AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Epidemiology and Immune Response of *Cryptosporidium parvum* Infection in Children in Tikrit City

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Citation: Alwan, H. A., Atha, E. K., Kurdy, R. S. Epidemiology and Immune Response of *Cryptosporidium parvum* Infection in Children in Tikrit City. American Journal of Biology and Natural Sciences 2026, 3(3), 196-201.

Received: 25th Jan 2026
Revised: 15th Feb 2026
Accepted: 10th Mar 2026
Published: 31st Mar 2026

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Abstract: This study aimed to identify the epidemiological factors associated with the spread of the Cryptosporidium parvum. Samples were collected from patients visiting Tikrit Teaching Hospital in Salah al-Din Governorate. Sample collection continued from September 2025 until December 2026. 150 stool samples were collected from patients whose ages ranged from (6 months to 10 years). *Cryptosporidium parvum* was detected using modified Ziehl-Neelsen staining in 38% of the samples upon microscopic examination. The infection rate was higher in males (42.1%) compared to females (27.3%). The highest prevalence was observed in the 3- 5year age group (42.1%), while the lowest was in the 6-10 year age group.(%7.3) The current immunological study recorded a highly significant increase in the level of Il-2, IL-10, and CCL20 in the affected children, with the average concentration of Il-12 (27.9 ± 2.6 Pg/ml), IL-10 (16.98 ± 1.9 Pg/ml), and CCL20 (231.6 ± 11.1 ng/mL) compared with the control group at p- value ≤ 0.05. Conclusion: The current study concluded a clear prevalence of *C. parvum* parasite in children, and also demonstrated a clear variation in the distribution of infection according to sex and age. The current study, through the evaluation of certain inflammatory immunological parameters in blood serum samples, concluded that the parasite stimulates an immune response resulting from its infection of intestinal cells in the small intestine, characterized by a highly significant increase in IL-10, IL-12, and CCL20.

Keywords: *Cryptosporidium parvum*, Demographic Study, IL-10, IL-12, and CCL20

Introduction

Cryptosporidium is a protozoan disease classified within the Phylum Apicomplexa. It induces a diarrheal condition termed cryptosporidiosis, which impacts the digestive system of mammals. *Cryptosporidium parvum* (*C. parvum*) infection is particularly concerning for immunocompromised persons [1]. This is due to the illness potentially causing severe diarrhea, with volumes reaching 10-15 liters per day [2]. Cryptosporidiosis was first documented in Iraq in 1996 by [3]. Infection may arise when an individual or animal encounters contaminated substances, including soil, water, or undercooked or cross-contaminated food that has been exposed to the excrement of an infected person or animal [4].The contaminated substance must next be conveyed to the oral cavity and ingested. This infection is most prevalent among individuals who frequently engage with fresh water sources, such

as swimming pools [4]. Additional potential sources of infection are untreated water supplies, infected food, or exposure to fecal matter [5]. Using a 100× oil immersion lens and a variety of staining procedures, such as Modified Ziehl–Neelsen (MZN) to identify the oocyst containing four mature sporozoites, a direct microscopic method is achieved [6]. The microscopic method is an efficient and economical tool; however, it requires proficient and experienced diagnosticians to reduce false-positive outcomes, which can be time-intensive, especially in instances of parasite species and co-infections. The microscopic examination is unable to identify these issues [7], [8].

Infection of small intestinal epithelial cells by *C. parvum* induces apoptosis and the secretion of several chemokines and pro-inflammatory cytokines, promoting the influx of inflammatory cells to the site of infection [9]. *C. parvum* exploits the host's biological processes for replication within epithelial cells, which subsequently engage the immune system by inducing acute mucosal inflammatory responses[10]. Consequently, epithelial cells are crucial to both defensive immune responses and parasite proliferation [11]. There are two types of immune responses that contribute to host resistance to C. parvum infection: innate and adaptive [12], [13]. Natural killer (NK) cells produce interferon-gamma (IFN-γ) during the early stages of Cryptosporidium infection, and they create more of it when stimulated by T-helper 1 (Th1) cytokines, such as interleukin (IL)-12, which help control *C. parvum* [14], [15], [16]. The primary protective immune response depends on interleukin-12, which increases the production of IFN-γ by T cells and NK cells by enhancing the Th1 pathway and indirectly promoting the antibacterial and antiparasitic actions of macrophages [17], [18], [19]. Numerous cells in the intestinal mucosa secrete interleukin-10, which triggers immunological and inflammatory reactions in the mucosa. Additionally, it might protect the body against overreaction to an infection [20]. CCL20, which is also referred to as macrophage infiltrating factor protein 3α, is a chemokine that is classified as a C-C chemokine. It produced by several cells as Neutrophils, B cells, T cell, NK, and several antigen-presenting cells, as macrophages, and dendritic cells [21], [22]. Epithelial cells that have been infected with *C. parvum* undergo apoptosis, which is accompanied by the production of a number of chemokines and a large number of proinflammatory cytokines. This process makes it easier for inflammatory cells to be recruited to the site of infection [23], [24].

Materials and methods

Samples collection

Samples were collected from patients visiting Tikrit Teaching Hospital in Salah al-Din Governorate. Sample collection continued from September 2025 until December 2026. 150 stool samples were collected from patients whose ages ranged from 6 months to 10 years, and 25 samples were collected from healthy children as a control. The stool samples were examined microscopically to detect the presence of oocysts.

Stool samples were collected in plastic containers. Blood samples were also taken from both infected and healthy children, in amounts of 5-10 ml, and placed in yellow test tubes containing a gelatinous medium. The serum was separated by centrifugation at 4000 rpm for 15 minutes, then placed in 10 ml plastic test tubes labeled with the appropriate information and stored in the freezer at -20°C until testing.

Microscopic Examination

The microscopic examination of the sample involved by using **hot method of MZN staining of fecal smears.** The technique done by fixing it through passing the glass slide over a flame using a gas burner [25].

Human IL-10, IL-12, CCL20 ELISA test

The level of **IL-10, IL-12, CCL20** was quantified via ELISA. The plate has been pre-coated with antibodies against human IL-10, IL-12, and CCL20. Upon the introduction of IL-10, IL-12, and CCL20 into the sample, a binding interaction occurs with the antibodies immobilized in the wells. Subsequently, the immune complex is treated with a human biotinylated conjugated IL-10, IL-12, and CCL20 antibody, which is then applied to the substrate. The quantification of absorbance at a wavelength of 450 nm.

Statistical Analysis:

Statistical analysis was performed with SPSS version 22. The continuous variable format was represented as means ± standard deviation (SD). The association between categorical factors was examined. P-values equal to or below 0.05 were considered significant.

Result

The study results utilizing the MZN stain indicated that the infection rate of the *C. parvum* was 55 samples (36.7%) positive, while 95 samples (63.3%) were negative, from a total of 150 stool samples analyzed, as presented in Table 1.

Table 1. Infection rates of *C. parvum* using MZN stain

Examined samples	Positive result	Percentage	Negative result	Percentage
150	55	36.7%	95	63.3%)

The oocyste of the *C. parvum*, following staining, manifested as round to oval shapes and exhibited a reddish-purple hue, whilst the remainder of the smear appeared blue, as depicted in Figure (1).

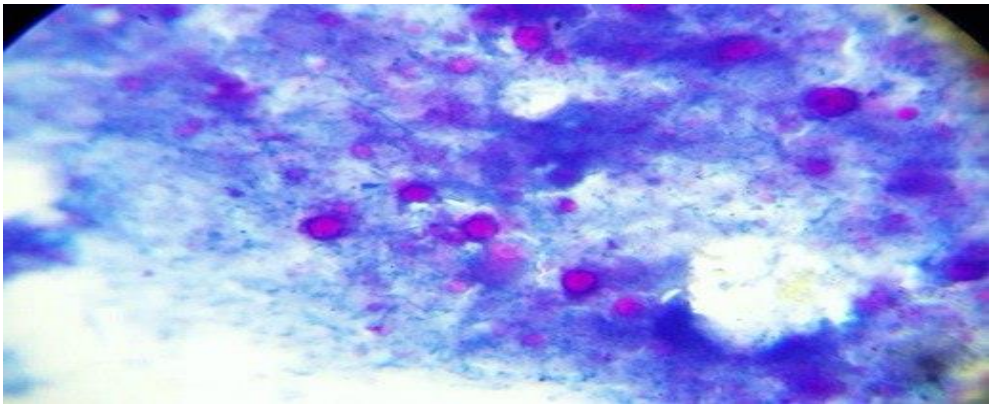

Figure 1. *C. parvum* oocyste stained with MZN, magnification 100x

The findings of the present investigation indicated that infection with *C. parvum* was identified in 40 males, accounting for 42.1%, and in 15 females, representing 27.3%, as illustrated in Table (2).

Table 2. Infection rates of *C. parvum* by sex of infected person

Sex	Examined samples	Positive result	Percentage
Male	95	40	42.1%
Female	55	15	27.3%

The findings of the present study indicated that the highest infection rate occurred in the age group of 3 to 5 years, with 24 positive samples (42.1%), whereas the lowest infection rate was observed in the age group of 6 to 10 years, with 3 positive samples (7.3%), as illustrated in Table 3.

Table 3. Infection rates of *C. parvum* according to age groups

Age	Examined samples	Positive result	Percentage
6 months -2 years	55	15	27.3%
3 -5 years	57	24	42.1%
6-10 years	38	11	7.3%

The present immunological investigation documented a significant elevation in the levels of IL-10, IL-12 and CCL20 in the affected children, with mean concentrations of IL-12 (27.9 ± 2.6 Pg/ml), IL-

10 (16.98 ± 1.9 Pg/ml), and CCL20 (231.6 ± 11.1 ng/mL) in comparison to the control group. At p- value ≤ 0.05 (Table 4).

Table 4. Level of IL-10, IL-12, and CCL20 in patients infected with *C. parvum*

Parameters	Control n=30	Patients infected with *C. parvum* (n:55)	P. value
IL-10 (Pg/ml)	12.31± 2.1	16.98± 1.9	p≤0.05
Il-12 (Pg/ml)	12.8 ± 1.7	27.9 ±2.6	p≤0.05
CCL20 ng/ml	111.3±20.5	187.9±15.2	p≤0.05

Discussion

The proliferation of intestinal parasites, notably the Cryptosporidium parasite, among the population is a significant health issue now being examined through surveys conducted in hospitals, urban areas, and educational institutions. These parasites are prevalent across all age demographics and in both urban and rural locales, which facilitate their transmission. The elevated transmission and infection rates of these parasites are due to the simplicity with which their infectious stages can be conveyed from one individual to another via contaminated water or food [26]. Sources of exposure to these parasites differ based on health culture, social conventions, interactions with animals, and dietary practices. Infection with certain parasites is exacerbated by opportunistic infections, particularly when the immunological state of the individual is compromised, notably in patients undergoing chemotherapy and those with AIDS [26].

The findings of the present study align with [27] conducted in Mosul that reported an infection rate of 35%. In Baghdad Province, [28] a rate of 47.33 percent was discovered.

Other study indicated a high infection rate of 92.2% [29]. These variations in infection rates may be due to differences in study design, location, number of samples tested, and laboratory methods used, all of which increase the chances of transmission and spread of the parasite [30]. This study was consistent with a study in Babylon Governorate, which found an infection rate of in males higher than females [31]. However, it differed from other studies, such as one conducted by [32], which showed an infection rate of females, higher than the males.

The results of the current study are consistent with a study conducted in Mosul, which recorded the highest infection rate in the 3-5 year age group at 49.4% [27]. However, these results do not align with those recorded by [33] in Ethiopia, which found the highest infection rate in the 9-10 year age group. The high infection rate under 5 year age group is likely due to the underdeveloped immune systems of young children, coupled with the absence of euglobulin, a factor found in adults that helps destroy the parasite.

The current investigation revealed markedly elevated levels of IFN-α, IL-12, and IL-10 cytokines in persons infected with Cryptosporidium compared to their uninfected counterparts, indicating the synthesis of both Th1 (IFN-α and IL-12) and Th2 (IL-10) cytokines in the infected population. The current study concurs with [34]. A previous study [35], indicated that a substantial proportion of infected immunocompetent individuals exhibited production of IFN-α and IL-2, primarily suggesting a Th1 response; however, Th2-type cytokine IL-10 production was also noted in a minority (20%) of immunocompetent individuals with cryptosporidiosis. Experimental research have yielded divergent findings concerning the functions of Th1 and Th2 cytokines. A study [36] endorse the involvement of Th1 cytokines, while others [37] propose that Th2 cytokines may also contribute to protection against cryptosporidiosis.

The current study demonstrated an elevation of CCL20 in individuals with *C. parvum*. In contrast to mucosal secretions, which have modest ion concentrations, intestinal fluids have approximately 150 mM of sodium ions, which has the potential to impede the complete operation of chemokines. Diarrhea reduces the Na+ concentration in the colon, which is expected to augment the antibacterial potency of CCL20 [38]. The study by [39] showed oral treatment of CCL20 significantly decreased the parasite load in infected neonates.

Conclusion

The current study concluded a clear prevalence of *C. parvum* parasite in children, and also demonstrated a clear variation in the distribution of infection according to sex and age. The current study, through the evaluation of certain inflammatory immunological parameters in blood serum samples, concluded that the parasite stimulates an immune response resulting from its infection of intestinal cells in the small intestine, characterized by a highly significant increase in IL-10, IL-12, and CCL20.

REFERENCES

[1] L. Xiao and V. A. Cama, "Cryptosporidium and cryptosporidiosis," in *Foodborne Parasites*, Springer Nature, 2018, pp. 73–117.

[2] I. A. Al-Ezzy and A. T. Khadim, "Spatial, environmental and sociodemographic risk factors associated with *C. parvum* infection in calves," *Diyala Journal for Veterinary Sciences*, vol. 1, no. 3, pp. 64–82, 2021.

[3] N. K. Mahdi, I. Al Sadoon, and A. T. Mohamed, "First report of cryptosporidiosis among Iraqi children," *Eastern Mediterranean Health Journal*, vol. 2, no. 1, pp. 115–120, 1996.

[4] H. Al-Fahdawi and O. Mohammed, "*Cryptosporidium parvum* as a causative agent of diarrhea in children: A study in Al-Ramadi and Falluja," *International Journal of Health Systems and Medical Sciences*, vol. 3, no. 3, pp. 283–291, 2024.

[5] G. Penakalapati *et al*., "Exposure to animal feces and human health: A systematic review and proposed research priorities," *Environmental Science & Technology*, vol. 51, no. 20, pp. 11537–11552, 2017.

[6] K. F. Omolabi, P. O. Odeniran, and M. E. Soliman, "A meta-analysis of *Cryptosporidium* species in humans from southern Africa (2000–2020)," *Journal of Parasitic Diseases*, vol. 46, no. 1, pp. 304–316, 2022.

[7] S. Paulos Viñas *et al*., "Comparative performance evaluation of four commercial multiplex real-time PCR assays for the detection of the diarrhoea-causing protozoa *Cryptosporidium hominis/parvum*, *Giardia duodenalis* and *Entamoeba histolytica*," *PLoS One*, 2019.

[8] M. S. Mahmood and W. A. Alobaidii, "Molecular detection of *Trypanosoma* species in sheep and goats in Mosul city," *Iraqi Journal of Veterinary Sciences*, vol. 36, no. 2, pp. 445–449, 2022.

[9] W. Guesdon *et al*., "*Cryptosporidium parvum* subverts antimicrobial activity of CRAMP by reducing its expression in neonatal mice," *Microorganisms*, vol. 8, no. 11, Art. no. 1635, 2020.

[10] M. Deng, M. S. Rutherford, and M. S. Abrahamsen, "Host intestinal epithelial response to *Cryptosporidium parvum*," *Advanced Drug Delivery Reviews*, vol. 56, no. 6, pp. 869–884, 2004.

[11] F. Laurent and S. Lacroix-Lamandé, "Innate immune responses play a key role in controlling infection of the intestinal epithelium by *Cryptosporidium*," *International Journal for Parasitology*, vol. 47, no. 12, pp. 711–721, 2017.

[12] D. S. Korbel, F. M. Barakat, J. P. Di Santo, and V. McDonald, "CD4+ T cells are not essential for control of early acute *Cryptosporidium parvum* infection in neonatal mice," *Infection and Immunity*, vol. 79, no. 4, pp. 1647–1653, 2011.

[13] C. A. Hunter and L. D. Sibley, "Modulation of innate immunity by *Toxoplasma gondii* virulence effectors," *Nature Reviews Microbiology*, vol. 10, no. 11, pp. 766–778, 2012.

[14] V. McDonald, D. Korbel, F. Barakat, N. Choudhry, and F. Petry, "Innate immune responses against *Cryptosporidium parvum* infection," *Parasite Immunology*, vol. 35, no. 2, pp. 55–64, 2013.

[15] J. R. Mead, "Prospects for immunotherapy and vaccines against *Cryptosporidium*," *Human Vaccines & Immunotherapeutics*, vol. 10, no. 6, pp. 1505–1513, 2014.

[16] D. L. Ivanova *et al*., "Innate lymphoid cells in protection, pathology, and adaptive immunity during apicomplexan infection," *Frontiers in Immunology*, vol. 10, Art. no. 196, 2019.

[17] C. Lang, U. Groß, and C. G. Lüder, "Subversion of innate and adaptive immune responses by *Toxoplasma gondii*," *Parasitology Research*, vol. 100, no. 2, pp. 191–203, 2007.

[18] V. Jakobi and F. Petry, "Humoral immune response in IL-12 and IFN-γ deficient mice after infection with *Cryptosporidium parvum*," *Parasite Immunology*, vol. 30, no. 3, pp. 151–161, 2008.

[19] M. Akdis *et al*., "Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and

TNF-α: Receptors, functions, and roles in diseases," *Journal of Allergy and Clinical Immunology*, vol. 138, no. 4, pp. 984–1010, 2016.

[20] D. Aboelsoued *et al*., "Evaluation of a vaccine candidate isolated from *Cryptosporidium parvum* oocyst in mice," *Veterinary World*, vol. 15, no. 12, Art. no. 2772, 2022.

[21] L. Zhao, J. Xia, X. Wang, and F. Xu, "Transcriptional regulation of CCL20 expression," *Microbes and Infection*, vol. 16, no. 10, pp. 864–870, 2014.

[22] Y. Lee, R. Eri, A. B. Lyons, M. C. Grimm, and H. Korner, "CC chemokine ligand 20 and its cognate receptor CCR6 in mucosal T cell immunology and inflammatory bowel disease: Odd couple or axis of evil?," *Frontiers in Immunology*, vol. 4, Art. no. 194, 2013.

[23] G. Auray, S. Lacroix-Lamandé, R. Mancassola, I. Dimier-Poisson, and F. Laurent, "Involvement of intestinal epithelial cells in dendritic cell recruitment during *C. parvum* infection," *Microbes and Infection*, vol. 9, no. 5, pp. 574–582, 2007.

[24] L. Lantier *et al*., "Intestinal CD103+ dendritic cells are key players in the innate immune control of *Cryptosporidium parvum* infection in neonatal mice," *PLoS Pathogens*, vol. 9, no. 12, Art. no. e1003801, 2013.

[25] R. A. Haidar, "Epidemiology of *Cryptosporidium parvum* in Basra city, Iraq in 2019," in *AIP Conference Proceedings*, AIP Publishing, 2022.

[26] Al-Mosa, *Epidemiological Study of the Parasitic Causes of Some Intestinal Disorders of Human in Al-Hilla City*, M.S. thesis, College of Medicine, University of Kufa, Iraq, 2002.

[27] S. H. Younis and F. M. Alkhasha, "Epidemiological study of *Cryptosporidium parvum* parasite in some districts of Nineveh province, with referring to histopathological effects in lab mice," *College of Basic Education Research Journal*, vol. 17, no. 4, pp. 1023–1048, 2021.

[28] Dabas, D. Shah, S. Bhatnagar, and R. Lodha, "Epidemiology of *Cryptosporidium* in pediatric diarrheal illnesses," *Indian Pediatrics*, vol. 54, no. 4, pp. 299–309, 2017.

[29] M. Salim, "Epidemiological study on *Cryptosporidium* among children in Basra Province-Iraq," in *Journal of Physics: Conference Series*, IOP Publishing, 2018.

[30] N. El-Helaly, M. Aly, and S. Attia, "Detection of *Cryptosporidium* infection among children with diarrhea," *New York Science Journal*, vol. 5, no. 7, pp. 68–76, 2012.

[31] M. J. B. U. Oleiwi, "Prevalence study for main protozoa diarrheal among patients by using microscopically and molecular methods in Babylon province," 2015.

[32] I. Al-Hindi, A. A. Elmanama, and K. J. A. Elnabris, "Cryptosporidiosis among children attending Al-Nasser pediatric hospital, Gaza, Palestine," *Turkish Journal of Medical Sciences*, vol. 37, no. 6, pp. 367–372, 2007.

[33] T. Hailegebriel, "Prevalence of intestinal parasitic infections and associated risk factors among students at Dona Berber primary school, Bahir Dar, Ethiopia," *BMC Infectious Diseases*, vol. 17, no. 1, Art. no. 362, 2017.

[34] K. Kaushik, S. Khurana, A. Wanchu, and N. Malla, "Lymphoproliferative and cytokine responses to *Cryptosporidium parvum* in patients coinfected with *C. parvum* and human immunodeficiency virus," *Clinical and Vaccine Immunology*, vol. 16, no. 1, pp. 116–121, 2009.

[35] M. Gomez Morales *et al*., "Cytokine profile induced by *Cryptosporidium* antigen in peripheral blood mononuclear cells from immunocompetent and immunosuppressed persons with cryptosporidiosis," *The Journal of Infectious Diseases*, vol. 179, no. 4, pp. 967–973, 1999.

[36] H. N. Ehigiator, N. McNair, and J. R. Mead, "*Cryptosporidium parvum*: The contribution of Th1-inducing pathways to the resolution of infection in mice," *Experimental Parasitology*, vol. 115, no. 2, pp. 107–113, 2007.

[37] S. A. McDonald *et al*., "Protection against the early acute phase of *Cryptosporidium parvum* infection conferred by interleukin-4-induced expression of T helper 1 cytokines," *Journal of Infectious Diseases*, vol. 190, no. 5, pp. 1019–1025, 2004.

[38] S. Yung and P. Murphy, "Antimicrobial chemokines," *Frontiers in Immunology*, vol. 3, Art. no. 276, 2012.

[39] W. Guesdon *et al*., "CCL20 displays antimicrobial activity against *Cryptosporidium parvum*, but its expression is reduced during infection in the intestine of neonatal mice," *The Journal of Infectious Diseases*, vol. 212, no. 8, pp. 1332–1340, 2015.