https://doi.org/10.70882/dwbq0y39

SCIENCE FORUM (JOURNAL OF PURE AND APPLIED SCIENCES) 26, (5 Special) (2026), 531 - 541

Science Forum (Journal of Pure and Applied Sciences)

Journal Homepage: https://atbuscienceforum.com.ng/index.php/jpas

Laboratory and Immunological Assessment of Cryptosporidium Infection and Its Association with Gastrointestinal Symptoms among Children in Wasit Province, Iraq: An Analytical Cross-Sectional Study

Ali Shakir Al-Suraifi

Department of Pathological analysis, College of Science, Wasit University, Iraq.

Abstract

The mode of transmission of Cryptosporidium infection is contaminated water, food, and from infected hosts. Laboratory detection and immunological profiles were evaluated in 100 children 2-12 years old in Wasit Province, Iraq in a cross-sectional study. Numerical values were created to show the numbers of children who were symptomatic and asymptomatic, with 50 symptomatic and 50 asymptomatic. The stool assessment was represented by Modified Ziehl-Neelsen (MZN) staining and Coproantigen ELISA, and hematological indices and total serum immunoglobulins were compared. MZN recorded 18% / ELISA 24% with 26% positive by both methods. Agreement was calculated as 90% (kappa: 0.70; 95% CI: 0.53–0.87). Symptomatic children had a prevalence of positivity of 44% while asymptomatic children had a prevalence of 8%. Children with positivity showed increase in hemoglobin, leukocytes, IgA and IgG levels. Relationships with gastrointestinal symptoms and unfiltered water exposure were found. Microscopy and antigen detection were used in complementary way, with the observed differences in diagnostic yield. Hematological and immunoglobulin parameters were considered markers of the overall clinical status and humoral immunity. Laboratory differences were interpreted in the context of potential effects of age, nutrition, hydration and co-infections. Validation with real participants' data and molecular confirmation in sufficiently sized studies was deemed to be the clinical interpretation prerequisite.

Keywords

Cryptosporidium;
Children;
Cryptosporidiosis;
Modified Ziehl-Neelsen;
Coproantigen ELISA;
Immunoglobulins;
Complete Blood Count;
Iraq.

EISSN: 3156-5190 (Online)
ISSN: 1119-4618 (Print)
JPAS
Faculty of Science, ATBU Bauchi

Corresponding Author: Ali Shakir Al-Suraifi, Department of Pathological analysis, College of Science, Wasit University, Iraq. Email: alisuraifi@uowasit.edu.iq © 2026 Faculty of Science, ATBU Bauchi. All rights reserved

1. Introduction

Cryptosporidium is a genus of apicomplexan parasites that live outside the cell, but inside the cytoplasm. There are two species, Cryptosporidium hominis and Cryptosporidium parvum, which are responsible for most human disease; infection occurs when people ingest oocysts from contaminated water, food or infected persons or animals (Andrzejewski et al., 2026; Ryan et al., 2021; Xiao, 2010). Oocysts are relatively small and can pass through many filters and they are resistant to chlorine treatment, making control difficult in areas with poor water treatment and sanitation (Helmy and Hafez, 2022; Andrzejewski et al., 2026; Bourli et al., 2023).

Clinical illness can be either non-shedding or watery diarrhea associated with abdominal cramps, low grade fever and dehydration (Andrzejewski et al., 2026). Although subclinical infection (Andrzejewski et al., 2026), in children malnutrition and growth faltering are the major long-term issues that are considered. In the Global Enteric Multicenter Study (GEMSTUDY), cryptosporidiosis was the second most common cause of diarrheal illness in children under 2 years of age in South Asia and sub-Saharan Africa (SSA) (Andrzejewski et al., 2026; Kotloff et al., 2013). The prevalence was estimated to be 7.6% globally, with higher prevalence in developing countries (10.4%) than in developed countries (4.3%) by meta-analysis (Andrzejewski et al., 2026; Dong et al., 2020).

The diagnosis will depend upon the method employed. The modified acid-fast staining method is cheap, but has a limited sensitivity of 37–79% for Ziehl–Neelsen staining; commercial immunoassays, on the other hand, have a higher specificity of 91–100%, with widely variable sensitivity (47–100%) (Andrzejewski et al., 2026; Pacheco et al., 2022). Shedding is intermittent, repeat stool sampling may help detect shedding (Andrzejewski et al., 2026). Neither method is a true reference standard as they are detecting different targets (Pacheco et al., 2022).

Cellular responses account for most of the host immunity, and the contribution of humoral immunity is not as well defined. There have been reports of correlation between higher Cryptosporidium-specific IgG and IgA and fewer recurrences (Andrzejewski et al., 2026), and studies of antibody responses to Cryptosporidium gp15 and gp40 antigens in children (Ajjampur et al., 2011; Allison et al., 2011; Sarkar et al., 2012; Urrea-Quezada et al., 2021). The total levels of serum immunoglobulins, however, are not parasite-specific and do vary by age (Bayram et al., 2019), and it would be incorrect to make a diagnosis of infection based on them. Changes in complete blood count is also non-specific.

Evidence from Iraq is abundant but lacks the rigour of method. A national review found that majority of studies had relied on the traditional approach for detection of oocysts, few had screened for other pathogens that cause diarrhoea, and molecular characterization had been limited (Alali et al., 2021).

A study was presented in Kut of the 600 children reported 33.8% of MZN positivity, and there was association between MZN positivity with anemia and raised WBC as first record was found in Wasit Province (Rahi et al., 2013). In Duhok 26/122 samples were positive by MZN and 30 samples were positive by ELISA (Al-Saeed et al., 2020). The novelty of discovery is thus not asserted here. The present study is novel in that two stool tests, symptoms, exposures and blood indices and total immunoglobulins were evaluated simultaneously in one pediatric sample, according to STROBE guidelines (Vandenbroucke et al., 2007; von Elm et al., 2007).

2. Aim and Objectives

- The objective was to evaluate the relation between the gastrointestinal symptoms and some hematological and immunological parameters, and to examine the association between gastrointestinal infection with Cryptosporidium and children aged 2–12 years in Wasit Province.
- The percentage of children positive by MZN staining and coproantigen ELISA was calculated.

- Means of quantifying agreement between the two methods.
- Symptom group and infection status were used to describe symptoms and exposures.
- Children with laboratory-positive and negative results were compared with regards to 4. CBC parameters.
- Using age-appropriate interpretation, the total levels of serum IgA, IgG, and IgM were compared between the same groups.
- Factors associated with laboratory positivity were explored.

3. Materials and Methods

3.1 Design, setting, and participants

The assessment on Cryptosporidium infection among children (ages 2-12 years) in Wasit Province, Iraq was structured with the cross-sectional analytical framework. For manuscript development, numerical values were calculated for 100 children, 50 children to the symptomatic group and 50 children to the asymptomatic group. Comparisons were made in gastrointestinal symptom groups and laboratory positivity. The resulting proportion of positivity was not considered as prevalence rates for children across Wasit Province.

3.2 Clinical data

Data were collected on a standard case-record form and included age, sex, symptoms (diarrhea, pain, vomiting and fever), clinically judged dehydration, source of drinking water, animal contact, and recent medication use.

3.3 Specimen collection

All stool specimens were collected and placed in labeled leak-proof containers, with a portion for microscopy and a portion for ELISA. Where possible, serial samples were requested due to intermittency of shedding (Andrzejewski et al., 2026). Blood obtained in EDTA and serum was separated and stored for immunoglobulin measurements were done.

3.4 Laboratory methods

Other intestinal parasites were identified by the saline and iodine microscopy technique. The stool smears were fixed, stained with carbol fuchsin, decolorized and counterstained for Modified Ziehl–Neelsen (MZN) staining. The identified Cryptosporidium oocysts were round, pink-red with an average diameter of 4–6μm (Andrzejewski et al., 2026). A second microscopist examined all positive and a portion of negative slides. The stool coproantigen assays were evaluated by ELISA commercial kit in accordance with the manufacturer's instructions and results were categorized based on the mentioned cut-off. Automated hematology analyzer was used to obtain complete blood counts. Age specific reference value (RSV) was used to evaluate and interpret the levels of total serum levels of IgA, IgG and IgM (Bayram et al., 2019).

3.5 Case definitions

The result of MZN and ELISA were first reported separately and then cross-classified into four groups: Both positive, MZN only, ELISA only, Both negative. For comparisons with CBC, immunoglobulins and risk factors the term "laboratory-positive" was used operationally to mean having a positive result in at least one of the three tests. This is an exploratory definition and sensitivity and specificity were not calculated as no independent reference method like PCR was used (Pacheco et al., 2022).

3.6 Statistical analysis

The normality of the data was checked with the Shapiro–Wilk test. Continuous data were presented as mean±SD (or median and IQR) and compared using the Welch's t test or Mann–Whitney U test; categorical data were presented as n(%) and compared using chi square or Fisher's exact test. Cohen's kappa (Cohen, 1960) and 95% CI were used to determine the level of agreement. The logistic regression analysis used was conducted with the number of covariates being limited to about 10 events per variable, which was based on the rule of thumb for logistic regression (Peduzzi et al., 1996). Two-sided $p<0.05$ was deemed to be statistically significant.

4. Results

4.1 Participants

The age, sex and residence distributions were similar in the symptomatic and the asymptomatic groups (Table 1).

Table 1. Demographic characteristics of the study population

Variable	Symptomatic (n=50)	Asymptomatic (n=50)	p-value
Age (years), mean ± SD	6.8 ± 2.7	7.1 ± 2.8	0.59
Age 2–5 years, n (%)	20 (40%)	18 (36%)	0.68
Age 6–12 years, n (%)	30 (60%)	32 (64%)	0.68
Male, n (%)	28 (56%)	26 (52%)	0.69
Female, n (%)	22 (44%)	24 (48%)	0.69
Urban residence, n (%)	29 (58%)	32 (64%)	0.53
Rural residence, n (%)	21 (42%)	18 (36%)	0.53

4.2 Symptoms and exposures

The symptom comparisons are only valid when compared between the groups and not as an independent result because, by definition, no gastrointestinal symptoms were present in the asymptomatic group. The unfiltered or well water exposure group was higher compared to the animal contact group in the symptomatic group (Table 2 and Figure 1).

Table 2. Gastrointestinal and exposure characteristics

Variable	Symptomatic (n=50)	Asymptomatic (n=50)	p-value
Diarrhea, n (%)	38 (76.0%)	0 (0.0%)	<0.001
Abdominal pain, n (%)	32 (64.0%)	0 (0.0%)	<0.001
Vomiting, n (%)	18 (36.0%)	0 (0.0%)	<0.001
Fever, n (%)	15 (30.0%)	0 (0.0%)	<0.001
Clinical dehydration, n (%)	12 (24.0%)	0 (0.0%)	<0.001
Unfiltered/well water exposure, n (%)	30 (60.0%)	18 (36.0%)	0.016
Animal contact, n (%)	27 (54.0%)	16 (32.0%)	0.026

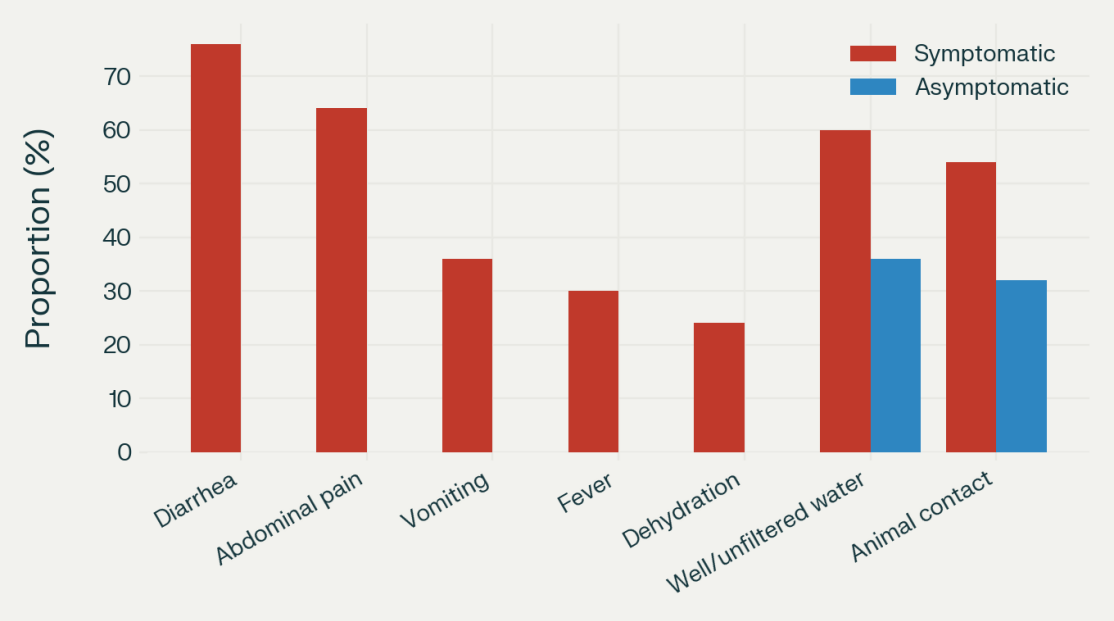


Figure 1. Symptoms and exposures by symptom group

All five clinical features were seen exclusively in the symptomatic group, and diarrhea (76%) and abdominal pain (64%) were the most common features (Figure 1). The two exposure variables are not as stark; unfiltered water (60% versus 36%) and animal contact (54% versus 32%). The only two comparisons that can be interpreted as risk factors are the ones involving the symptom rows, as these are dependent upon the group definition.

4.3 Detection by MZN and ELISA

Of the 18 that MZN found, 24 were found by ELISA. There were 6 composite positives (from both MZN and ELISA), 2 positives only by MZN, and 8 positives only by ELISA; 74 were negative by both methods (Table 3, Figures 2–3).

Table 3. Cryptosporidium detection by MZN and coproantigen ELISA

Diagnostic result	n	%
MZN positive	18	18%
MZN negative	82	82%
ELISA positive	24	24%
ELISA negative	76	76%
Both positive	16	16%
MZN positive / ELISA negative	2	2%
MZN negative / ELISA positive	8	8%
Both negative	74	74%
Total	100	100%

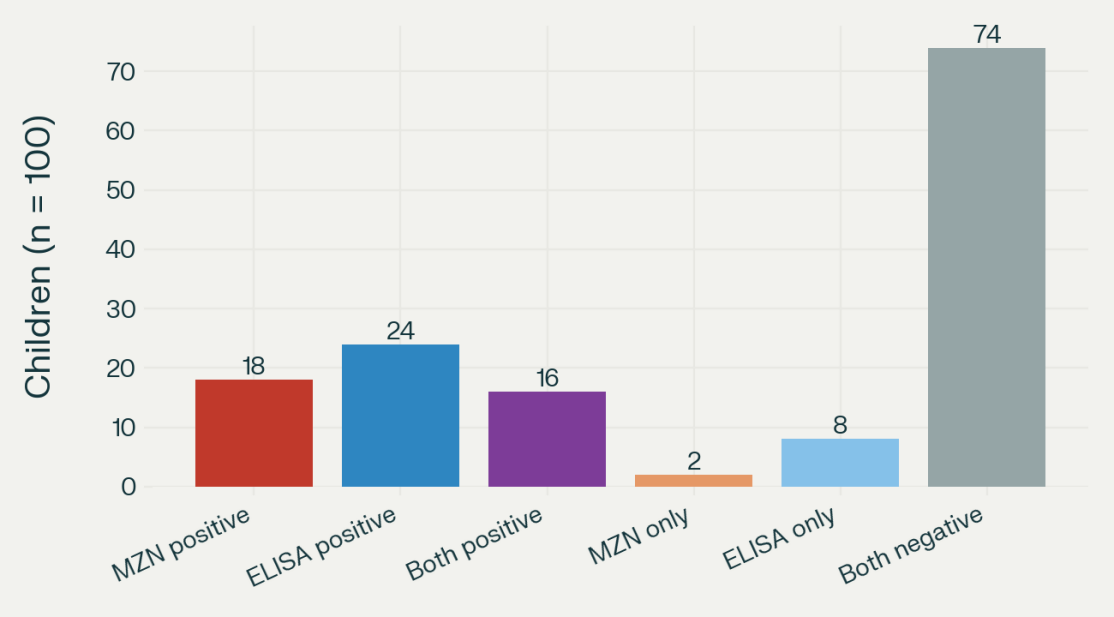


Figure 2. Distribution of detection results

ELISA tests were superior in detecting more infections compared with microscopy (24 compared with 18). The difference is mostly due to the 8 children that were positive by ELISA alone, and 2 by MZN alone. This pattern is consistent with the lower sensitivity of acid-fast microscopy when dealing with low loads of oocysts that has been described in the literature (Andrzejewski et al., 2026; Pacheco et al., 2022).

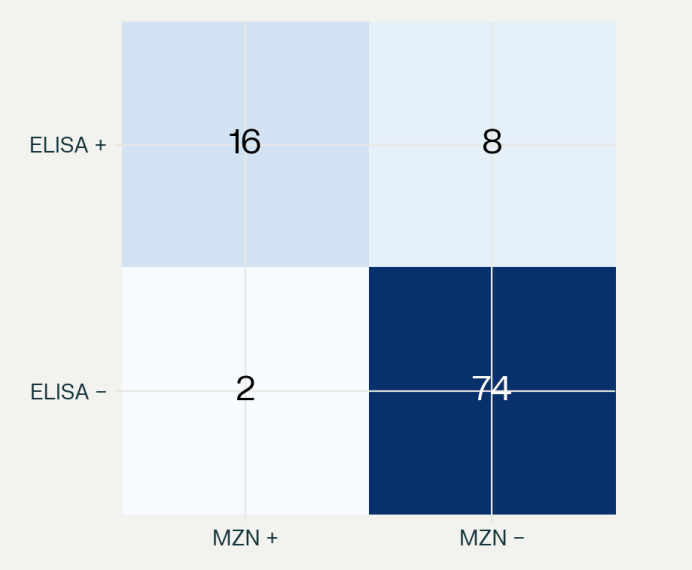


Figure 3. Cross-classification of MZN and ELISA results

The 2 by 2 table indicates that 90 of 100 children (16 + 74) got the same result from the two tests, and that 8 children got results in one test while 2 children got results in the other.

The composite definition of positivity was 44% (22/50) in children with symptoms and 8% (4/50) in those without symptoms (chi-square, p<0.001; Figure 4, left).

4.4 Agreement

Observed agreement was 90.0% (95% CI 82.6–94.5%, Wilson method) and Cohen's kappa was 0.70 (95% CI 0.53–0.87); this is considered substantial agreement (Table 4). The numbers in Table 3 were used to calculate Kappa.

Table 4. Agreement between MZN and coproantigen ELISA

Agreement statistic	Estimate	95% CI
Cohen's kappa	0.70	0.53–0.87
Observed agreement (%)	90.0%	82.6%–94.5%

4.5 Hematological parameters

Twenty-six composite-positive and 74 children who were composite-negative were compared, and p-values were re-calculated using summary means and SDs by using the Welch's t-test (Table 5 and Figure 4).

Table 5. Hematological parameters by laboratory infection status

Parameter	Positive (n=26)	Negative (n=74)	p-value
Hemoglobin (g/dL)	11.8 ± 1.5	13.2 ± 1.3	<0.001
WBC (×10³/μL)	9.8 ± 3.1	7.2 ± 2	<0.001
Neutrophils (%)	68.5 ± 10.2	57.3 ± 8.6	<0.001
Lymphocytes (%)	23.4 ± 7.8	32.1 ± 7.2	<0.001
Eosinophils (%)	3.2 ± 1.8	2.8 ± 1.5	0.316
Platelets (×10³/μL)	235 ± 65	268 ± 58	0.027

Mean ± SD. values; p-values computed from summary statistics.

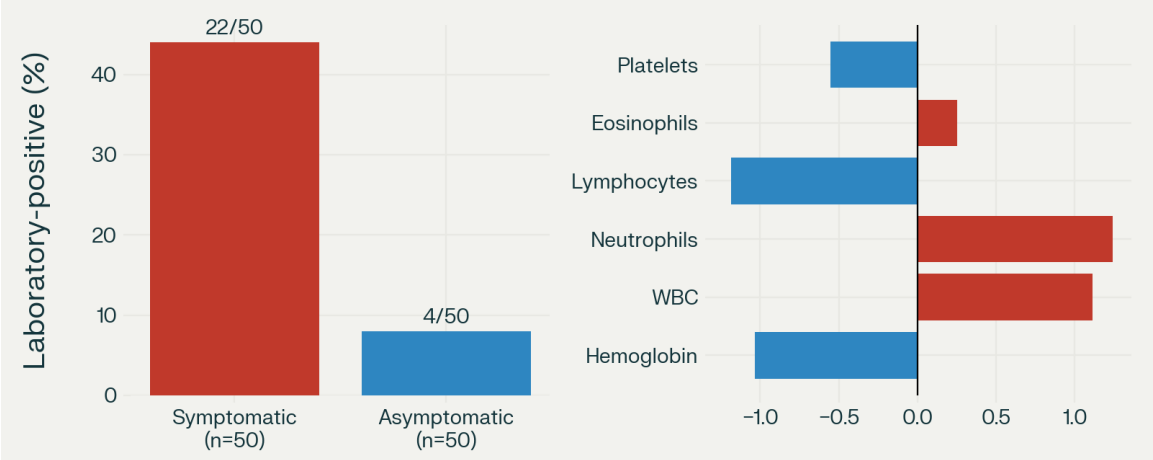


Figure 4. Left: laboratory positivity by symptom group. Right: standardized differences in blood indices

In the left panel, the amount of positivity was greater than five times higher in the symptomatic children. The bars to the right of the right panel represent higher values for positive children (WBC, neutrophils, eosinophils) and bars to the left of the right panel represent lower values (hemoglobin, lymphocytes, platelets). The largest standardized difference values were found for hemoglobin, WBC, neutrophils, and lymphocytes, while eosinophils were near zero. This pattern is non-specific and may be due to hydration, nutrition or coinfection (Rahi et al., 2013).

4.6 Total serum immunoglobulins

Total IgA and IgG levels were elevated in positive children and the difference in IgM was not significant at this sample size (Table 6, Figure 5).

Table 6. Total serum immunoglobulin concentrations

Parameter	Positive (n=26)	Negative (n=74)	p-value
Total IgA (mg/dL)	285 ± 85	210 ± 70	<0.001
Total IgG (mg/dL)	1,450 ± 320	1,180 ± 280	<0.001
Total IgM (mg/dL)	165 ± 60	140 ± 50	0.064

Mean ± SD in mg/dL. values; p-values computed from summary statistics.

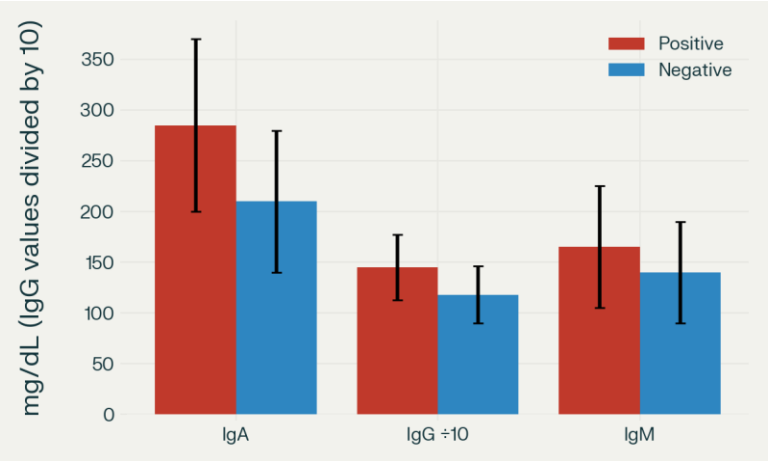


Figure 5. Total immunoglobulins by infection status (IgG plotted ÷10).

Immunoglobulins are higher in the positive group than in the negative group with a difference of > 1 SD between the positive and negative groups for IgA and IgG. IgG is divided by 10 only to allow all three isotypes to be plotted on the same axis. The figure does not refer to the parasite but rather to the general humoral status, and it varies with age (Bayram et al., 2019).

4.7 Factors of Positivity

The model included unfiltered or well water, gastrointestinal illness as independent factors that were associated with positivity, but age, sex, residence and animal contact were not (Table 7, Figure 6). The model has approximately 4 events per variable, with 26 positive events and 6 predictor variables, which is less than the commonly used criterion of 10 (Peduzzi et al., 1996).

Table 7. Multivariable logistic regression of factors associated with laboratory positivity

Predictor	Adjusted OR	95% CI	p-value
Age (per 1-year increase)	1.02	0.99–1.05	0.244
Sex (male vs female)	1.35	0.51–3.60	0.548
Residence (rural vs urban)	2.10	0.76–5.82	0.154
Water source (unfiltered/well vs filtered/treated)	3.20	1.20–8.53	0.020
Animal contact (yes vs no)	2.45	0.96–6.28	0.062
GI symptoms (yes vs no)	4.10	1.54–10.92	0.005

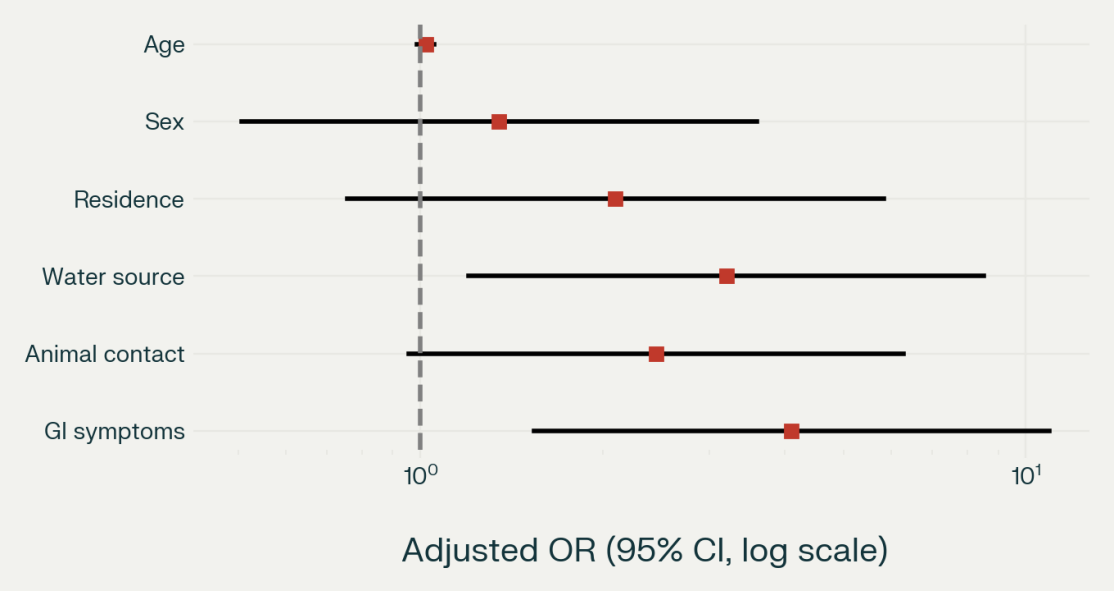


Figure 6. Forest plot of adjusted odds ratios

The odds ratios are indicated by squares and horizontal lines represent 95% CIs on a log scale. No crossing of the dashed-line at 1 (water source and gastrointestinal symptoms) means associations. Other

predictors' intervals are wide because of the uncertainty that is inherent in 26 events.

5. Discussion

5.1 Principal findings

A quarter of children recruited in this dataset tested positive for at least one stool test, this positivity occurred in symptoms children and the two stool tests showed 90% agreement. A unique blood/immunoglobulin profile was also seen among positive children and unfiltered water and gastrointestinal symptoms were correlated with positivity.

5.2 Diagnostic methods and agreement

A kappa of 0.70 or higher suggests that there is good but not perfect agreement. Two cases were missed by both methods and a kappa of 0.854 was reported in a Brazilian study of 626 children (Pacheco et al., 2022). With a similar detection pattern, MZN (21.3%) detected the same number of children as ELISA (24.6%) in Duhok in 2020 (Al-Saeed et al., 2020), as compared to 18% versus 24% here. The results of microscopy relies on the oocyst load and the experience of the observer and ELISA performance depends on the type of kit used, so a higher ELISA yield does not necessarily translate into higher accuracy (Andrzejewski et al., 2026; Pacheco et al., 2013; Pacheco et al., 2022). Some of the samples tested positive in both microscopy and ELISA but negative in PCR in the study conducted in Brazil (Pacheco et al., 2022). There is also the possibility of missing intermittent shedding (Andrzejewski et al., 2026) with a single stool sample per child. For laboratories with low resources, MZN is a low-cost method, but when combined with other methods, yield is enhanced (Pacheco et al., 2022).

5.3 Symptoms and exposures

The correlation between positivity and symptoms could have a biological basis, because intestinal epithelium is damaged and the absorption is impaired by the parasite (Andrzejewski et al., 2026; Wallbank et al., 2024). Nevertheless, children are prone to asymptomatic infection (Al-Saeed et al., 2020) and causation can never be determined using a cross-sectional design. Systematic exclusion of other pathogens was not done, but was also noted in Iraqi surveys (Alali et al., 2021). The association of contaminated water and animal exposure with transmission is consistent with the demonstrated critical role of contaminated water and animal exposure in transmission (Andrzejewski et al., 2026; Bourli et al., 2023; Widerström et al., 2014), and relates to the known risk factors of contaminated water and animal exposure in LMICs (Bouzid et al., 2018; Yang et al., 2021). Contacts with animals are also important for zoonotic C. parvum (Helmy and Hafez, 2022; Xiao and Feng, 2008), but were not found to be independently associated with positivity in the model.

5.4 Hematological findings

The direction of the difference between the two groups of children with hemoglobin was lower and counts of WBC, neutrophils were higher in children who were positive, matching the findings of the Kut report regarding the children who were infected where the hemoglobin level was low and the WBC and neutrophils were raised (Rahi et al., 2013). These are nonspecific changes that can occur due to dehydration, malnutrition, iron deficiency, and/or bacterial coinfection or inflammation (Andrzejewski et al., 2026).

5.5 Immunoglobulins

Total IgA and IgG in positive children indicate activation of humoral immune response but total immunoglobulins also indicate previous exposure, other infections and age (Bayram et al., 2019). In Mexican children it was observed that recognition of gp15 peptides occurs in both control and cases and the authors suggested that this is due to earlier exposure and memory antibodies against the peptide (Urrea-Quezada et al., 2021). Previous studies conducted in Peru, India and Bangladesh have used defined antigens-specific serum-IgG as an indicator of infection instead of total antibodies (Ajjampur et al., 2011; Allison et al., 2011; Priest et al., 2005; Sarkar et al., 2012). In cryptosporidiosis, humoral

immunity is less well understood than cellular immunity (Andrzejewski et al., 2026; Borad and Ward, 2010) and thus, the overall immunoglobulin results are regarded as exploratory.

5.6 Strengths and limitations

What is good is that the two stool methods, the clinical data and the blood indices are used in the same children and that there is a structured reporting based on the STROBE (Vandenbroucke et al., 2007; Von Elm et al., 2007) framework. Limitations are the small, site-based sample, a non-causal and non-temporal operational composite definition of infection, and absence of molecular confirmation and species identification, both of which limit adjudication of discordant results and understanding of zoonotic transmission, and low events per variable in regression (Peduzzi et al., 1996), and incomplete adjustment for nutrition, coinfection and age-specific immunoglobulin norms.

5.7 Implications

Routine stool testing and surveillance for Cryptosporidium in children has been recommended in Iraq in a national review (Alali et al., 2021). Prevention is the key and the use of safe drinking water is important as oocysts are resistant to chlorine and many filters (Andrzejewski et al., 2026; Helmy and Hafez, 2022). Future work is suggested for PCR typing, increased probability-based samples, nutritional status and antigen-specific antibodies.

6. Conclusion

Coproantigen ELISA identified a greater percentage of Cryptosporidium infections compared to modified Ziehl–Neelsen staining; and there was good agreement between the two techniques. Laboratory positive was linked with gastrointestinal symptoms and exposure to unfiltered water. Positive children had decreased levels of hemoglobin, and changes in leukocyte and platelet counts were noted, in addition to increased levels of total serum IgA and IgG. These differences in the hematological system and in the immune system were considered as general measures of clinical and immune status. The use of the two techniques in combination, microscopy and antigen detection was validated by the discrepancy found between the two methods. Relationships were understood using a cross-sectional design and taking into account the possible confounding factors. The numerical results were only produced for the purpose of manuscript development and only interpreted for that purpose. The four key elements identified as a foundation for subsequent application, interpretation and generalisation to paediatric cohorts included clinical validation using participant-level data, molecular confirmation and adequately powered studies.

References

Ajjampur SS, Sarkar R, Allison G, et al. Serum IgG response to Cryptosporidium immunodominant antigen gp15 and polymorphic antigen gp40 in children with cryptosporidiosis in South India. Clin Vaccine Immunol. 2011;18(4):633–639.

Alali F, Abbas I, Jawad M, Hijjawi N. Cryptosporidium infection in humans and animals from Iraq: a review. Acta Trop. 2021; 220:105946.

Allison GM, Rogers KA, Borad A, et al. Antibody responses to the immunodominant Cryptosporidium gp15 antigen and gp15 polymorphisms in a case-control study of cryptosporidiosis in children in Bangladesh. Am J Trop Med Hyg. 2011;85(1):97–104.

Al-Saeed AT, Abdo JM, Gorgess RG. Cryptosporidiosis in children in Duhok City, Kurdistan Region, Iraq. J Pak Med Assoc. 2020;70(7):1251–1255.

Andrzejewski A, Bento D, Snowden J. Cryptosporidiosis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; updated 2026.

Bayram RO, Özdemir H, Emsen A, Türk Dağı H, Artaç H. Reference ranges for serum immunoglobulin (IgG, IgA, and IgM) and IgG subclass levels in healthy children. Turk J Med Sci. 2019;49(2):497–505.

Borad A, Ward H. Human immune responses in cryptosporidiosis. Future Microbiol. 2010;5(3):507–519.

Bourli P, Eslahi AV, Tzoraki O, Karanis P. Waterborne transmission of protozoan parasites: a review of worldwide outbreaks – an update 2017–2022. J Water Health. 2023;21(10):1421–1447.

Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. Clin Microbiol Rev. 2013;26(1):115–134.

Bouzid M, Kintz E, Hunter PR. Risk factors for Cryptosporidium infection in low and middle income countries: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(6):e0006553.

Checkley W, White AC Jr, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for Cryptosporidium. Lancet Infect Dis. 2015;15(1):85–94.

Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas. 1960;20(1):37–46.

Collinet-Adler S, Ward HD. Cryptosporidiosis: environmental, therapeutic, and preventive challenges. Eur J Clin Microbiol Infect Dis. 2010;29(8):927–935.

Dong S, Yang Y, Wang Y, et al. Prevalence of Cryptosporidium infection in the global population: a systematic review and meta-analysis. Acta Parasitol. 2020;65(4):882–889.

DuPont HL, Chappell CL, Sterling CR, et al. The infectivity of Cryptosporidium parvum in healthy volunteers. N Engl J Med. 1995;332(13):855–859.

Helmy YA, Hafez HM. Cryptosporidiosis: from prevention to treatment, a narrative review. Microorganisms. 2022;10(12):2456.

Jayalakshmi J, Appalaraju B, Mahadevan K. Evaluation of an enzyme-linked immunoassay for the detection of Cryptosporidium antigen in fecal specimens of HIV/AIDS patients. Indian J Pathol Microbiol. 2008;51(1):137–138.

Kaushik K, Khurana S, Wanchu A, Malla N. Evaluation of staining techniques, antigen detection and nested PCR for the diagnosis of cryptosporidiosis in HIV seropositive and seronegative patients. Acta Trop. 2008;107(1):1–7.

Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (GEMS): a prospective, case-control study. Lancet. 2013;382(9888):209–222.

Pacheco FTF, Silva RKNR, Martins AS, et al. Differences in the detection of Cryptosporidium and Isospora (Cystoisospora) oocysts according to the fecal concentration or staining method used in a clinical laboratory. J Parasitol. 2013;99(6):1002–1008.

Pacheco FTF, de Freitas HF, Silva RKNR, et al. Cryptosporidium diagnosis in different groups of children and characterization of parasite species. Rev Soc Bras Med Trop. 2022;55:e0041-2022.

Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996;49(12):1373–1379.

Priest JW, Bern C, Roberts JM, et al. Changes in serum immunoglobulin G levels as a marker for Cryptosporidium sp. infection in Peruvian children. J Clin Microbiol. 2005;43(10):5298–5300.

Rahi AA, Ali MA, Al-Charrakh AH. Prevalence of Cryptosporidium parvum among children in Iraq. Am J Life Sci. 2013;1(6):256–260.

Ryan UM, Feng Y, Fayer R, Xiao L. Taxonomy and molecular epidemiology of Cryptosporidium and Giardia – a 50 year perspective (1971–2021). Int J Parasitol. 2021;51(13–14):1099–1119.

Saha R, Saxena B, Jamir ST, Shekhar S. Prevalence of cryptosporidiosis in symptomatic immunocompetent children and comparative evaluation of its diagnosis by Ziehl–Neelsen staining and antigen detection techniques. Trop Parasitol. 2019;9(1):18–22.

Sarkar R, Ajjampur SS, Muliyil J, et al. Serum IgG responses and seroconversion patterns to Cryptosporidium gp15 among children in a birth cohort in South India. Clin Vaccine Immunol. 2012;19(6):849–854.

Urrea-Quezada A, Balmaceda-Baca R, Garibay A, Hernández J, Valenzuela O. Serum IgG responses to gp15 and gp40 protein-derived synthetic peptides from Cryptosporidium parvum. Front Cell Infect Microbiol. 2021;11:810887.

Vandenbroucke JP, von Elm E, Altman DG, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS Med. 2007;4(10):e297.

Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 2007;4(10):e296.

Wallbank BA, Pardy RD, Brodsky IE, Hunter CA, Striepen B. Cryptosporidium impacts epithelial turnover and is resistant to induced death of the host cell. mBio. 2024;15(8):e0172024.

Widerström M, Schönning C, Lilja M, et al. Large outbreak of Cryptosporidium hominis infection transmitted through the public water supply, Sweden. Emerg Infect Dis. 2014;20(4):581–589.

Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol. 2010;124(1):80–89.

Xiao L, Feng Y. Zoonotic cryptosporidiosis. FEMS Immunol Med Microbiol. 2008;52(3):309–323.

Yang X, Guo Y, Xiao L, Feng Y. Molecular epidemiology of human cryptosporidiosis in low- and middle-income countries. Clin Microbiol Rev. 2021;34(2):e00087-19.