



RESEARCH ARTICLE

Molecular Characterization of *Hydatigera taeniaeformis* Recovered from Rats: An Update from Pakistan

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ABSTRACT

Rodents are one of the most abundant and successful mammals on the planet. The rats harbor the metacystode stage of *Hydatigera taeniaeformis* serving as the intermediate hosts, whereas cats act as definitive hosts of the parasite. The incidence of the larval stage in rats has been documented in various regions of the world. To best of our knowledge, the mainstream studies on the genetic diversity of *H. taeniaeformis* are based on the *cox1* gene which precludes an in-depth analysis of genetic variation in the parasite. To provide insights about *nad1* gene-based genetic variation, in continuation of our previous work, we thereby in this study report *H. taeniaeformis* infection in the urban murine population from the Faisalabad district of Pakistan and presented genetic polymorphism using the *nad1* gene. Out of 38 isolates investigated in the study, a total of 13 haplotypes were found with high haplotype diversity ($H_d = 0.909$), while the nucleotide diversity was found to be 0.02340 in the study population. The neutrality study found a significant level of nucleotide polymorphism, indicating a rise in low-frequency polymorphism, which might be attributed to global population growth of the parasite. Construction of phylogenetic comprising isolates from Kazakhstan, Finland, Turkiye, Canada, Germany, France, and China revealed that Pakistani isolates of *H. taeniaeformis* are distinct and formed a separate cluster. Further research utilising full-length multiple mitochondrial genes is required to understand the molecular epidemiology of *H. taeniaeformis* on the global scale.

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INTRODUCTION

The Taeniidae family (Order Cyclophyllidea) possesses four genera, namely *Hydatigera*, *Taenia*, *Echinococcus*, and *Versteria*, according to a recent revision. The genus *Hydatigera* contains four species: *Hydatigera taeniaeformis*, *Hydatigera parva*, *Hydatigera kamiyai*, and *Hydatigera krepkogorski* (Catalano *et al.*, 2019). Members of the genus *Taenia* and *Echinococcus* primarily cause production losses in animals.

Hydatigera taeniaeformis inhabits the intestinal tracts of felids (definitive host), and become infected with the larvae of *H. taeniaeformis* by consuming the liver of mice and rats (intermediate host) (Moudgil *et al.*, 2016). The definitive hosts spread eggs of *H. taeniaeformis* into the environment through feces that are taken up by the intermediate host, resulting in the development of cysts in the liver. The feeding habits between felines and rodents are responsible for the parasite life cycle to be completed (Jia *et al.*, 2012).

Infections caused by helminths, especially cestodes, pose a huge threat to animals as the majority of these infections remain latent. Mature *H. taeniaeformis* in the gut of cats and dogs seldom causes the onset of clinical symptoms, but if present, clinical signs vary depending on the severity of the infection, and the host's age, health, and breed (Kahn and Line, 2010). In rats, subclinical infection results in the creation of tumor-like growths on the liver (Kumar *et al.*, 2006) whereas chronic infections lead to gastric disorders like gastric hyperplasia and gastric enteropathy (Konno *et al.*, 1999). Praziquantel is considered to be the drug of choice for this parasite. However, the irrational use of anthelmintics leads to the development of resistance in helminths.

Rodents infected with *H. taeniaeformis* have been reported from different continents, namely Asia (India, Korea, China, Malaysia, and Pakistan), Europe (Serbia and Switzerland), Africa (Nigeria and West Indies), North America (Mexico), South America (Colombia) and Oceania (Australia) (Seong *et al.*, 1995; Chikweto *et al.*, 2009; Kataranovski *et al.*, 2010; Malsawmtluangi *et al.*, 2011; Burlet *et al.*, 2011; Duque *et al.*, 2012; McInnes *et al.*, 2014; Premaalatha *et al.*, 2016; Onoja *et al.*, 2017; Medina-Pinto *et al.*, 2019; Guo, 2020; Alvi *et al.*, 2021). Human cases of *H. taeniaeformis* infection have been reported in a number of countries, including Japan, Sri Lanka and Argentina (Ekanayake *et al.*, 1999; Hoberg, 2002).

Although many measures were devised to prevent and control cestode infection, strategies to reduce and overcome the infection are continuously influenced by the diversity of tapeworm species and variation among species. There is a significant gap in the molecular characterization of several members of the Taeniid family in underdeveloped countries. To best of our knowledge, the mainstream studies on the genetic diversity of *H. taeniaeformis* are based on the *cox1* gene which precludes an in-depth analysis of genetic variation in the parasite. To provide insights about *nad1* gene-based genetic variation, in continuation of our previous work, we thereby in this study report *H. taeniaeformis* infection in the urban murine population from the Faisalabad district of Pakistan and presented genetic polymorphism using the *nad1* gene.

MATERIALS AND METHODS

Study locale: District Faisalabad is one of the most famous agricultural and industrial cities in Pakistan. It has an extensive canal system and rivers that make this land highly fertile, seeking the attention of people towards husbandry practices and agriculture farming. As markets are the main source of food for rodents in the central part of the city, their population has significantly increased in this region. In continuation of research collaboration between the CMS Department of University of Agriculture, Faisalabad, Pakistan, and Lanzhou Veterinary Research Institute, Lanzhou, China, *H. taeniaeformis* isolates recovered from urban rats in a previous study performed by Alvi *et al.* (2021) were used in the current investigations and all samples were collected from district Faisalabad.

DNA extraction and amplification of the *nad1* gene: Already extracted DNA was used as a template to amplify the *nad1* gene portion using a pair of primers as reported

by Bowles *et al.* (1992). Briefly, the twenty-five microliter reaction volume consisted of 12.5 µl Premix, 10 pmol of each of reverse and forward primers, 0.5 µl genomic DNA, and RNase free water up to final volume. Agarose gel was prepared and stained with GelRed™. 5 µl of amplicon in each well of agarose gel were visualized under an ultraviolet light transilluminator. A DNA ladder (2000bp length) was run to estimate the sizes of each amplicon. Then PCR products were sent to the Chinese collaborators for sequencing.

Molecular analyses: DNA sequences were examined for any misread nucleotide with the aid of UGENE software. After alignment, the confirmation of each isolate was achieved through the BLASTn tool. The population diversity and neutrality indices were calculated using DnaSP. Phylogenetic analysis was carried out using a dataset of *nad1* (220 bp) gene sequences (representative haplotype) and other *Taenia* species. MrBayes software was used to construct the phylogenetic tree based on Bayesian method.

RESULTS AND DISCUSSION

Infectious diseases including parasitic infestations are important health problems in both animals and humans, which cause economic losses and severe illness (Pinilla *et al.*, 2022; Nawaz *et al.*, 2022; Dahab *et al.*, 2022). Parasites are responsible for causing diseases that lead to heavy economical losses in terms of decreased productivity and illness (Mo'awad *et al.*, 2022; Mahmoud *et al.*, 2022). *Hydatigera taeniaeformis* has two transmission cycles: a sylvatic cycle comprising wild rodents and felines, and a synanthropic or urban cycle involving domestic felids and rats (Tull *et al.*, 2021). The study area is the main industrial zone of the country, and its agricultural land is very productive due to the vast canal system and the presence of rivers on the periphery. In this study, urban cycle transmission was found as the major source of the parasite dissemination to urban rats, with food and water contaminated with cat excrement regarded as the main sources of spread that is according to the research conducted by Medina-Pinto *et al.* (2019) and declared that no transmission cycle was associated with wild rodents. Maintenance of the *H. taeniaeformis* life cycle may be seriously influenced by the continuously changing habitat of wild rodents, their density, and relative population dispersion (Sosa-Escalante *et al.*, 1997; Tovar and Villanueva, 2009). In the future, investigations concentrating on non-industrialized parts of Pakistan with different wildlife populations are recommended to gain a better understanding of involvement of wild animals in the life cycles, as their existence in India has already been verified (Malsawmtluangi *et al.*, 2011). As the record of *H. taeniaeformis* distribution in rodents was restricted to a single zone, the high prevalence identified in rats cannot be extrapolated to other parts of the country to explain why the prevalence rate is so high (Alvi *et al.*, 2021).

Following successive alignment of the 38 sequences, a total of 12 parsimony informative sites were discovered, yielding 13 haplotypes. Tajima's D (-1.85891) and Fu's Fs (-3.022) values were found non-significant and the diversity indices are mentioned in Table 1.

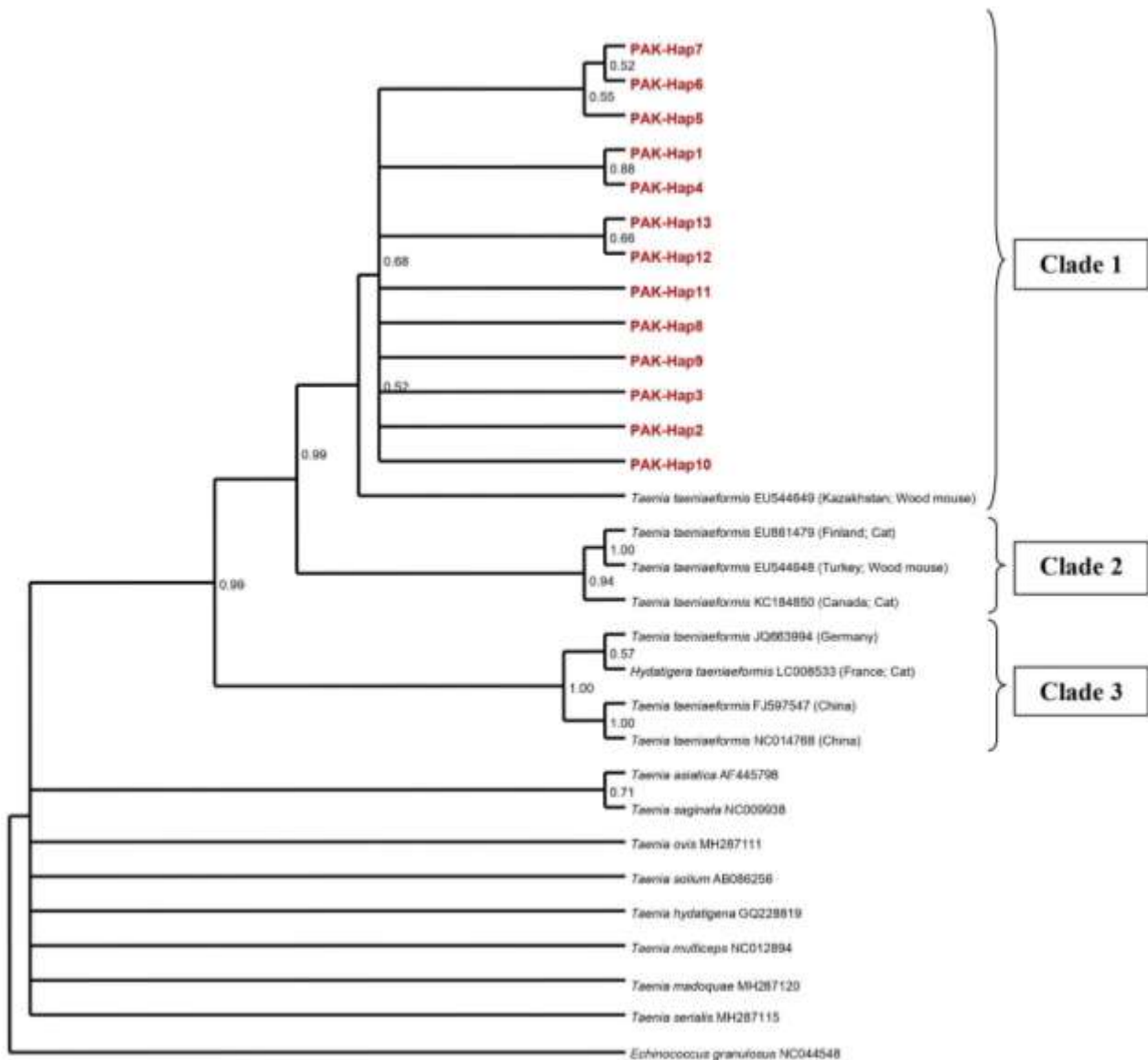


Fig. 1: Bayesian phylogeny of Pakistani isolates of *Hydatigera taeniaeformis* inferred from *nad1* gene.

Table 1: Population indices for *H. taeniaeformis* based on *nad1* gene sequences

Indices	<i>nad1</i> (220 bp)
Number of isolates	38
Number of mutations	36
Total parsimony informative sites	12
Number of haplotypes	13
Overall haplotype diversity (Hd)	0.909
Overall nucleotide diversity (π)	0.02340
Tajima's <i>D</i>	-1.85891
Fu's <i>F_s</i>	-3.022

Mitochondrial genomic DNA (mtDNA) is commonly used to research intraspecific variations in metazoans due to its lack of recombination, conserved structure, and rapid evolutionary rate (Wei *et al.*, 2010; Shen *et al.*, 2010; Gasser, 2006; Liu *et al.*, 2011). As a result of some of the detected nucleotide modifications indicated in Table 2a, a few substitutions in amino acids were identified (Table 2b). The neutrality investigation discovered considerable nucleotide polymorphism, indicating an increase in low-frequency polymorphism that could be attributable to world population expansion. The genetic population structure of *H. taeniaeformis* is likewise comparable to that of other

parasitic species previously studied (Carmena and Cardona, 2014; Alvi *et al.*, 2020). The availability of information on *H. taeniaeformis* neutrality and polymorphism indices is limited internationally, which has hampered a comprehensive investigation of these measurements.

As the observed amino acid alterations did not display a clear trend, the cause might be the examination of partial gene sequences. However, a new nucleotide and amino acid arrangement might imply that *H. taeniaeformis* has been genetically classified by geographic area. The importance of these variations at molecular levels is yet unknown. However, research on *Echinococcus granulosus sensu lato* has revealed the impact of genetic diversity on epidemiology, host infectivity and control techniques (Carmena and Cardona, 2014). Mitochondrial genome analysis of *T. saginata* and *T. solium* has also depicted intraspecific variations that might influence the pathogenicity of disease in various host species (Rostami *et al.*, 2015).

Phylogenetic analysis implies that *H. taeniaeformis* might be divided into regionally different species. Pakistani isolates were grouped at an acceptable distance near the

isolates of Asian, European, and North American countries namely Kazakhstan, Finland, Turkey, and Canada while isolates from Asian and European countries (Germany, France, and China) formed an independent cluster (Fig. 1). As shown by the Bayesian phylogeny and previously documented by Lavikainen *et al.* (2016), Asian strains have significant haplotype diversity, whereas non-Asian isolates have modest diversities (Lavikainen *et al.*, 2016). Interestingly, Pakistani haplotypes (cluster 1) were found closer to European and American haplotypes (cluster 2) instead of the Chinese haplotypes (cluster 3).

The representative sequences of this study have been submitted in the NCBI GenBank database under accession numbers MZ868741-MZ868753. *Echinococcus granulosus* (accession number NC044548) was used as an outgroup that validated the authenticity of the constructed tree.

The isolates obtained in this study were supposed to fall under the *H. taeniaeformis* sensu stricto (s. s.) lineage rather than *H. kamiyai* which was further confirmed through sequencing analysis and by the fact that *H.*

kamiyai lineage was found to occur predominantly in northern Eurasia infecting arvicoline rodents (voles) and mice of the genus *Apodemus* whereas *H. taeniaeformis* was identified in members of subfamily Murinae (rats and mice) having Asian origin and now it has a worldwide distribution (Lavikainen *et al.*, 2016).

In this study, the diversity of the partial *nad1* gene marker among isolates of *H. taeniaeformis* was investigated. As far as we know, the majority of information on the genetic diversity of *H. taeniaeformis* is based on incomplete *cox1* gene sequences, which prohibits a full investigation of the genotypic diversity situation. Investigations into genetic variants have been reported to be influenced by the length of the gene under investigation (Yanagida *et al.*, 2012; Romig *et al.*, 2015). It is recommended that longer DNA fragments, rather than full-length genes, should be sequenced to improve the resolution of genetic analyses. As a result, more research is needed to understand the molecular epidemiology of *H. taeniaeformis* on global scale.

Table 2: Mitochondrial *nad1* gene based nucleotide sequence polymorphism
Table 2a: *nad1* mutation sites

Haplotype	<i>nad1</i> DNA mutation sites																	
	14	15	34	45	47	50	54	73	87	91	98	108	111	112	113	117	118	121
PAK-HAP1	A	T	C	C	C	C	C	C	T	T	C	C	C	C	C	C	C	C
PAK-HAP2	.	.	G	A	T	T	A	T	.	.	.	T	T	T	T	T	A	T
PAK-HAP3	.	.	G	A	T	T	A	T	.	.	T	T	T	T	T	T	A	T
PAK-HAP4	.	.	.	A	T	T	A	T	.	.	.	C	C	T	T	T	A	T
PAK-HAP5	.	.	.	A	T	T	A	T	.	C	.	T	T	T	T	T	A	T
PAK-HAP6	.	.	G	A	T	T	A	T	.	.	.	T	T	T	T	T	A	T
PAK-HAP7	.	.	G	A	T	T	A	T	.	.	.	T	T	T	T	T	A	T
PAK-HAP8	.	.	G	A	T	T	A	T	.	.	T	T	T	T	T	T	A	T
PAK-HAP9	.	.	G	A	T	T	A	T	.	.	.	T	T	T	T	T	A	T
PAK-HAP10	.	C	G	A	T	T	A	T	.	.	.	T	T	T	T	T	A	T
PAK-HAP11	.	.	G	A	T	T	A	T	C	.	.	T	T	T	T	T	A	T
PAK-HAP12	.	.	G	A	T	T	A	T	.	C	.	T	T	T	T	T	A	T
PAK-HAP13	C	.	G	A	T	T	A	T	.	C	.	T	T	T	T	T	A	T

Haplotype	<i>nad1</i> DNA mutation sites																	
	127	136	156	164	173	177	178	180	186	187	193	197	203	204	205	211	212	217
PAK-HAP1	T	T	C	C	C	T	C	C	T	C	A	T	G	T	T	T	C	C
PAK-HAP2	.	.	G	G	.	.	T	T	.	T	A	G
PAK-HAP3	.	.	G	G	.	C	T	T	.	T	A	G
PAK-HAP4	.	.	.	G	.	C	.	.	.	T	.	.	C	.	C	.	A	.
PAK-HAP5	.	C	G	G	.	C	T	T	C	T	G	.	.	C	.	C	A	G
PAK-HAP6	.	.	G	G	T	C	T	T	.	T	G	A	.
PAK-HAP7	.	.	G	G	.	C	T	T	.	T	G	A	G
PAK-HAP8	.	.	G	G	.	.	T	T	.	T	A	G
PAK-HAP9	C	.	G	G	.	.	T	T	.	T	.	C	A	.
PAK-HAP10	.	.	G	G	.	.	T	T	.	T	A	G
PAK-HAP11	.	.	G	G	.	.	T	T	.	T	A	.
PAK-HAP12	.	C	G	G	.	.	T	T	.	T	A	G
PAK-HAP13	.	.	G	G	.	.	T	T	.	T	A	G

Table 2b: *nad1* amino acid substitution

Haplotype	<i>nad1</i> amino acid substitution											
	5	12	16	17	36	38	40	41	46	66	68	71
PAK-HAP1	D	R	T	P	D	P	H	H	C	F	G	S
PAK-HAP2	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP3	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP4	.	.	M	L	G	F	N	Y	.	.	A	Y
PAK-HAP5	.	.	M	L	G	F	N	Y	R	.	.	H
PAK-HAP6	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP7	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP8	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP9	.	G	M	L	G	F	N	Y	.	S	.	Y
PAK-HAP10	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP11	.	G	M	L	G	F	N	Y	.	.	.	Y
PAK-HAP12	.	G	M	L	G	F	N	Y	R	.	.	Y
PAK-HAP13	A	G	M	L	G	F	N	Y	.	.	.	Y

Conclusions: The results of the *nad1* gene sequence analysis revealed a considerable genetic polymorphism, which is further supported by population studies of isolates from Asia, Europe, and North America. Further studies are highly recommended for better conceptualization regarding the distribution and prevalence of *H. taeniaeformis* in both final and all possible intermediate hosts as well as intraspecies diversity and relationships with other populations. In this study, we amplified only a segment of the *nad1* gene. It is recommended that longer DNA fragments, rather than full-length genes, should be sequenced to improve the resolution of genetic analyses. Therefore, further investigations are needed to comprehend the global molecular epidemiology of *H. taeniaeformis*.

Authors contributions: MAA, HBY, AA, IR, and MS conceptualized the study. The methodology was designed by MAA, AAB, MS, and RMAA while MAA, RMAA, MSh, KA, LL, and WQ carried out validation. Writing—original draft preparation was done by MAA and RMAA while review and editing and performed by HA, HBY, NA, BQF, and WZJ. Funds were acquired by HBY and WZJ. All authors have read and agreed to the published version of the manuscript.

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