

Diagnosis of Pediatric Gastrointestinal Nematode Infections by Multiplex Polymerase Chain Reaction

Dear Editor,

Globally, gastrointestinal parasites significantly impact children under 12 years due to their developing immune systems and environmental exposure, leading to issues ranging from mild disturbances to severe malnutrition and developmental problems. However, understanding their full impact is challenging due to diagnostic limitations. Historically, detection relied on microscopic stool sample analysis, which, while insightful, is limited by operator dependency and reduced sensitivity, especially in low parasite density cases.^[1]

The scientific community has increasingly shifted towards molecular techniques like multiplex polymerase chain reaction (PCR) for their enhanced sensitivity and specificity. This method allows for simultaneous amplification of multiple target sequences in a single reaction, advantageous in parasitology due to the variety of potential pathogens. Multiplex PCR provides a comprehensive infection overview, enabling targeted therapeutic interventions. Studies demonstrate its superiority over conventional methods in sensitivity and turnaround time. It is particularly effective in identifying mixed infections, which microscopic methods might overlook, leading to incomplete treatment and potential symptom recurrence.^[2,3]

This study aimed to use multiplex PCR to investigate gastrointestinal parasites in children under 12 years, exploring the prevalence and diversity of infections and validating the utility and efficacy of multiplex PCR as a superior diagnostic tool in pediatric parasitology. In conclusion, integrating advanced methodologies like multiplex PCR in pediatric gastrointestinal parasitology is essential as the global scientific community strives to enhance healthcare and diagnostics. This research is expected to provide valuable insights for future studies and clinical applications in this field.

One hundred stool samples were collected from patients presenting with severe digestive complaints, acute abdominal pain, and diarrhea at Diwaniyah Teaching Hospital and private clinics in Diwaniyah City, Iraq, between March and December 2023. The multiplex PCR technique was employed to detect *Enterobius vermicularis*, *Ancylostoma duodenale*, and *Hymenolepis nana* in human stool samples, targeting the mitochondrial cytochrome gene.

The data were analyzed by Statistical Package for the Social Sciences version 22 (IBM Corp., Armonk, NY, USA). A chi-square test compared the prevalence values with the mitochondrial cytochrome oxidase subunit *Cox1* gene for the detection of *E. vermicularis*, *A. duodenale*, and *H. nana* genes. Confidential intervals at 95% and $P < 0.05$ were considered levels of significance.^[4]

The present study has shown 25 positive samples of *E. vermicularis* (25.00%), 17 positive samples of *A. duodenale* (17%), and 7 positive samples of *H. nana* (7%) were detected among pediatric gastrointestinal nematode infections [Table 1; Figure 1].

Multiple molecular investigations have yielded empirical support linking to the pathogenesis of gastrointestinal disorders. As a result, this study evaluates the effectiveness of a PCR assay in detecting a diverse range of gastrointestinal parasites, encompassing both protozoa and helminthes. The assay's performance was compared to traditional microscopy methods. These studies showed that a considerable quantity of parasites could be identified by PCR assays, even without specialized expertise in parasitology. Numerous studies have shown that real-time PCR detects intestinal parasites better than microscopy.^[5]

Compared with microscopy, real-time PCR detects A with improved sensitivity and specificity. Since most of the population is *E. vermicularis* and *H. nana*, several PCR methods used in this investigation were more sensitive than microscopy at detecting parasites in asymptomatic patients. This component will help manage neglected tropical diseases by identifying the reservoir, assessing transmission, and assessing deworming efficacy.^[6,7]

Table 1: Rate of intestinal helminth infections by using molecular examination

Parasite	Total of samples	Positive	%
<i>Enterobius vermicularis</i>	100	25	25.00
<i>Ancylostoma duodenale</i>	100	17	17.00
<i>Hymenolepis nana</i>	100	7	7.00
<i>E. vermicularis</i> + <i>H. nana</i>	32	0	0.00
<i>E. vermicularis</i> + <i>A. duodenale</i>	42	2	4.76
<i>A. duodenale</i> + <i>H. nana</i>	24	1	4.16
χ^2		20.059	
P value		0.00122S	

S: Significant difference at $P < 0.05$

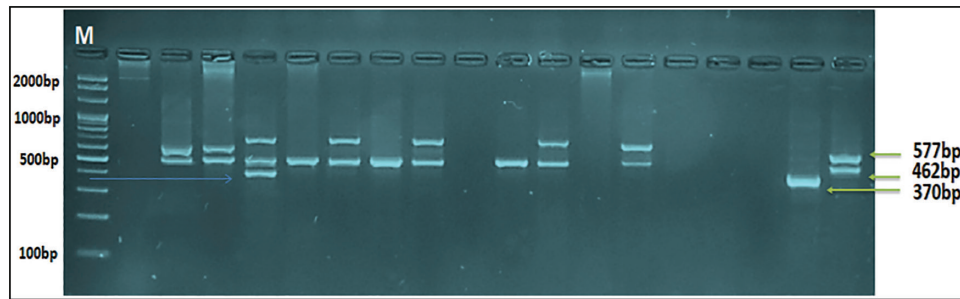


Figure 1: Agarose gel electrophoresis showcases the multiplex polymerase chain reaction (PCR) product analysis targeting the *Cox1* gene for the detection of *Enterobius vermicularis*, *Ancylostoma duodenale*, and *Hymenolepis nana* in human stool samples. Lane (M) represents the deoxyribonucleic acid marker ladder. Lanes (1–24) display some positive samples for *E. vermicularis*, *A. duodenale*, and *H. nana*, with PCR product sizes at 462, 577, and 370 bp, respectively

The assay results for the sensitivity and specificity of *E. vermicularis* were determined to be 36.23% and 42.75%, respectively. The sensitivity testing for *A. duodenale* demonstrated a percentage of 53.125%, whereas the specificity was 50.59%. The sensitivity testing of *H. nana* yielded a percentage of 53.125%, but the specificity assay resulted in a lower percentage of 50.59%. The importance of examining the relationship between age, gender, and place of residency in diagnosing and treating parasites in the Al-Diwaniyah Governorate is underscored.

The present study examines *E. vermicularis*, *A. duodenale*, and *H. nana*. The intestinal parasites were the most frequently encountered across all techniques employed. There exist significant concerns about the possible pathogenicity of *E. vermicularis*, *A. duodenale*, and *H. nana*, the initial organism identified with a prevalence rate of the presence of intestinal parasites has been verified, thereby corroborating previous findings that establish this parasite as a significant public health issue in Iraq.^[8]

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Conflicts of interest

There are no conflicts of interest.

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