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Microscopic Detection and Prevalence of *Cryptosporidium parvum* Oocysts in Al–Muthanna Province

Abthal Ali aaqul [1], Yassir Dakheel Kremsh Alasadiy*[2]

[1,2]Department of Biology, College of Education for Pure Sciences, University of Al-Muthanna, Iraq, ibtehal.msc@mu.edu.iq

*Corresponding author email: dr.yassiralasadiy@mu.edu.iq

Abstract

The parasite *Cryptosporidium parvum* is one of the most important primary intestinal parasites causing water- and foodborne diseases globally, as it causes cryptosporidiosis, which is characterized by the occurrence of diarrhea and gastrointestinal disorders, particularly in children and individuals with weakened immunity, and the current study aimed to collect, diagnose and isolate oocysts for intestinal parasites from fecal samples, and preserved under suitable conditions to preserve their vitality for use in subsequent laboratory experiments. The study included the collection and examination of (200) human fecal samples from patients suffering from diarrhea and some intestinal symptoms, and the samples were collected during the period from November 2025 to May 2026 . The samples were subjected to microscopic examination to detect the oocysts of *C. parvum* parasite, as the Modified – Ziehl – Neelsen stain technique was used to diagnose the parasite and distinguish the oocysts, and the flotation method was used to isolate the oocysts and increase their detection efficiency. The samples were kept under suitable laboratory conditions at a temperature of 20 C° degrees until all tests were completed. The results of the study showed that the total infection rate of *C. parvum* was recorded (41.5%) as 83 positive samples were diagnosed out of 200 examined fecal samples, and the results also

showed that there were significant differences in the infection rate according to gender, where males recorded infection rate of 61% (compared to females where they scored 22%) at the level of statistical significance ($P<0.05$). . With regard to the age groups, the results showed that the age group (1-9) years recorded the highest infection rate (27%), while the lowest infection rate was recorded in the age group (55-63 years (7%). The results of the study indicate that there is a significant prevalence of *C. parvum* infection among patients with diarrhea in Muthanna Governorate, with a high rate of infection among males and children, and these results confirm the health importance of the parasite and the need to adopt effective programs for early detection and the promotion of preventive measures to reduce the spread of infection.

Key words : *cryptosporidium* , Modified Ziehl – Nelseen , Human, intracellular intestinal parasitic , Flotation Method ,

1. Introduction

The cryptosporidium parvum is a primitive intestinal single-celled, obligate intracellular parasitic protozoa and belongs to eukaryota (Checkley et al., 2015). It is of great health and economic importance, as it represents one of the intestinal pathogens for humans and animals, and it is widely spread globally, and the severity of its disease effect varies according to the type of host, the strain of the parasite, and the site of infection (Beiting and John, 2022). These parasites infect epithelial cells in the gastrointestinal tract of humans and animals, causing latent sporesis Parasitic infections can also ،be acquired through contaminated water, contaminated food, or direct contact with infected people or animals (Efstratiou et al., 2017). It has been identified as one of the essential organisms found in contaminated drinking water, as it has a high ability to resist traditional drinking water treatment methods (Pignata et al., 2019). The parasite has a wide range of hosts where it infects humans, domestic animals, and wild animals (Okojokwu et al., 2016). A better understanding of epidemiology, sources of infection, and modes of transmission is key to improving control programs, and providing more data on exposure to cases will contribute significantly to a better understanding of pathology and epidemiology, leading to

the development of better preventive measures (Roellig and Xiao, 2020).

The clinical symptoms of the disease vary from person to person depending on the immune status of the host, the severity of the infection, and the extent to which it affects the epithelial cells lining the mucous layer of the affected organ, and diarrhea is one of the most prominent and important signs observed in those affected (Davies and Chalmers, 2009). In immunocompromised people, especially children, the infection is characterized by the occurrence of severe watery diarrhea accompanied by intestinal colic caused by gastroenteritis, as well as nausea, vomiting, and high body temperature, and the condition usually lasts for up to two weeks (Juranek, 2002; Huang et al., 2004). Infection is accompanied by headache and loss of appetite, which may be followed by weight loss and in some cases feeling sick, and dehydration is another clinical symptom besides tremors, poor absorption, bloating, and occurrence of allergic reactions as well as the appearance of signs similar to food poisoning (Go ddard et al., 2000). The current research aims to collect, diagnose and isolate the oocysts of the parasite (*Cryptosporidium parvum)*) of fecal samples, and preserved under appropriate conditions to preserve their vitality for use in subsequent laboratory experiments.

2. Methods

2.1 Collection and examination of samples

In this study, a parasite (*Cryptosporidium parvum)* was isolated and diagnosed from the clinical samples collected from children and adults of both sexes for visitors to some Province hospitals (Women's and Children's Hospital and Al-Hussein Teaching Hospital) with diarrhea and intestinal symptoms. The diagnostic study was conducted in the Graduate Studies Laboratory of the Department of Biology / College of Education for Pure Sciences / Muthanna University, the study included the examination of 200 fecal samples of patients of both sexes and the samples collected in hospitals were saved before being transferred to a laboratory with a pre-prepared 2.5% potassium dichromate solution, then placed at a temperature of 20 C° to conduct the necessary laboratory tests.

2.2 Isolation and diagnosis of a parasite (*Cryptosporidium parvum*)

The samples preserved in potassium dichromate were examined in the graduate laboratory of the laboratories of the College of Education for Pure Sciences / Muthanna University / Department of Biology. The sample was examined in two stages:

2.2.1 Macroscopic examination:

Macroscopic examination of stool samples was performed immediately after they were collected to observe phenotypic traits such as smell, color and consistency of sample (Forbes et al., 2007).

2.2.2 Microscopic examination:

Microscopy of the sample included:

First: Diagnosis using the modified Ziehl-Nelseen stain:

Where the Ziehl-Neelsen stain method was adopted to detect the oocysts of cryptosporidium, the Ziehl -Nelsen stain is mainly based on the high affinity of Carbol fuchsin stain to bind to fatty substances and fatty acids, such as Mycolic acid present within the structure of the parasite's oocysts wall, and this strong bond leads to the oocysts retaining red color and its resistance to the decolorization solution (HCI) is unable to remove the associated stain, which increases the sensitivity of this technique in detection, and the use of the heating method in the stain (hot method) contributes to increasing the permeability of the stain on the inside of the parasite's oocysts and enhances its binding strength to it (Sevinc et al., 2005), Table (1) .

Table (1) :Components of the Detection Kit for Zel-Nelsen Modified stain (MZN)

Volume	Materials
100ml	Stain Carbol Fuchsine
100 ml	Stain Methylene Blue
100 ml	Sulfuric Acid Concentrate H_2SO_4

According to the following steps :

A quantity of the feces of a person with diarrhea was placed on a glass slide and the sample was brushed along the area of the slide and left to dry and fixed using heat. The slide was immersed in Carbol fuchsine stain for 5 minutes. Then the slice washed with distilled water until the color was removed.

Shorten the color with a concentrated sulfuric acid until the stain is removed for one minute and then gently rinse the slice with distilled water, The slice was soaked in methylene blue and left on for a minute and then washed with distilled water, let the slice sit for 10 minutes in the slice holder and at room temperature. The swab was then examined by light microscope after adding an oil drop under 40x magnification (Garcia, 2016).

Second: Isolation by Flotation Method:

It is one of the most effective ways to concentrate intestinal parasites in fecal samples, as it allows accurate detection of oocysts even when they are present in small quantities that may not appear in direct examination , this method relies on the use of solutions with a higher qualitative weight than parasites and their phases, such as zinc sulfate solution, where the oocysts float on the surface of the specimen while impurities and heavy substances remain at the bottom. After centrifugation, the supernatant is collected and examined microscopy, which increases the detection sensitivity (Garcia, 2016).

According to the following steps :

2 mg of fecal sample was weighed and placed in a centrifugal tube.7 ml of zinc sulfate solution was added to it and the sample was mixed well, then it was placed in a centrifuge at a speed of 2000 rpm, with a balancer tube placed on the opposite side to ensure balance. Using a pipette, one drop of supernatant liquid was taken and placed on a microscopic slide. Slides were stain with Carbol-fuchsine for 5 minutes and then washed with distilled water. After using concentrated sulfuric acid, the color was removed and washed with distilled water. Then it was dyed with methylene blue dye for one minute. Count it and put it in a rack to air dry at room temperature. Then examined the slides with 40x and 100x magnification. (Wahed , 2021).

2.3 Statistical Analysis

The results of the statistical analysis shown in the table (2) that there were significant differences ($P \leq 0.05$) in the infection rate between males and females, as males recorded the highest incidence rate of(61%) 61 compared to females, where the Prevalence rate was(22%) 22. This result indicates that the sex of the affected person is still an influential factor in the incidence rate. Table (2).

Table (2): Percentage of C. *parvum* infection by sex

Gender	Number of Samples	Infection rate	Standard deviation	T-value	Statistical analysis
Male	100	61	7.378	12.113	$P \leq 0.05$
Female	100	22	5.888		

3. Results

The results of this study were based on the examination of (200) stool samples collected from different age groups of both sexes and visitors to some government hospitals in Muthanna Governorate who suffer from intestinal disorders and diarrhea. The results of microscopy showed the presence of parasitic infections (*Cryptosporidium parvum)* in varying proportions among the examined samples.

Table (3) shows that the infection of *C. parvum parasite* reached 83 positive samples (41.5%), while 117 negative samples (58.5%) were recorded, out of a total of 200 fecal samples that were tested.

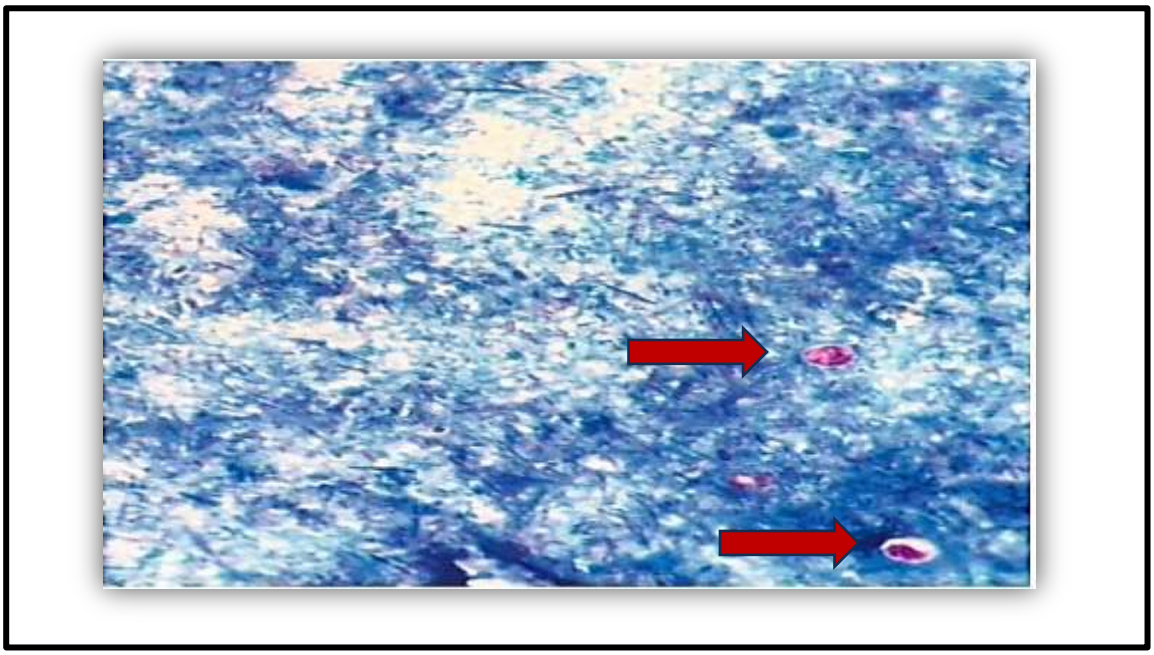
The results of the statistical analysis revealed a significantly higher infection rate of *C. parvum* among males compared with females. The highest prevalence was recorded in males, and the differences in infection rates between the two sexes were statistically significant at the probability level ($P \leq 0.05$), Table (3).

Table (3): Infection rates of *C. parvum* parasite using the modified Zel-Nelsen stain

Number of Samples Examined 200 Samples	Number of positive samples	Number of negative samples
	83	117
Percentage %	41.5%	58.5%

3.1 Isolation and detection *C. parvum* Using Ziehl-Neelsen Staining

Oocysts showed the parasite after being stained with the modified Neelsen stain with a purple-red spherical shape, while the rest of the feces appear blue, making it easier to distinguish and diagnose the Oocysts microscopicly, Figure (1).



Method as shown in the following.

The results of isolation showed that it was successful in separating and concentrating Oocysts from stool samples, as it was possible to obtain clear Oocysts suitable for use in subsequent examinations and experiments, figure(2)

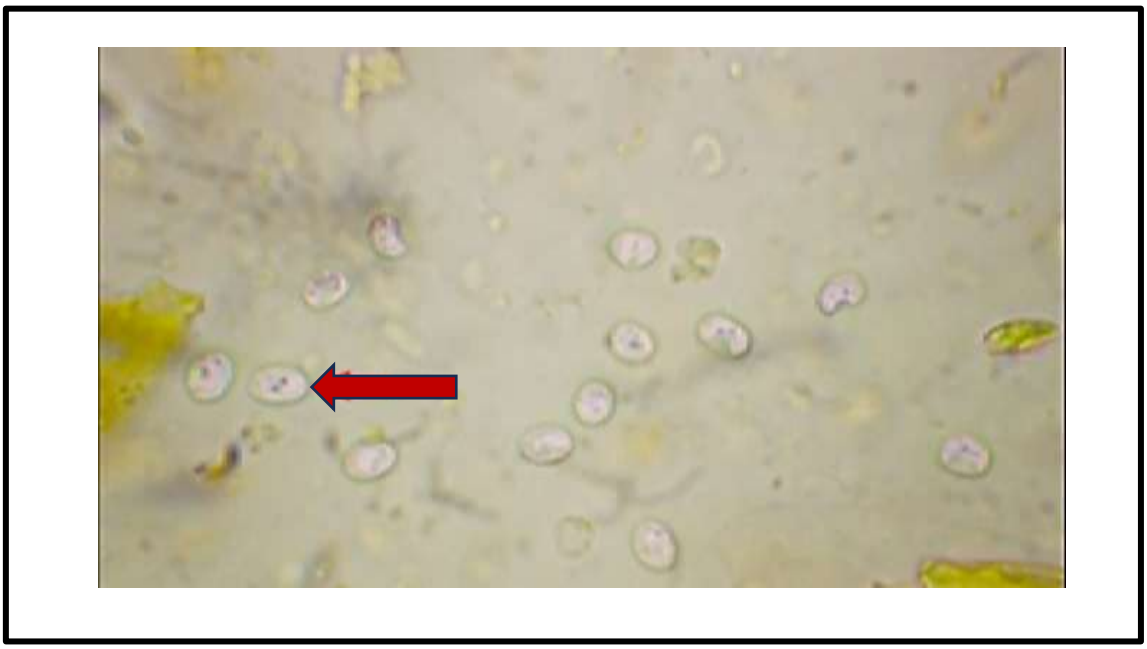


Figure (2): Swab of C. *parvum* oocysts isolated by 100x circumnavigation method

4. Discussion

The ability of *C. parvum* oocysts to retain the dye after staining reflects the distinctive structural characteristics of their outer wall, which allows them to be clearly distinguished from other components of the sample. The importance of the Ziehl -Neelsen modified stain is highlighted in increasing the accuracy of microscopic diagnosis of the parasite, especially in specimens with a small number of oocysts, which supports its use as an effective diagnostic tool in detecting infections and studying the spread of the parasite.

The Flotation Methods one of the most common techniques in isolating Oocysts of intestinal parasites from fecal samples, as it relies on the principle of specific density difference between Oocysts and other components, and this method contributes to increasing the efficiency of microscopic detection by reducing the amount of impurities and organic matter, which facilitates the observation and

diagnosis of egg cysts with higher accuracy. They are also easy to apply and low cost compared to some other technologies.

The infection rate recorded in the current study (41.5%) indicates a significant spread of the parasite among the individuals included in the study, which may be attributed to several environmental and health factors that contribute to the transmission of the parasite's Oocysts, especially the contamination of water and food sources and the ease of transmission between individuals. The results of the current study are consistent with the findings of Alkashab and Younis (2021) in the city of Mosul, where they recorded (70) positive samples out of (200) fecal samples with an infection rate of (35%).

On the other hand, the results of the current study differed from many previous studies, including the one conducted in Basra, where an infection rate of (4) positive samples was recorded (7.2%) out of a total of (55) fecal samples (Mahdi and Ali, 2002). Also, with a study conducted at Samarra General Hospital, where the Prevalence rate was (8.2%) out of a total of (300) fecal samples (Al-Jubouri and Shahed, 2015). It also differed with the results of Al-Murshidi (2021) in Karbala Governorate, which recorded (26) positive samples out of a total of (320) fecal samples (8.1%). This disparity in the rates of infection recorded between the different studies is likely due to the difference in the size of the studied samples and The sites where the procedure was performed, environmental and health conditions, as well as the different laboratory methods used, and the chances of transmission and spread of the parasite may increase as a result of the simplicity of its direct life cycle, as it is completed in one host, which is human, where the parasite produces two types of Oocysts, namely thick oocysts and thin oocysts , and the latter type is responsible for the occurrence of autoinfection. Which increases the chances of infection continuing and spreading (EI-Helaly et al., 2012).

Differences in infection rates between males and females may be related to the degree of exposure to environmental risk factors affecting the transmission of the parasite, as increased exposure to potential sources of infection may contribute to an increased risk of infection, and differences in daily

activities and environmental exposure patterns can lead to a variation in the prevalence of infection between the sexes.

5. conclusion

The results of the present study demonstrated a notable prevalence of *Cryptosporidium parvum* among individuals suffering from gastrointestinal symptoms, indicating the potential role of this parasite in causing digestive disorders and diarrheal diseases, and highlighting its importance as one of the pathogenic agents associated with intestinal infections. The findings also showed that the Modified Ziehl–Neelsen stain is one of the fundamental and effective diagnostic techniques for the microscopic detection of the parasite's oocysts, owing to its ability to clearly visualize parasitic structures, thereby enhancing the efficiency and accuracy of laboratory diagnosis. Furthermore, the study revealed that the Flotation Technique represents an important approach for the isolation and concentration of parasitic oocysts from fecal samples. This technique is based on the principle of differences in specific gravity between oocysts and other sample components, which increases detection sensitivity, reduces impurities, improves the accuracy of microscopic examination, and consequently enhances the reliability of diagnostic results.

6. Conflict of Interest

The authors have no financial conflict of interest or personal relationships that could have influenced the content of this study ..

7. Fund

The authors have self – financed this study . There was no financial support for the research from governmental , corporate , or non – profit entities. .

8. data Availability Statement

The original images , raw data , and statistical analyses of this study , as well as The statistical analyses of The study , were deposited in the figshare repository..

9. Authors' contributions

The sample collection and laboratory work was performed by graduate student Abthal Ali aaqul , while Prof. Yassir

Dakheel Kremsh Alasadiy provided supervision for this work .

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