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Effect *Cryptosporidium parvum* on Fecal Lactoferrin Level in Children in Tikrit Province / Iraq

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ABSTRACT: Cryptosporidium spp. is a group of coccidian parasites of significant pathogenicity to both animals and humans. Cryptosporidiosis is understood to be the underlying factor behind the development of severe and protracted watery diarrhea in individuals diagnosed with acquired immunodeficiency syndrome. This study aimed to diagnosis of infection with the parasite *Cryptosporidium parvum* in children by microscopic examination of stool samples using the Modified Ziehl-Nelsoon stain and ELISA, as well as detection level of Lactoferrin in samples of patients infected with *Cryptosporidium parvum***.** A total 300 samples children aged 5 months - 5 years, of both sexes, who visited some government hospitals (Tikrit Teaching Hospital, Salah al-Din General Hospital, Baiji General Hospital, Sharqat General Hospital) and some private medical clinics in some areas of Salah al-Din Governorate who suffered from diarrhea and some intestinal symptoms during the period from July 1, 2022 to September 1, 2023. Based on the analysis of 300 stool samples, the present investigation employing MZN revealed an infection rate of 70 (23.33%) positive samples and 230 (76.67%) negative samples. The ELISA technique used in the current study recorded 130 positive samples and a total infection rate of **72.22**% with a mean concentration of **2.54±1.13** pg/mL), while the number of negative samples was 50 (**27.78%**) with a mean concentration of **(27.78%)** pg/mL out of 90 samples. This study showed increased incidence of infection with cryptosporidium in male than female that were 82(63.08%) and 48(36.92%) respectively, at p-value<0.05. The study observed a significant increase in the average concentration of ***fecal LF*** in infected infants (506.125 ± 99.143 pg/mL) compared to the control group (104.183 ± 21.368pg/ml). This study concluded that Modified Ziehl Neelsen stain is the best staining method used in diagnosing infection with the parasite. Furthermore, this study concluded increase level inflammatory immune parameters as lactoferrine in stool samples of children infected with the *C. parvum* parasite

Keywords: Diarrhea, *Cryptosporidium parvum*, Modified Ziehl-Nelsoon, ELISA, Lactoferrin

1. INTRODUCTION

Cryptosporidium parvum is an obligate intracellular parasite of the eukaryota phylum Apicomplexa, which has health and economic impacts as it is an intestinal pathogen for humans and animals and is widely spread all over the world and the severity of its effects varies according to the type of host, the type and strain of the parasite, and the site of infection (1). Cryptosporidiasis occurs by eating food or drink contaminated with the parasite's egg sacs or by handling or contacting animal

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feces or the feces of infected people (2). Epidemiological studies have revealed the existence of several transmission routes, direct contact from one person to another or through human contact with animals, and contaminated water sources as primary sources of disease transmission (3). The parasite of cryptosporidiosis is diagnosed through a number of techniques, the most important of which is microscopic examination of stool samples using the modified Ziehl-Neelsen stain method, which is the most commonly used to detect parasite egg cysts due to its ease, low cost, and availability of the dye(1). Therefore, microscopic diagnosis depends on staining the swab taken from the stool of the infected person, and diagnosis using the immunochromatographic assay technique depends on the interaction between the antigens present in the stool to be detected and the antibodies coated on the test plate(4). Diagnosis using the enzyme-linked immunosorbent assay technique Copro-antigen assay ELISA depends on the qualitative and quantitative measurement of the concentration of the parasite or parasite antigens in the stool sample, as it is one of the most important methods used in diagnosing the parasite, because it has high sensitivity and specificity in detecting and assessing the quality and load of the parasite in the samples, as this technique depends on or The method of examining the coating of antigens or antibodies to the parasite within the holes of the technique(5).

C. parvum is one of the opportunistic protozoan parasites that infect the epithelial cells of the small intestine and cause watery diarrhea in both immunodeficient children (6). Lactoferrin (LTF) is a natural immunomodulatory cytokine that has the ability to modulate and influence the response of both the innate and adaptive immune systems, being a first-line defense mechanism of the host against invading pathogens(7). Furthermore, by stimulating innate response mediators, it triggers signaling pathways that influence the function of adaptive immune cells(7). It affects the immune system in several ways, including increasing the activity of natural killer cells, enhancing the function of neutrophils by promoting phagocytosis and activating mast cells and limiting the spread of intracellular pathogens (6). It has antimicrobial activity against many microbial pathogens, for example parasites. Antimicrobial activity has been recorded against a wide range of parasites, including the Cryptosporium parasite (7, 8).

2. MATERIALS AND METHODS

2.1 Sample collection

A total 300 stool samples children aged 5 months - 5 years, of both sexes, who visited some government hospitals (Tikrit Teaching Hospital, Salah al-Din General Hospital, Baiji General Hospital, Sharqat General Hospital) and some private medical clinics in some areas of Salah al-Din Governorate who suffered from diarrhea and some intestinal symptoms during the period from July 1, 2022 to September 1, 2023.

2.2 Diagnosis *C. pavium* by using Modified Ziehl-Neelsen stain

Modified Ziehl-Neelsen staining method used for *C. pavium* examination according to Omoruyi *et al*., (9). The stool samples were examined microscopically to detect the presence of oocysts. The

samples positive for microscopic examination and those suspected were frozen at -20°C until the diagnostic with ELISA.

2.3 Diagnosis *C. pavium* by using ELISA technique

Sandwich-ELISA was used in this assay (Company: EDI Kit - Germany), and the plate was pre-coated with *C. parvum* parasite antigens. Samples with particular antibodies have been added. After binding the antigen to anti-cryptosporidium or standard solutions, the specialized enzyme conjugated Hydrogen peroxide (HRP) for *C. parvum* antigen was applied to all wells and incubated. Only the wells with the HRP conjugated enzyme and the protein to be identified were added the coloured solution, which turned blue and subsequently yellow after adding the stop solution. The optical density (OD) was measured at 450nm by spectrophotometry. The OD value is proportional to the sample standards' concentrations. Comparing the sample OD to the standard curve determined the standard concentrations.

2.4 Estimation of the concentration of Fecal Lactoferrine Human in the stool of children infected with the parasite

The method adopted in this test is Sandwich-ELISA, based on the leaflet attached to the kit by using (Company: Sunlong-China), the plate provided in this kit is pre-coated with an antibody specific to FLTF. Samples and standard solutions are added to the specified wells and bound to the specific antibody, then the specialized enzyme conjugated Hydrogen peroxide (HRP) for FLTF is added to all wells and incubated. The colored solution is added to each well only the wells containing the HRP conjugated enzyme and the protein to be detected appear blue and then turn yellow after adding the stop solution. The optical density (OD) is measured by spectrophotometry at a wavelength of 450nm. The OD value is proportional to the concentration of each of the standards to be measured in the samples. The concentration of each of the standards in the samples was calculated by comparing the OD of the samples to the standard curve.

Statistical analysis

Statistical analysis performed using SPSS for windows TM version 17.0 (10).

3. RESULTS AND DISCUSSION

Based on the analysis of 300 stool samples, the present investigation employing MZN revealed an infection rate of 70 (23.33%) positive samples and 230 (76.67%) negative samples. These results are presented in Table (1) and Figure 1).

Table 1: Percentage of *C.pavium* by using MZS

Assay	Total number of examined sample	Negative result (-ve)	Positive result(+ve)
Modified Ziehl-Neelsen stain	300	230 (76.67) %	70 (23.33) %

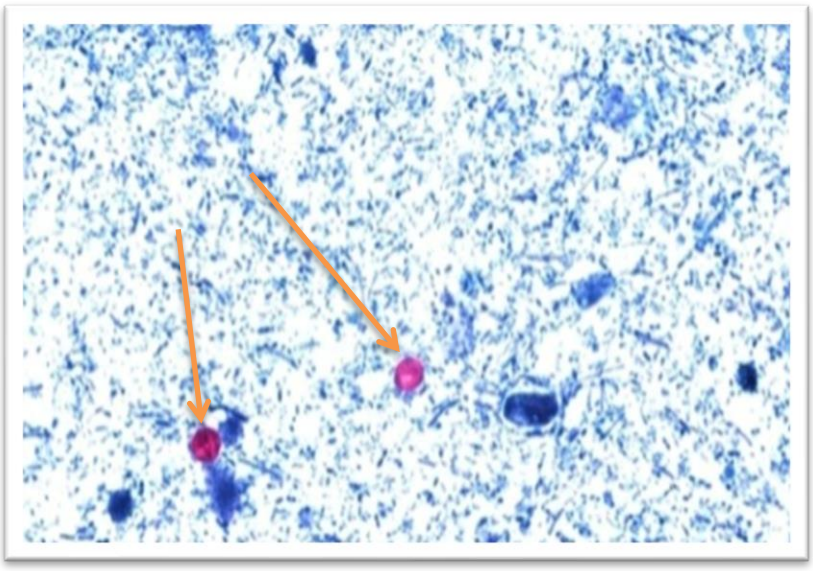

Figure 1: Oocysts of Cryptosporidium spp by using modified Ziehl-Neelsen stain (100X).

The ELISA technique used in the current study recorded 130 positive samples and a total infection rate of 72.22% with a mean concentration (mean ± SD) of 2.54±1.13 pg/mL), while the number of negative samples was 50 (27.78%) with a mean concentration (mean ± SD) of (27.78%) pg/mL out of 90 samples. As shown in Table (2)

Table 2: Percentage of *C.pavium* by using ELISA

Assay	Total number of examined sample	Negative result (-ve)	Pg/ml	Positive result(+ve)	Pg/ml
ELISA copro- antigen	180	50 (27.78%)	1.19±0.81	130 (72.22%)	2.54±1.13

This study showed increased incidence of infection with cryptosporidium in male than female that were 82(63.08%) and 48(36.92%) respectively, at p-value<0.05, as shown in Table (3).

Table 3: The incidence of *C. parvum* parasite infection according to the gender of the patient

Sex	Infected samples	Percentage %
Male	82	%63.08
Female	48	%63.08
Total	130	%100
p<0.05		

The results of the current study indicated that the highest rate of infection with the cryptosporidium was for the age group from 5 months to 1 year with 25.6% (46) compared to other age groups 1–2-year, 2–3-year, 3-4 year, and 4-5 year were 13.33% (24), 10% (18), 11.11% (20), and 12.22% (22) respectively, as shown in Table (4).

Table 4: The incidence of *C. parvum* parasite infection according to the age of the patient under study

Age groups	Number of samples tested	Positive samples	% of positive samples
5 months -1 year	50	46	25.56%
1-2 year	34	24	13.33%
2-3 year	30	18	10%
3-4 year	32	20	11.11%
4-5 year	34	22	12.22%
Total	180	130	**72.22%**

The study observed a significant increase in the average concentration of fecal lactoferrin in infected infants (506.125 ± 99.143 pg/mL) compared to the control group (104.183 ± 21.368pg/ml). As shown in Table (5).

Table 5: Comparison of lactoferrin concentration levels between control and patients infected with *C.parvum*

lactoferrin pg/mL	Patients N = 70	Control N = 20	P Value
Mean ± SE	506.125 ± 99.143	104.183 ± 21.368	*0.0001*
P Value is high significant at P ≤ 0.05			

There are more effective techniques, such as the Modified Ziehl-Neelsen stain, for identifying *Cryptosporidium oocysts* in feces accompanying diarrhea (11). Known for its exceptional sensitivity and specificity, this approach is the principal test in our clinical laboratory for patient testing, especially when the organism load is small (11).

The Modified Ziehl-Neelsen stain method is a more efficient and less labor-intensive approach for detecting *C. parvum*, requiring less technical expertise for accurate interpretation (12). The results indicated that oocysts exhibited red bodies dyed against a dark blue background and a distinct halo surrounding the oocyst. These findings are consistent with previous research(13), that showed the prevalence of *C. parvum* infection, as assessed by Ziehl-Neelsen, was 21%. These findings align with previous studies conducted in Mosul (20.52%)(14) , Bashra province-Iraq (8.6%) (15), Wasit province-Iraq (33.83%) (16) , and Kirkuk (61.81%) (17). The variation in infection percentage may be attributed to factors such as the number of patient samples in the screening study, differences in the characteristics of the regions, patient age, and the diagnostic protocol employed(18).

Recent research has identified enzyme-linked immunosorbent assays (ELISA) as a sensitive, cost-effective, straightforward, and fast technique for detecting *Cryptosporidium* in stool samples (19).

The results of the current study showed similarity with the results in South Africa, which was (240)74.3% of the total 323 stool samples (20). The copro-antigen ELISA test is attributed to the strong association between *C. parvum* antigens and the added stool sample containing specific antibodies to the parasite(10). This technique is characterized by high sensitivity and specificity in accurately diagnosing *C. parvum* exclusively(10). The results of this study differ from previous studies and can be attributed to reasons such as lifestyle, hygiene, immunotherapy, food and environmental pollution inside and outside the home, health education about infectious diseases, clean drinking water, and economic status(21).

Furthermore, the hygiene habits of youngsters of both genders are similarly fundamentally comparable (22). This study agree with (23) ,reporting that 61% of males were infected parasites including C.parvum while the rest 39% of infected children were females. Sweden (24) concurs with the findings of the present investigation, stating that the prevalence of cryptosporidium infection was more significant in males (45.1%) than in females (34.5%).

Children in different countries are exposed to *C. parvum* in the first period of their lives within a few weeks after birth, up to the first year. Despite maternal protection through breastfeeding, the current study recorded the highest infection rate for the age group from 5 months to 1 year due to the weakness of their immune system.

This study demonstrated increase lactoferrin in children infected with *C. parvum* that agree with (25). Lactoferrin has antimicrobial activity against many microbial pathogens, such as parasites, fungi, viruses and bacteria (26).

It is secreted or produced by neutrophils, where this increase is due to the presence of LTF receptors on a wide range of immune cells and their ability to bind the molecule, as it plays an important role in regulating the innate immune response, being a first-line defense mechanism of the host against invading pathogens, moreover, by stimulating innate response mediators, where it triggers signals of pathways affecting the function of adaptive immune cells, as it affects the immune system in several ways, including increasing the activity of natural killer cells, enhancing the function of neutrophils by enhancing phagocytosis and activating strict cells and limiting the spread of intracellular pathogens (7) .

4. CONCLUSION

This study concluded that Modified Ziehl Neelsen stain is used in diagnosing infection with the parasite. While The ELISA copro-antigen technique characterized by high sensitivity and specificity for detecting and identifying the *C. parvum* parasite in stool samples. Male more infected than female. Furthermore, this study concluded increase level inflammatory immune parameters as lactoferrine in stool samples of children infected with the *C. parvum* parasite.

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