominant central haplotype by 1–2 mutational steps only. Since the median-joining haplotype network is capable of depicting reticulation events derived from non-vertical inheritance processes [26], it significantly allows to study the genetic relationship and diversity between sequences that belong to the same phylogenetic group. The star-shaped configuration of median-joining haplotype network depicted either a recent population expansion around a central node/founder population or a selective sweep due to purifying selection. Subsequently, purifying selection leads to reduced genetic differentiation in related genotypes or genes among populations inhabiting different geographical locations [27].

A low haplotype diversity observed in the present study for both genetic markers was quite evident from the number of very few haplotypes (n = 6 and 2 for the *cox1* and *nad1* genes, respectively) recorded in relation to the total number of sequences (n = 73 and 5 for the *cox1* and *nad1* genes, respectively). Whereas low nucleotide diversity values indicate only small nucleotide differences between haplotypes, and same was evident from median-joining haplotype networks [28]. The significant negative values recorded for the neutrality indices of *T. asiatica cox1* gene evinced either a recent expansion of the circulating *T. asiatica* population or a purifying selection that might have resulted in an excess of rare polymorphisms to neutral expectations in sequences [29,30]. The finding was in concordance with the observations of median-joining haplotype network.

5. Conclusions

Taeniois is a 'biological indicator' of the social or economic well-being of a community. Various contributory factors, including open defecation, poor sanitary and hygiene practices, culinary habits (uncooked, partially-cooked or smoked pork/pig liver consumption), free-roaming, open access of pigs to human excreta, and no separate place/sties for pigs in economically weaker communities, could play

significant roles in the transmission of infection. Albeit, a single animal was found positive for the zoonotic neglected parasite, it is worth noticing that *T. asiatica* is expanding its horizon in India. The findings of the present study are of significant medical importance considering an emerging global public health threat of the neglected tapeworm *T. asiatica* and warrant further detailed studies in definitive and intermediate hosts in different geographical regions of India.

CRediT authorship contribution statement

Aman D. Moudgil: Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Anil K. Nehra:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Pallavi Moudgil:** Writing – review & editing, Writing – original draft, Supervision, Methodology.

Data availability

Data will be made available on request.

Ethics statement

No studies involving laboratory animals or invasive techniques were conducted. The samples were collected from slaughtered animals at abattoirs.

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Declaration of competing interest

The authors declare that they do not have any competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2024.101562.

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