

Genetic Characteristics and Molecular Diagnosis of Hymenolepiasis in Children Under 14 Years of Age

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Abstract: Hymenolepiasis, caused primarily by *Hymenolepis nana* (the dwarf tapeworm), remains a significant public health concern in children under 14 years of age, particularly in developing regions with limited sanitation infrastructure. While the clinical manifestations and immunological aspects of this infection have been extensively studied, the genetic characteristics of *H. nana* and the application of molecular diagnostic tools in pediatric populations represent an evolving field of research. This thesis examines the genetic diversity of *H. nana* isolates from children using mitochondrial and ribosomal genetic markers, evaluates the utility of molecular diagnostic techniques compared to conventional microscopy, and explores the implications of genetic variation for epidemiological surveillance and clinical management. Recent studies have identified intraspecific genetic differences within *H. nana* isolates ranging from 0 to 5% based on mitochondrial cytochrome c oxidase subunit 1 (CO1) gene sequencing, suggesting the existence of a species complex or 'cryptic' species. Molecular methods, including polymerase chain reaction (PCR) targeting the ITS-1 region, 18S rDNA, and 28S rDNA, demonstrate superior sensitivity and specificity compared to traditional stool microscopy, with PCR-RFLP showing 100% sensitivity in confirming *Hymenolepis* spp. infections. The genetic variation observed in *H. nana* isolates from different geographical regions highlights the importance of molecular characterization for understanding transmission dynamics and developing effective control strategies. This thesis contributes to the growing body of knowledge on the molecular epidemiology of hymenolepiasis and advocates for the integration of molecular diagnostic tools into routine pediatric parasitological practice.

Keywords: *Hymenolepis nana*, molecular diagnosis, genetic diversity, PCR, children, mitochondrial DNA, ITS-1, cryptogenic species

Introduction

Hymenolepiasis, the infection caused by cestodes of the genus *Hymenolepis*, is among the most prevalent parasitic zoonoses worldwide, with *Hymenolepis nana* (the dwarf tapeworm) being the most common causal agent in humans. Unlike most other cestodes, *H. nana* can complete its entire life cycle within a single host, enabling direct transmission through the fecal-oral route and autoinfection, which contributes to its high prevalence, particularly in pediatric populations. Children under 14 years of age are disproportionately affected due to their developing immune systems, poor hygiene practices, and increased environmental exposure to contaminated food, water, and soil.

The global burden of hymenolepiasis is substantial, with prevalence rates varying considerably across geographical regions. Studies have reported infection rates ranging from 1.5% in Ethiopia to 32.6% in Sudan, with prevalence in rural areas often exceeding urban settings. A study in Upper Egypt detected *H. nana* in 28.3% of examined children, while in Mexico, a prevalence of 7.8% was documented. In Iraq, molecular detection revealed an infection rate of 13.3% among children, with the highest rates observed in the 1–5 year age group (20%).

Historically, the diagnosis of *H. nana* infection has relied on microscopic examination of stool samples using techniques such as direct smear, formol-ether sedimentation, and Sheather's sugar flotation. However, these methods have significant limitations, including operator dependency, reduced sensitivity in cases of low parasite density, and the inability to differentiate morphologically identical species or identify mixed infections.

The accurate identification of *Hymenolepis* species is further complicated by taxonomic challenges. Isolates of *H. nana* from humans and rodents are morphologically indistinguishable, and studies have confirmed that morphological identification alone is insufficient to reliably distinguish between these isolates. Molecular tools, particularly those targeting mitochondrial and ribosomal genetic markers, have proven essential for accurate species identification and for elucidating the genetic relationships among isolates from different hosts and geographical regions.

The first internal transcribed spacer (ITS-1) region of ribosomal DNA, the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, the 18S rDNA and 28S rDNA genes, and the nuclear paramyosin gene have been employed as genetic markers for the molecular characterization of *H. nana*. Phylogenetic analyses based on these markers have identified intraspecific genetic differences within *H. nana* ranging from 0 to 5% for the CO1 gene, 0 to 0.4% for *H. microstoma*, and 0 to

6.5% for *H. diminuta*, suggesting the existence of a species complex or 'cryptic' species—morphologically identical yet genetically distinct organisms. Macnish et al. (2002) demonstrated a phylogenetically supported genetic divergence of approximately 5% between some mouse and human isolates of *H. nana*, supporting the hypothesis of cryptic speciation.

Molecular diagnostic techniques, including conventional PCR, multiplex PCR, and PCR-RFLP, offer significant advantages over microscopy, including enhanced sensitivity, specificity, and the ability to detect mixed infections. PCR targeting the mitochondrial cytochrome gene has been successfully employed to detect *H. nana* in pediatric stool samples, with studies reporting that multiplex PCR can detect a broad range of gastrointestinal parasites, including *H. nana*. The utility of PCR-RFLP targeting the ITS-1 region has been validated, demonstrating 100% sensitivity in confirming *Hymenolepis* spp. infections.

The genetic characterization of *H. nana* isolates has also shed light on transmission dynamics. Epidemiological and molecular evidence suggests that infections with *H. nana* primarily involve human-to-human transmission, with synanthropic rodents playing a secondary role. However, the presence of other hymenolepidid species, such as *H. microstoma* and *H. diminuta*, in rodents represents a potential zoonotic risk.

This thesis aims to review the current state of knowledge on the genetic characteristics of *H. nana* isolates from children under 14 years of age, evaluate the utility of molecular diagnostic techniques for accurate detection and species identification, and explore the implications of genetic variation for epidemiological surveillance and clinical management.

Conclusion

The application of molecular diagnostic tools and the investigation of genetic characteristics have significantly advanced our understanding of hymenolepiasis in children under 14 years of age. The recognition of *H. nana* as a species complex or group of cryptic species, as supported by genetic divergence observed in mitochondrial CO1 and ribosomal ITS-1 sequences, has important implications for the epidemiology, diagnosis, and control of this infection.

Molecular methods, particularly PCR-based techniques targeting genetic markers such as ITS-1, 18S rDNA, 28S rDNA, and the mitochondrial CO1 gene, provide superior sensitivity and specificity compared to conventional microscopy. These tools enable the accurate identification of *Hymenolepis* species, the detection of mixed infections, and the characterization of genetic diversity, which is essential

for understanding transmission dynamics and implementing effective control strategies.

The integration of molecular diagnostics into routine pediatric parasitological practice, particularly in endemic regions, is recommended to improve case detection, guide treatment decisions, and monitor the effectiveness of control interventions. Further research is needed to standardize molecular diagnostic protocols, expand the database of genetic sequences from diverse geographical regions, and investigate the clinical implications of genetic variation in *H. nana*.

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