

Detection of *Cryptosporidium* spp. in Lake Water in Shah Alam

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Structured Abstract

Background: *Cryptosporidium* species are waterborne protozoan parasites that raise significant public health concerns due to their resilience to environmental stress and potential to cause gastrointestinal illness. The presence of *Cryptosporidium* oocysts in recreational and drinking water sources presents a risk of cryptosporidiosis, particularly in urban areas. This study aimed to identify the presence of *Cryptosporidium* species in lake water samples obtained from various regions of Shah Alam Lake Garden using microscopy and molecular techniques.

Methods: A total of six lake water samples were obtained from different region sites within Shah Alam Lake Garden and processed using zinc sulphate flotation to concentrate protozoan oocysts. Subsequently, the samples underwent examination through direct wet mount microscopy and Ziehl-Neelsen staining to identify the morphological characteristics of *Cryptosporidium* oocysts. Freeze-thawing was utilized as a pre-treatment to disrupt the oocyst walls, followed by genomic DNA extraction using a commercial extraction kit. A conventional PCR targeting the 18S rRNA gene was performed using specific primers (AWA995F and AWA1206R) to confirm the presence of *Cryptosporidium* DNA.

Results: Three out of six water samples (50%) were found to contain *Cryptosporidium* oocysts when examined under light microscopy. These oocysts exhibited refractile, spherical bodies measuring approximately 4–6 µm in diameter, consistent with previously reported characteristics. Subsequent PCR analysis of Sample 1 confirmed the presence of *Cryptosporidium* DNA, evidenced by a distinct amplification band at around 250 bp. Furthermore, NanoDrop quantification revealed that the DNA concentration in Sample 1 surpassed 100 ng/µL. These results conclusively validated the detection of *Cryptosporidium* at both the microscopic and molecular levels.

Conclusion: In conclusion, the findings of this study demonstrate the successful detection of *Cryptosporidium* spp. in lake water using microscopy and PCR methods. The findings underscore the importance of integrating molecular tools with traditional microscopy for reliable environmental surveillance. Regular monitoring of recreational waters is recommended to mitigate potential public health risks.

Keywords: *Cryptosporidium*, Lake Water, 18S rRNA, PCR, Waterborne Parasite

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