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Development of Multiplex Pcr Primers for The Detection of Waterborne Parasites

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Structured Abstract

Background: Water is a vital resource for life to sustain ecosystems and supports human, plant, and animal growth. Water is often polluted, posing health risks from microorganisms. Parasites transmitted through contaminated water, like *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum*, cause diseases like amebiasis, giardiasis, and cryptosporidiosis, leading to severe health issues and millions of deaths annually. Efforts to ensure water purity are essential to prevent waterborne parasitic infections and protect global health. Detecting waterborne diseases is challenging, hence developing multiplex primers that identify the parasite's genus is crucial for specific detection in water. These primers are to be employed in the PCR process for analyzing water samples, aiding accurate identification of the three waterborne parasites.

Methods: First, 18S rRNA gene sequences for *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium parvum* were retrieved from NCBI Genbank. Next, multiplex primers were designed using Multiple Alignment Sequence and OligoCalc. Lastly a phylogenetic analysis was constructed using MEGA11 based on DNA sequences obtained from GenBank, following BLAST analysis on the multiplex primers' sequences.

Results: This study shows that the multiplex primers were successfully developed based on the three parasites. Additionally, the developed multiplex primers were able to detect all three parasites via BLAST analysis. The phylogenetic analysis shows a tree grouped and categorized parasite sequences, illustrating their evolutionary relationships, divergence, and common ancestry characteristics obtained from the BLAST results.

Conclusion: The study shows the potential of developed multiplex primers in identifying waterborne parasites of *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium parvum*. BLAST analysis shows high similarity between the parasites' sequences in Genbank. These primers can be employed for water sample screening and quality monitoring.

Keywords: *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum*, multiplex primers, 18S rRNA

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