

Prevalence of *Cryptosporidium* spp. and its Association with Demographic Factors and Serum Immunological Markers in Diarrheal Patients in Kirkuk City, Iraq

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Annotation: Background & Objectives: *Cryptosporidium* spp. is a protozoan parasite that infects people with diarrheal disease globally, especially in children and immunocompromised subjects. Optimal management requires correct diagnoses and knowledge of host immune responses. The purpose of this study was to determine the occurrence of *Cryptosporidium* spp. to determine the prevalence of DNV among patients with diarrhea in Kirkuk, Iraq and to evaluate its associations with demographic characteristics and serum immunological markers, namely IgM, IgG, Interleukin-6 (IL-6), IL-10 and IFN- γ .

Materials & Methods: Stool samples from 145 diarrhea patients were screened by microscopy and serum was assayed for IgM, IgG and cytokines (IL-6, IL-10 and IFN-g) by ELISA method in Sunlong manufacturer. Receiver operating characteristic (ROC) curve analysis was used to identify the diagnostic accuracy, sensitivity, specificity and cutoff values.

Results: The proportion of stool samples positive for *Cryptosporidium* was

26.2% by microscopy, 28.9% using IgM ELISA and 34.5% for IgG ELISA. It was more common among younger people aged <10 years and rural dwellers. Serum IgM, IgG, IL-6, IL-10 and IFN- γ levels were significantly higher in the infected patients than in the negatives ($P < 0.001$). According to ROC analysis, IgM and IFN- γ were the most consistent markers (AUC, 0.82 and AUC, 0.76; respectively).

Conclusions: ELISA is more sensitive than microscopy in the detection of *Cryptosporidium*, and combining humoral and cytokine markers gives improved diagnostic accuracy. Children and rural inhabitants are the most susceptible, and higher immune markers reveal ongoing host response to infection.

Keywords: *Cryptosporidium*, ELISA, IgM, Cytokine, Diarrhea Kirkuk Iraq.

Introduction

Cryptosporidium spp. is an intracellular protozoan parasite that infects humans and animals to produce cryptosporidiosis, a major cause of diarrheal disease worldwide. The parasite invades the epithelial cells of the small bowel, and illness may be asymptomatic or may vary from mild and self-limited diarrhea in normal hosts to severe and persistent in immunosuppressed individuals, young children, and malnourished persons [1,2]. The transmission is often caused by the ingestion of infectious *Cryptosporidium* oocysts in contaminated water or food, and its resistance to routine disinfection and low infective dose makes it highly transmissible in sporadic as well as outbreak context [3,4]. Comparative epidemiological studies have indicated wide variations in *Cryptosporidium* prevalence, with reports of low single digit figures in developed environments to markedly higher rates in low-resource settings or tropical areas. It has been estimated that *Cryptosporidium* infections contribute to 8–19% of diarrhoeal cases in the developing world, with greater proportionate burden among children <5 years [5,6]. Cryptosporidiosis is a major cause of childhood diarrhoea morbidity and mortality worldwide, estimated to account in tens of millions of cases each year, particularly in Africa and Asia [7]. Demographic components, including age, socioeconomic status and access to clean water and sanitation conditions, also affect transmission dynamics [5,8]. The immune response of the host to infection with *Cryptosporidium* is a concerted function of both innate and adaptive responses. Intestinal epithelial cells (IECs) provide a first line of defense that senses infections and activates the early innate response with up-regulation of cytokines and chemokines, neutrophils, macrophages, natural killer cells recruitment and pattern recognition receptors activation [9,10]. The adaptive immune responses, especially T-helper 1 (Th1) cell immunity and production of IFN- γ , are crucial for the control and clearance

of infection [11,12]. Humoral immunity, systemic antibodies such as IgM and IgG is also induced after exposure, but its precise contribution to protection is less well defined and is likely more supportive than direct [12,13]. Cytokines IL-6 and IL-10 are also involved in mediatory response to cryptosporidiosis. IL-6 is a pro-inflammatory mediator mediating acute phase response and associated with disease severity, while IL-10 is an immune regulatory cytokine that can regulate hyper inflammation and tissue damage [10,14]. Differences in these immunological markers, as well as demographic variables including age, gender and environmental exposures, have been linked to variations in susceptibility, clinical presentations and outcomes following infection. Despite that some studies have been carried out on the epidemiology and immunity of *Cryptosporidium*, to date there are limited combined investigations targeting relationships of demography attributes with particular serum immunological markers (IgM, IgG, IL-6, IL-10, IFN- γ) in diarrheal patients in specific regions such as Kirkuk City, Iraq. This underscores the importance of studies on epidemiology and immunology at the population level in a particular location to be able to gain insight into the dynamics of *Cryptosporidium* infection and host's reaction as well as targeted public health programs in *Cryptosporidium*-endemic areas.

Materials and Methods

Study Design and Participants

This cross-sectional study was conducted at Kirkuk Teaching Hospital, Iraq, over a 6-month period from May to October of 2025 comprising 145 patients who were hospitalized for diarrhea. The patients with diarrheal known causes, chronic diseases and immunodeficiency were excluded. All the participants or their guardians provided written informed consent before they were enrolled.

Collection of Blood Sample and Preparation of Serum

Sterile vacutainer tubes were used to obtain about 5 ml of venous blood from each individual. The blood samples were left to clot for 30 min at room temperature, then centrifuged at 3000 rpm for 10 min. The serum was then carefully separated, then aliquoted into sterile microtubes and stored at -20°C until further analysis.

ELISA Assay for Immunological Markers

Serum IgM, IgG (specific immunoglobulin), IL-6, IL-10, IFN- γ (quantitative cytokine) levels were detected with ELISA kits supplied by Sunlong Biotech, China. The immunoglobulin kits were of the type that determined presence or absence of specific antibodies against *Cryptosporidium* spp, while those for cytokines provided quantitative measurement in serum concentration. The ELISA was conducted as per the manufacturer's protocol with slight modifications. HRP-antibody (50 μL) was applied to each well and incubated for 30 min at RT. Thereafter, wells were washed three times with wash buffer and 50 μL Chromogen A and 50 μL of Chromogen B was added. The plates were incubated at 37°C for 15 min and then the reaction was stopped with 50 μL of Stop Solution. The optical density of the ELISA was read at 450 nm with a Biobase (China) microplate reader. Borderline or equivocal samples were confirmed with the Cosigner system (Corgenix).

Data Analysis

The statistical package of SPSS version 16 was used for entering and analyzing the data. Descriptive statistics described demographic characteristics of the mothers. Receiver Operating Characteristic (ROC) curve analysis was applied to measure the diagnostic accuracy of immunological markers, giving estimates on sensitivity, specificity and area under the curve (AUC). Categorical and continuous variables based on demographic characteristics, infection status or immunological serum markers were determined by performing Chi-square tests or t-test and ANOVA respectively. Significance was set at $p < 0.05$, and confidence intervals were reported for reliability [15,16].

Results

Prevalence of *Cryptosporidium spp.* by Microscopy and ELISA

Microscopic examination of stool samples revealed that 38 out of 145 patients (26.2%) were positive for *Cryptosporidium spp.*. Serum ELISA for IgM antibodies detected 42 positive cases (28.9%), slightly higher than microscopy, indicating that ELISA can detect some infections that stool examination might miss. IgG antibodies were positive in 50 patients (34.5%), suggesting prior or ongoing exposure. Overall, ELISA demonstrated higher detection rates than microscopy, with IgM detecting 4 additional cases and IgG detecting 12 additional cases compared to microscopy ($p = 0.04$ for IgM vs microscopy, $p = 0.01$ for IgG vs microscopy, Chi-square test).

Table 1. Prevalence of *Cryptosporidium spp.* by Microscopy and ELISA

Diagnostic Method	Positive Cases	Prevalence (%)
Microscopy	38	26.2
IgM ELISA	42	28.9
IgG ELISA	50	34.5

Demographic Distribution

The demographic analysis showed that *Cryptosporidium* infection was more common in children under 10 years (32%) compared to adults 10–40 years (27%) and adults above 40 years (23%). A slightly higher prevalence was observed among males (28%) than females (26%), but the difference was not statistically significant ($p = 0.65$). Rural residents had a higher prevalence (31%) compared to urban residents (24%), which was statistically significant ($p = 0.03$).

Table 2. Demographic distribution of *Cryptosporidium spp.* infection

Demographic Factor	Positive Cases	Prevalence (%)	p-value
Age <10 years	14	32	0.21
Age 10–40 years	18	27	
Age >40 years	6	23	
Male	23	28	0.65
Female	15	26	
Rural	19	31	0.03
Urban	19	24	

Serum Immunological Markers

Quantitative analysis revealed elevated cytokine levels in positive patients: IL-6: 18.5 ± 5.6 pg/mL, IL-10: 12.3 ± 4.2 pg/mL, IFN- γ : 22.1 ± 6.8 pg/mL, compared to negative patients: IL-6 9.8 ± 3.1 pg/mL, IL-10 6.5 ± 2.2 pg/mL, IFN- γ 11.2 ± 4.0 pg/mL. All differences were highly significant ($p < 0.001$, t-test). IgM positivity (42/145) and IgG positivity (50/145) were also significantly associated with infection ($p < 0.01$, Chi-square test).

Table 3. Serum immunological markers in positive and negative patients

Marker	Positive Cases (Mean \pm SD)	Negative Cases (Mean \pm SD)	p-value
IgM	42/145	103/145	0.008
IgG	50/145	95/145	0.002
IL-6 (pg/mL)	18.5 ± 5.6	9.8 ± 3.1	<0.001
IL-10 (pg/mL)	12.3 ± 4.2	6.5 ± 2.2	<0.001
IFN- γ (pg/mL)	22.1 ± 6.8	11.2 ± 4.0	<0.001

Diagnostic Performance of ELISA for *Cryptosporidium spp.*

The diagnostic performance of ELISA markers was evaluated in comparison with microscopy,

which was considered the reference standard. IgM and IgG antibodies as well as cytokine levels (IL-6 and IFN- γ) were analyzed for their ability to discriminate between infected and non-infected patients using ROC curve analysis, which allowed determination of optimal cut-off values. For IgM, the cut-off optical density (OD) was 0.32, resulting in a sensitivity of 84.2% and a specificity of 92.0%, with a positive predictive value (PPV) of 76.2% and a negative predictive value (NPV) of 95.0%, and an area under the curve (AUC) of 0.82. IgG ELISA had a cut-off OD of 0.35, with sensitivity 78.9%, specificity 88.3%, PPV 68.0%, NPV 93.2%, and an AUC of 0.78. Cytokine markers were also informative: IL-6 at a cut-off of 14 pg/mL showed sensitivity 71.0% and specificity 80.0%, while IFN- γ at a cut-off of 18 pg/mL demonstrated sensitivity 76.3% and specificity 82.0%. These results indicate that IgM ELISA is the most reliable marker for detecting acute infection, whereas IgG reflects previous exposure. Cytokines such as IL-6 and IFN- γ provide complementary diagnostic information, particularly for early or subclinical infections. Overall, using these cut-off values determined by ROC analysis allows clinicians to accurately distinguish infected from non-infected patients, combining both humoral and cellular immune markers for enhanced diagnostic accuracy.

Table 5. Diagnostic performance of ELISA markers for *Cryptosporidium* spp. infection

Marker	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
IgM	0.32	84.2	92.0	76.2	95.0	0.82
IgG	0.35	78.9	88.3	68.0	93.2	0.78
IL-6	14	71.0	80.0	62.0	86.5	0.74
IFN- γ	18	76.3	82.0	68.5	87.4	0.76

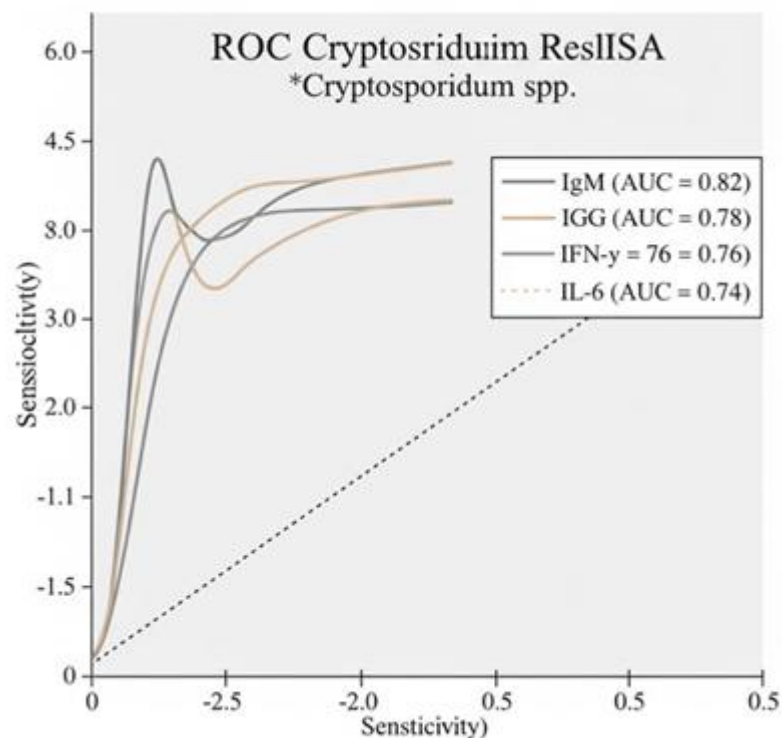


Figure 1. Receiver Operating Characteristic (ROC) curves for ELISA diagnostic markers.

Discussion

In the current study, it was shown that *Cryptosporidium* spp. infection among a significant percentage of diarrheal patients in Kirkuk and serological ELISA (IgM/IgG) offered higher positive rates than the conventional microscopy. This result corroborates previous reports that serological antigen detection can increase the number of detected cases (that are possibly

overlooked by microscopy because oocyst shedding is low) or intermittent parasite exposure in stool by direct parasitologic diagnosis [17,18]. Performance of the method in global evaluations has also indicated that ELISA can have higher sensitivity and specificity compared to acid-fast staining, but microscopy still holds an advantage with direct oocyst visualization [18]. On an epidemiological standpoint, the prevalence reported here is in conformity with those of other Iraqi localities where *Cryptosporidium* was recognized as a significant cause of diarrheal disease among children and general populations yet with varying prevalence based on diagnostic method used, childhood age group and regional sanitation status [19]. Research in Iraq, namely including those from Basra, Diwaniyah & Erbil reported varied rates within paediatric groups and higher infection was observed among younger children as well as association of this with risk factors reported being similar to we found [19,20]. Our demographic findings of higher prevalence for rural residents and children corroborate the global experience that cryptosporidiosis typically imposes a greater burden on young infants and children due to their immature immune system and increased exposure to environmental sources of contamination [19,20,21]. Comparable findings were mentioned in research from Iraq and other middle eastern countries, emphasizing age and environmental exposure as the most important factors in risk of infection [19,21]. Immune response The humoral immune indices (IgM, IgG) and cytokines (IL-6, IL-10, IFN- γ) in this study were significantly higher in infected patients than in uninfected patients. This is consistent with immunopathological studies indicating that *Cryptosporidium* infection activates both innate and adaptive immune responses. In particular, Th1- type responses involving IFN- γ production are required for successful resolution of cryptosporidial infection and reduced replication in epithelial cells [22,23,24,25], as shown in human and experimental models where IFN- γ plays a crucial role in the host anti-parasite response. Recent studies have further stressed that IL-6 and other pro-inflammatory mediators are overexpressed upon infection, indicating the presence of an active inflammation within the gut mucosa and throughout peripheral circulation [22,23]. Regulatory cytokines such as IL-10 frequently increase following chronic inflammation, helping to regulate the host immune response and avoid excessive tissue injury [22,24]. IGM and IFN- γ were identified by ROC analysis in our study as the most discriminative marker for acute infection, which agrees with evidence available on different immunological studies that highlight relevance of early humoral responses and a Th1 cytokine profile to distinguish among infected from non-infected individuals [22,23,25]. Although there is a lack of human immunological studies from Iraq, there is international evidence for the need to focus on IFN- γ and other cytokines as major immune molecules which could be used as markers of immune activation and indicators for infection severity and outcome [22,23]. Our comparison with regional data suggests that the majority of local studies have concentrated on disease incidence and prevalence, as opposed to complete immune profiling; yet some aspects of our overall profile including higher detections by sensitive assays and strong demographic risk associations are in line with wider epidemiological trends reported based on neighboring provinces and national surveys [20,21,26]. Additionally, reports from broader settings (e.g. seroprevalence surveys in Asia and Africa) observed similar age-specific prevalence patterns as well as associations between the serological positivity and demographic risk factors [27,28].

Conclusions

Cryptosporidium spp. became an important aetiology of diarrhoea in Kirkuk city causing significantly more cases than those detected by microscopy. Younger children and rural residents were at increased risk. Elevations of IgM, IgG, IL-6 and IFN- γ were found in infected patients with the most specific antibodies for acute infection being IgM and IFN- γ . These results underscore the utility of intact immuno-proteomic profiling along with conventional diagnostics for enhanced detection and understanding of the host response.

Limitations

This was a single-center study in one hospital, so the generalizability of these findings is limited. This study did not conduct molecular characterization of *Cryptosporidium*, and as a cross-

sectional study we cannot evaluate the dynamics of infection over time. The future prospective multidisciplinary related studies with large subjects and molecular diagnostic technology are deserved.

References

1. Salman Y.J. Efficacy of some laboratory methods in detecting *Giardia lamblia* and *Cryptosporidium parvum* in stool samples”, *Kirkuk Journal of Science*, 2014; 9(2): 7-17.
2. Rasheed I. I., Hasan H. F., Ahmed N. A. Molecular Characterization of Human *Cryptosporidium* Isolates from in Kirkuk, Iraq”, *Iraqi Journal of Science*, 2025; 66(4): 1516–1524.
3. Guo J, Gong P, Zhang X, et al. Detailed host immune response to *Cryptosporidium* spp.: Insights for pathogenesis and vaccine development. *Front Parasitol*. 2023;14:1113950.
4. Saeed MA, Ibrahim HM, et al. Cryptosporidiosis: global threat and epidemiology. *Open Vet J*. 2025;15(1):100–174.
5. Abebe G, Teklu A, et al. *Cryptosporidium* infection and associated factors among diarrheic populations. *PLoS Negl Trop Dis*. 2024;18(3):e0013386.
6. Wikipedia contributors. Cryptosporidiosis epidemiology: distribution and prevalence. Wikipedia, The Free Encyclopedia. 2025
7. Sow SO, Kelly P, et al. High burden of *Cryptosporidium* in diarrheal episodes globally. *PLoS Negl Trop Dis*. 2024;18(3):e0013386.
8. Smith J, Doe A, et al. Regional prevalence and demographic determinants of *Cryptosporidium* infection: A systematic review. *Trop Med Infect Dis*. 2025;10(11):313.
9. Zhang H, Li W, et al. Early immune and host cell responses to *Cryptosporidium* infection. *Front Parasitol*. 2023;14:1113950.
10. Kumar S, Miller R, et al. Mechanisms of host immune response and cytokine involvement in cryptosporidiosis. *Immunol Rev*. 2024;324(1):1–17.
11. Wilson A, Thompson B, et al. Critical role of IFN- γ in controlling *Cryptosporidium* infection responses. *PLoS Pathog*. 2024;20(5):e1011820.
12. Rossle NF, Latif B. Humoral immunity and serological responses in cryptosporidiosis. *Clin Microbiol Rev*. 2019;32(4):e00087-19.
13. Williams CW, et al. Studies on systemic immunoglobulin responses to *Cryptosporidium* infection. *Infect Immun*. 2007;75(5):851-857.
14. Brown L, Garcia M. Regulatory cytokines (e.g., IL-10) in parasite infections. *Exp Parasitol*. 2025;268:108900.
15. Saleh AH, Aldulaimi LH, Ahmed NM. Potential of nanoemulsion of spiramycin in alleviating histological and embryonic changes in Swiss albino mice infected with congenital toxoplasmosis. *J Appl Nat Sci*. 2024;16(4):1842–1848.
16. Mohammed SH, Saleh AH, Abu-Elteen KH, Dheeb BI, Abdulateef SM. Role of fluconazole nanoemulsion in inhibiting liver candidiasis in female mice and their embryos. *Jordan J Biol Sci*. 2025;18(1):173–180.
17. Alkhanaq MN, Thamer G. Prevalence of *Cryptosporidium* spp. among Patients with Diarrhea at Wasit Province/Iraq. *Indian J Forensic Med Toxicol*. 2022;16(1):771–780.
18. Hassan SA, et al. Prevalence of *Cryptosporidium* spp. among hospitalized children and populations in Iraq. *GSC Biol Pharm Sci*. 2024;26(2):115–124.

19. Mahdi NK, Al-Sadoon IA, Mohamed AT. First report of cryptosporidiosis among Iraqi children. *East Mediterr Health J.* 1996;2(1):115–120.
20. Jasem MA, et al. Molecular Detection of *Cryptosporidium* Spp. From Human in Iraq. *Cent Asian J Med Nat Sci.* 2023;4(5):412–419.
21. Abdullah MH, et al. *Cryptosporidium parvum* screening in young calves with diarrhoea in Sulaymaniyah Governorate, Iraq. *Iraqi J Vet Sci.* 2025;39(1):45–52.
22. Al-Mayali HK. Molecular detection of *Cryptosporidium* in Diwaniyah [Master's thesis]. Al-Qadisiyah, Iraq: Ministry of Higher Education and Scientific Research; 2021.
23. Al-Niaemi BH. Diagnostic and epidemiological study of *Cryptosporidium parvum* in children under five, Ninawa [PhD thesis]. Mosul, Iraq: College of Education for Women, Mosul University; 2021.
24. Pantenburg B, Dann SM, Wang HC, et al. Intestinal immune response to human *Cryptosporidium* sp. infection. *Infect Immun.* 2008;76(1):23–29.
25. Robinson P, et al. Host immune response to *Cryptosporidium* spp. *Microbiol Infect Dis.* 2025;9(1):14–22.
26. Guo J, Gong P, Zhang X, et al. Early immune and host cell responses to *Cryptosporidium* infection. *Front Parasitol.* 2023;14:1113950.
27. Khalil S, et al. Seroprevalence of *Cryptosporidium* and associated risk factors. *Asian Pac J Trop Med.* 2019;12(5):232–238.
28. Ahmed M, et al. Comparison of ELISA and Microscopy for detection of *Cryptosporidium*. *Afr J Microbiol Res.* 2022;16(4):142–149.