Journal of Biomedical Sciences | ISSN: 2960-9356
Research Article Peer-review, Open Access

Molecular Characterization of Cryptosporidium Infection among Students of Abu Ghraib Schools During the Academic Year 202502026

Alaa Hussein Alzaidi [1]

Abstract

This investigation aimed to determine the prevalence of Cryptosporidium in children in Baghdad, specifically among students in Al-Karkh primary schools. DNA was extracted from stool samples that had tested positive for Cryptosporidium oocysts via microscopic examination. The extracted DNA was then analyzed using 18S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) testing.

In contrast, the use of the PCR system demonstrated that this technique is a more specific and sensitive method for the detection and identification of protozoan parasites like Cryptosporidium. Overall, the results confirmed that C. parvum was frequently the species demonstrated in Baghdad.

[1] Ministry of Education / Open College of Education

Introduction:

Cryptosporidium, one of the most widely distributed intestinal parasites globally [1], was first defined as a human pathogen in 1976 [2]. Human infections with *Cryptosporidium* are predominantly caused by *C. hominis* and *C. parvum* [3]. Belonging to the *Coccidia* class within the phylum Apicomplexa, Cryptosporidium possesses distinct features that distinguish it from other members of the *Coccidia* group [4]. *Cryptosporidium* infection is mainly transmitted through the fecal-oral route and can occur due to contact with animals, feces, or contaminated water or food [5]. Respiratory secretions and aerosolised droplets from coughing can also serve as means of transmissions, leading to pulmonary infections [6]. The pathogen poses a significant threat to the waterindustry [7].

Cryptosporidium infection can lead to damage to intestinal epithelial cells in both humans and other mammals. This damage disrupts the tight junctions of the intestinal epithelium, affecting intestinal barrier function. It also hinders the development of villi and reduces the absorptive capacity of theintestinal surface, leading to malnutrition, dehydration, and diarrhea [8]. The diarrheal symptoms associated with cryptosporidiosis pose a major threat to human health [9]. Malnourished children are particularly susceptible to *Cryptosporidium* infection, which can lead to recurrent or persistent infections and even

death [10, 11].

This cycle perpetuates the spread of *Cryptosporidium*, making it a significant cause of waterborne disease. Recently molecular diagnostic tools have been used for the precise identification of Cryptosporidium species [12-13]. Fundamentally, examining genotypic variation in Cryptosporidium is important for comprehending its biology, how it spreads, and how it evolves over time. A deeper understanding in these areas can ultimately help guide approaches for preventing, containing, and managing cryptosporidiosis [14]. However, little attention has been paid to genotyping diversity of *Cryptosporidium* species in Iraq. Therefore, the current study aimed to characterize the prevalence and genotype variation of cryptosporidiosis in fecal samples of human in Baghdad, Iraq.

2. Materials and Methods

2.1 Collection of samples:

A total of 100 fecal samples *Cryptosporidium* in children in Baghdad, specifically among students in Al-Karkh primary schools. , were collected between 1 October/ 2025 to 30 November/ 2025 .

2.2 Staining of oocysts

The fecal samples were smeared on to glass slide, stained with modified Ziehl- Nielsen stain for the identification of oocysts by microscopy [15].

2.3 Extraction of DNA

DNA was extracted using (Stool DNA extraction package Favorge, Korea) according to the manufacturer's protocol. A 20 mg frozen stool sample was incubated with 30 ml of lysis buffer (pH 8.0, 10 mM EDTA, 10 mM Tris-HCl, 1 mg/ml K-protease) at 65°C for 2 hours to solubilize it, followed by heating to 100°C for 30 minutes to denature the enzyme. Cell wreckage and proteins were removed by centrifugation (10,000 × g, 4°C, 10 min). The portion of the supernatant Contains DNA were used for PCR, and 5 µl add DNA solution to PCR mix. Assess the purity of DNA samples using optical density on a Nanodrop spectrophotometer. The purity of DNA samples was assessed by optical density using a Nanodrop spectrophotometer[16].

2.4 PCR Protocol

The PCR amplification was performed using the primers forward (GGAAATCCGTCTATCAGTGG) and reverse (CCCTCACGGTACTTGTTTGC), which were the previously reported to specifically amplify of the18SrRNA gene of C. parvum . The PCR reaction mixture (25µl) containing 10mM Tris-HCI, 0.2 mM of each deoxy nucleoside triphosphate, 25 pmol of each appropriate primer, and 2.5 U of Taq DNA polymerase were used amplified using gradient Perkin- Elmer thermocycler following thermal profile which Including 32 cycles (pre-incubation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and further incubation at 72°C for 10 minutes). A 10 µL aliquots of the PCR amplification products were analyzed via electrophoresis on a 1% agarose gel using Tris-borate EDTA (TBE X) running buffer containing 0.045 M Tris-borate and 1 mM EDTA [17]. The PCR product bands were visualized under UV - light after staining the gel with 0.2 mg/mL ethidium bromide. This allowed separation and detection of the amplified DNA fragments by size.

3. Results and Discussion

The prevalence of infection of the *Cryptosporidium* species as demonstrated by the direct method of 100 children stool examination are shown in Table 1. Of all samples examined were found to be positive for cryptosporidiosis. The extraction of total DNA from all microscopically positive stool samples were used as templates PCR reaction. The results revealed that the small subunit 18SrRNA gene was amplified from 7 (7%.) samples . The results of this study investigation are in agreement with results obtained in other provinces in Iraq, Babylon, Kirkuk, and neighboring countries Nigeria and Vietnam, England, Iran [16-

17]. The variation in infection rates may be attributed to factors such as the characteristics of the surveyed district, the level of personal hygiene and sanitation, the number of stool samples examined, and whether the children examined schools.

Table (4-1) Total infection rate with cryptosporidiosis in childrens

PCR positive microscopy positive	No. of samples examined	Positive samples	%
Cryptosporidium parvum	**100**	**7**	**7**

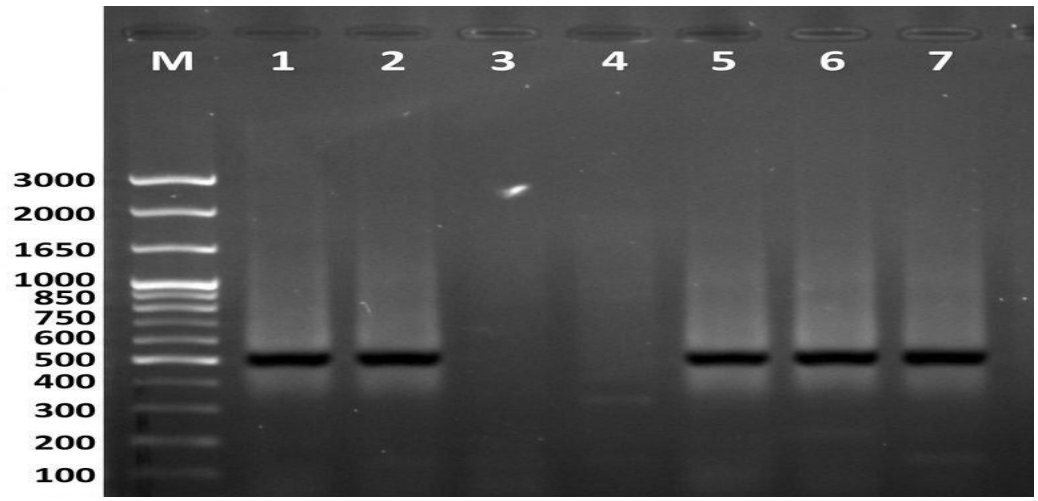

Figure1: Agarose gel electrophoresis analysis for PCR products stained with 0.2 mg/ml of ethidium bromide for18SrRNA gene using primers species specific for C. parvum obtained from human stool samples. Positive samples reveal 500 bp bands. M represent (100-2000) pb DNA Lader Marker.

Conclusion:

Ultimately, our findings validated that the Polymerase Chain Reaction (PCR) technique exhibits the highest sensitivity for the diagnosis of *Cryptosporidium*. However, alternative methods such as Ziehl-Neelsen staining and Enzyme-Linked Immunosorbent Assay (ELISA) can also be employed for the detection of this parasitic organism.

References:

1. **Shirley, D. A. T., Moonah, S. N., & Kotloff, K. L. (2012).** Burden of disease from cryptosporidiosis. Current Opinion in Infectious Diseases, 25(5), 555–563.
2. **Nair, G., & Neogi, P. (2021).** Cryptosporidiosis: A review. International Journal of Current Microbiology and Applied Sciences, 10(01), 3122–3133.
3. **Feng, Y., Ryan, U. M., & Xiao, L. (2018).** Genetic diversity and population structure of Cryptosporidium. Trends in Parasitology, 34(11), 997–1011.
4. **Helmy, Y. A., & Hafez, H. M. (2022).** Cryptosporidiosis: From infection to treatment, a narrative review. Microorganisms, 10(1), 172.
5. **Ryan, U., Hijjawi, N., & Xiao, L. (2018).** Foodborne cryptosporidiosis. International Journal for Parasitology, 48(1), 1–12.
6. **Gerace, E., Lo Presti, V. D. M., & Biondo, C. (2019).** Cryptosporidium infection: Epidemiology, pathogenesis, and differential diagnosis. European Journal of Microbiology & Immunology, 9(4), 119–123.

7. **Chalmers, R. M. (2012).** Waterborne outbreaks of cryptosporidiosis. Annali dell'Istituto Superiore di Sanità, 48(4), 429–446.

8. **Klein, P., Kleinová, T., Volek, Z., & Simůnek, J. (2008).** Effect of Cryptosporidium parvum infection on the absorptive capacity and paracellular permeability of the small intestine in neonatal calves. Veterinary Parasitology, 152(1-2), 53–59.

9. **Kaur, S., Kumar, K., & Singla, L. D. (2017).** Cryptosporidium and cryptosporidiosis: An update on epidemiology and control. Journal of Entomology and Zoology Studies, 5(5), 1836–1844.

10. **Checkley, W., White, A. C., Jaganath, D., Arrowood, M. J., Chalmers, R. M., Chen, X. M., Fayer, R., Griffiths, J. K., Guerrant, R. L., Hedstrom, L., Huston, C. D., Kotloff, K. L., Kang, G., Mead, J. R., Miller, M., Petri, W. A., Priest, J. W., Roos, D. S., Striepen, B., ... Houpt, E. R. (2015).** A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for Cryptosporidium. The Lancet Infectious Diseases, 15(1), 85–94.

11. **Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., Wu, Y., Sow, S. O., Sur, D., Breiman, R. F., Faraque, A. S., Zaidi, A. K., Saha, D., Alonso, P. L., Tamboura, B., Sanogo, D., Onwuekuba, U., Manna, B., Ramamurthy, T., ... Levine, M. M. (2013).** Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. The Lancet, 382(9888), 209–222.

12. **Mahdavi Poor, B., Rashedi, J., Asgharzadeh, M., Fallah, E., Hatam-Nahavandi, K., & Dalimi, A. (2015).** Molecular characterization of Cryptosporidium species in children with diarrhea in North West of Iran. International Journal of Molecular and Cellular Medicine, 4(4), 235–239.

13. **Ghaffari, S., & Kalantari, N. (2012).** Molecular analysis of 18S rRNA gene of Cryptosporidium parasites from patients living in Iran, Malawi, Nigeria and Vietnam. International Journal of Molecular and Cellular Medicine, 1(3), 153–161.

14. **Faruque, A. S., Sur, A. S., & Saha, S. (2013).** Aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. The Lancet, 382(9888), 209–222.

15. **Osaki, A., Taguchi, M., & Ichikawa, H. (2011).** Molecular characterization of Cryptosporidium spp. in children with diarrhea in Kathmandu Valley, Nepal. The American Journal of Tropical Medicine and Hygiene, 84(1), 121–126.

16. **Checkley, W., White Jr, A. C., Jaganath, D., Arrowood, M. J., Chalmers, R. M., Chen, X. M., ... & Houpt, E. R. (2015).** A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for Cryptosporidium. The Lancet Infectious Diseases, 15(1), 85-94.

17. **Adamu, H., Petros, B., Hailu, A., Petry, F., & Gebrehiwot, A. (2010).** Molecular characterization of Cryptosporidium isolates from humans in Ethiopia. Acta Tropica, 115(3), 252-256.