Original Article

Cryptosporidium parvum parasite microscopic and molecular diagnostics in the Holy Karbala/ Iraq.

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DOI: 10.47750/pnr.2022.13.S02.41

Abstract

The present study aimed to diagnose the *Cryptosporidium* parasite and identify the extent of the parasite's spread in people who suffer from diarrhea and its relationship to epidemiological factors in the holy Karbala Province using three different diagnostic methods. Microscopic examination, which includes staining the samples with the modified acid dye, as well as using the molecular examination using the technique of polymerase chain reaction, the collection of samples was carried out in Al-Hussein Medical City and Karbala Teaching Hospital for children. So, in the laboratories of the College of Veterinary Medicine at the University of Karbala for the period from the beginning of September 2020 until the end of February 2021, 320 stool samples were collected from persons suspected of having the parasite (those with diarrhea and not infected) and who came to Imam Hussein Medical City and Karbala Teaching Hospital. For children, of both sexes, and with age groups from (1-50) years, this was done. The first half of each sample was utilized to produce reasonably thick smears for evaluation with the modified acid dye, and the second part was preserved at -20°C for subsequent use in the molecular study., as the results of the microscopic examination using the modified acid dye showed that the number of infected samples reached 26 samples as the number of Males have 15 samples, while the number of females reached 11 samples and the overall infection rate was 8.1%, while the infection rate was lower by using immunological tests by 6.8%.

The incidence rate was affected by epidemiological factors below the probability level P≤ 0.05, as males recorded a higher rate of infection compared to females, as the infection rate reached 10% and 6.47%, respectively, while the infection rate recorded significant differences according to the type of housing, as the infection rate reached 12.2% in the countryside and in The city, the infection rate was 6.5%. As the current study recorded different infection rates according to the months of the year, the highest rate of infection was in the month of December by 16%, while the lowest injury rate was concentrated in the month of October and November by 1.8%, 4.7%, and the study recorded. The highest rate of infection was in the age group (1-10) by 14.1%, while the current study did not record any infection in the age group (40-31). Drinking water sources played a role in the spread of the *Cryptosporidium* parasite, the infection rate for persons who drink tap water was 18.9%, whereas the lowest rate for people who drink sterile water was 4.8 percent, according to the research. As for the companion parasites, the current study recorded that the most associated parasite is *Entamoeba histolytica* by 3.4%, while the appearance of *Girdia lamblia* parasite was less by 2.1%.

A molecular assay was used on the second part of the samples, which was kept at a temperature of -20, for the purpose of confirming infection in the positive samples and others, and to determine the accurate diagnosis of this parasite. Specific primers for the oocyst wall protein (*COWP*) gene were used, *Cryptosporidium* oocyst wall protein. Polymerization, the first stage was the polymerase chain reaction (PCR), where the DNA was amplified using specific primers and the reaction was accomplished using a thermocycler and under optimal conditions. The results revealed a single package (Singal band in agarose gel) with weight Molecular 769 base pairs of nitrogenous bases in the DNA extracted from stool samples of people with *Cryptosporidium*, which is an indication of the patient's infection with the parasite, and the second stage in which the technique of nested polymerase chain reaction is used. By using two types of specific primers in the reaction (*COWP F* and *COWP R*), the DNA was amplified using specific primers and it was The reaction was carried out using a thermocycler device and under optimal conditions, the results showed the presence of one packet in the acarose gel with a molecular weight of 553 pairs of nitrogenous bases in the DNA extracted from the stool samples of people with *Cryptosporidium*, an indication of the patient's infection with the parasite, and the current study showed that the sensitivity The microscopy examination was less than the molecular examination, It was concluded from this study that the *Cryptosporidium* parasite is one of the parasites that cause diarrhea in humans, especially children in Karbala Province. It may be diagnosed using a variety of ways, with the polymerase chain reaction approach being one of the most effective.

Key words: *Cryptosporidium parvum*, children, diarrhea, Molecule, immune system.

INTRODUCTION

Cryptosporidium parvum is one of the obligatory unicellular parasites that parasitize inside cells. It is called *cryptosporidium* [1]. because it is difficult to find inside the egg sac, and its size is smaller than the size of a red blood cell, its size is (5-4). The parasite does not use insects as intermediate hosts for infection, as it can complete its life cycle within one host and produce egg sacs that are released with faeces or with a wet cough and transmission to a new host [2]. This parasite causes Cryptosporidiosis, a common intestinal pathogen in humans and animals worldwide, and one of the opportunistic diseases common to humans and animals. It is the world's fourth leading cause of diarrhea, mainly in immunocompromised patients [3], but it is unsatisfactory or asymptomatic (Asymptomatic) in immunocompetent people, resulting in an unclear illness incidence [4]. This parasite is a major cause of diarrhea in children under the age of five and people with weakened immunity [5]. It causes a group of diseases of the digestive and respiratory system and is called intestinal cryptosporidiosis or respiratory cryptosporidiosis [6].

The modes of transmission of the parasite include either indirectly through the mouth by drinking water and eating food contaminated with oocytes. The presence of egg sacs in water poses a great danger to public health and may lead to epidemics in different regions of the world. It is transmitted through water pollution in developed countries, and this parasite has caused the largest spread of water-borne diseases [7].

The parasite was first isolated from an infected mouse in 1907 [8], and the first case of cryptospora was diagnosed in 1976 [9]. This parasite is a parasite that has the ability to attack the epithelial cells lining the intestines, causing watery diarrhea that can last up to 3 weeks and can lead to malnutrition, nausea, high temperature and abdominal pain, especially in patients who They suffer from immunodeficiency [10], and the disease may turn into a chronic type in people who suffer from suppression of the immune system, such as AIDS patients, transplant recipients, patients with kidney disease, pregnant women, as well as those receiving chemotherapy for cancer, which leads to death as a result of infection With this disease [11], *Cryptosporidium* infection is characterized as being associated with the development of the parasite within the villi layer of the intestinal epithelial cells of the host's digestive system, leading to villi atrophy and a variable increase in white blood cells.

Several factors are involved in the success of the interaction between oocysts and host epithelial cells including excystation, glidin motility, attachment, invasion, parasitophorous vacuole formation, and parasitic vacuole formation. Host cell damage [12].

Cryptosporidium species may infect up to 170 different hosts, including reptiles, birds, amphibians, mammals, and fish, as well as humans [13]. More than 27 species of Cryptosporids have been identified that infect a wide range of mammals, reptiles and birds. and fish [14].

MATERIAL AND METHODS

320 samples Was collected from the governorate and who suffer from diarrhea, intestinal colic, and abdominal pain and the methods was fellawed the following steps.

MODIFIED ZEIL NELSEN STAIN

The first method of staining with a modified Zell Nelson stain is done through a part of the stool is taken as a woody stick and brushed on the glass slide to make a light smear and leave it to dry at room temperature, then the smear is fixed by adding drops of methanol and left for several minutes and after that drops of Carbol Foxin dye were added to glass slide and expose it to a calm flame and leave for a period 20 minutes, then rinse with tap water and finally blue malachite dye and leave for 5 minutes and examine under the force of magnification 100 .

MOLECULAR EXAMINATION

Cryptosporidium parasite DNA is investigated using Nested PCR technique from stool samples of study groups based on amplification of the parasite gene *COWP* according to the method described by [15].

RESULTS AND DISCUSSION:

The results:

The overall percentage of infection with the Cryptosporidium parasite was 8.1 percent, according to the findings of microscopic and molecular tests of 320 diarrhea samples and 20 control samples, according to the results of the microscopic examination, using the modified acid-stain dye, and it reached 5.6%, according to the results of the molecular examination using the polymerase chain reaction (PCR) technology, and as shown in table (1)

Table 1: The results are based on three diagnostic methods:

laboratory tests	The number of positive samples	Percentage (%)
Microscopy	26	8.1
Molecular examination	18	5.6
X^2=1.562	P > 0.05 degrees of freedom = 2	

1- Results based on microscopic examination

The results of the microscopic examination of the stool samples by using the acid-resistant dye showed the presence of eggs of the *Cryptosporidium* parasite, which was in the form of a purple-colored ball with a blue background as in Figure 1

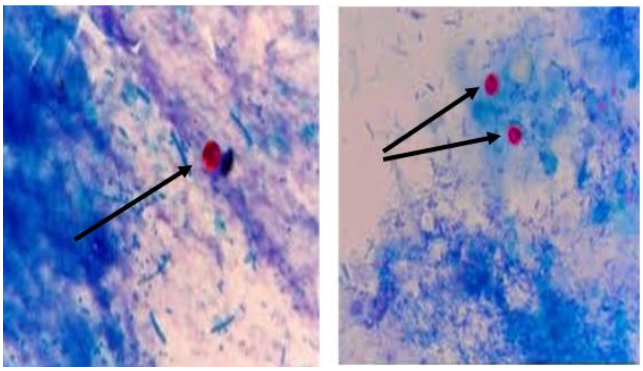

Fig 1: Ziehl Neelsen stained smear of *C. parvum* oocysts

Cryptosporidium infection rate by sex

The results of the current study indicated that the incidence of males is high compared to females, as the incidence of males was 10% and the incidence of females was 6.4%. Under the probability threshold of P 0.05, the statistical analysis verified that there were no significant differences between the sexes. and as shown in table (2)

Table (2): Percentages of Cryptosporidium infestation by sex.

Gender	Examined number	Infected number	Percentage %
Male	150	15	10
Female	170	11	6.4
total summation	320	26	8.1
X^2=1.33	P > 0.05	Degree of freedom1 =	

Cryptosporidium infection rate by age groups.

The current study found that the age groups 1-10 years had the greatest percentage of Cryptosporidium parasite infection, with a rate of.114 percent, and that the age groups 31-40 years had no infection, as statistical analyzes confirmed the existence of significant differences in infection rates between the studied age groups in favor of the ages under Age 10 years and under the probability level of $P \leq 0.05$ and what percentage of the table is presented (3)

Table (3): Percentages of people infected with Cryptosporidium by age group

Various age groups	Examined number	Number infected	Percentage %
10 -1	155	22	14.1*
20 -11	50	2	4
30 -21	60	1	1.6
40 -31	40	0	0
50 -41	15	1	6.6
total summation	320	26	8.1
degrees of freedom = 4	$P \leq 0.05$	X^2=15.719	

Percentage of those infected with Cryptosporidium over the study's months.

The findings of the microscopic inspection revealed that the rate of Cryptosporidium parasite infection varied throughout the year, as the percentage of infection reached its peak during the month of December at 16% and the lowest was in the month of October at .81% and the statistical analyzes confirmed the existence of significant differences during the months of the study in the rates of infection The parasite is under the probability level $P \leq 0.05$, as shown in Table (4).

Table (4): Percentages of infection with *Cryptosporidium* during the months of the study

Months of preparation	The number was examined.	Infected number	Percentage %
September 2020	23	2	8.7
October	55	1	1.8
November	42	2	4.7
December	75	12	16*
January 2021	65	6	9.2
February	60	3	5
total summation	320	26	8.1
degrees of freedom = 5	$P \leq 0.05$	X^2=10.699	

Cryptosporidium infection rate according to the presence of some parasites accompaniment.

The results of the current study showed the presence of some parasites accompanying the Cryptosporidium, the tissue-lytic amoeba parasite, with a rate of 3.4 percent, had the largest percentage of these associated parasites. While the percentage of infection associated with Giardia lamblia was 2.1%, as shown in Table (5).

Table 5: Percentages of Cryptosporidium infestation by associated parasites

accompanying parasites	Infected number	Percentage %
Tissuelolytic amoeba + Cryptosporidium	11	42.3
Giardia lamblia + Cryptosporidium	7	26.9
Cryptosporidium only	8	30.8
total summation	26	8.1
X^2= 0.23	P > 0.05	degrees of freedom = 1

MOLECULAR STUDY

Molecular study results using polymerase chain reaction technology Polymerase Chain Reaction (PCR)`

For the purpose of confirming the results that appeared in the microscopic and immunological study, a molecular study was used to detect the percentage of infection with the crypt spore parasite, which relied on the technology of polymerase chain reaction (PCR) to detect the egg wall protein (COWP) cell Outer Wall Protein (COWP) gene, as the DNA was amplified using specific primers The reaction was accomplished using a thermal circulator and under optimal conditions, the results showed the presence of one band (Singal band in agarose gel with a molecular weight of 769 base pairs) in the DNA extracted from stool samples of people with cryptosporidiosis, which is Indication of the infection of the patient with the parasite, on the contrary, the packet did not appear in the DNA samples extracted from people who were not infected with the cryptosporidium parasite or the so-called control samples, which is an indication that they were not infected with the parasite. It is shown in Figure (2).

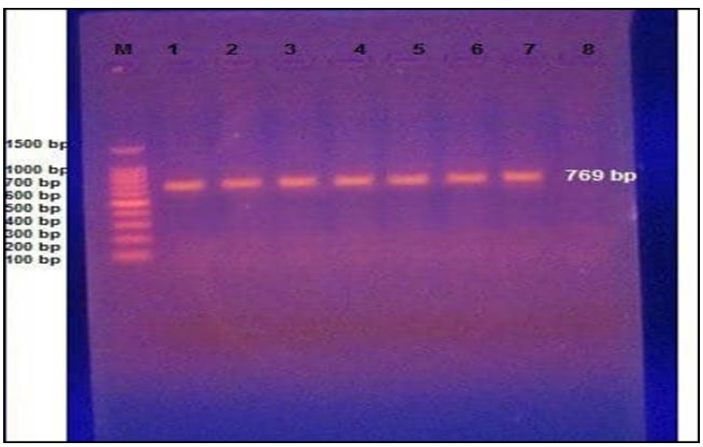

Figure 2: Electrophoresis of the PCR product Cryptosporidium parvum COWP gene) on agarose gel at a voltage of 70 volts for 1.5 hours using ethidium bromide stain. Some samples show a bundle of 769 pairs of nitrogenous bases, which is an indication of the parasite's DNA. Path M: DNA lader, lanes 1-7: DNA isolated from stools of patients with diarrhea due to cryptosporidiosis (in which the band appears) Path 8: DNA isolated from stools of non-diarrhea patients, defined as control samples.

Molecular Study Results Using Nested PCR

At this stage, the genotype of the COWP gene is analyzed by using two types of specific primers in the reaction (COWP F and COWP R). Acarose gel has a molecular weight of 553 base pairs in DNA extracted from stool samples of people infected with *Cryptosporidium*, which is an indication of infection of the patient with the parasite, on the contrary, the band did not appear in DNA samples extracted from people without *Cryptosporidium* infection or the so-called parasite In the control samples, which is an indication that they were not infected with the parasite, the statistical analyzes confirmed that the infection with the *Cryptosporidium* parasite in the current study amounted to 5.6%, as shown in Figure (3).

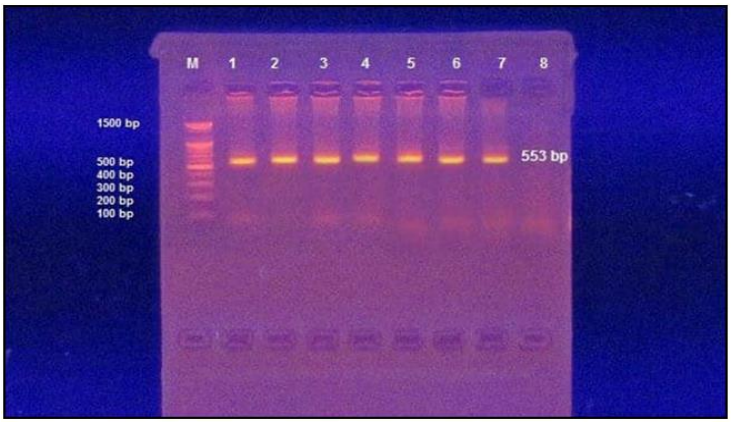

Figure 3: Electrophoresis of the Nested PCR product of the Cryptosporidium parvum COWP gene on agarose gel at a voltage of 70 volts for 1.5 hours using ethidium bromide dye. Some samples show a bundle of 553 pairs of nitrogenous bases, which is an indication of the parasite's DNA. Path M: DNA lader, lanes 1-7: DNA isolated from stools of patients with diarrhea due to cryptosporidiosis (in which the band appears) Path 8: DNA isolated from stools of non-diarrhea patients, defined as control samples.

THE DISCUSSION:

The modified acid stain method is the most popular method for diagnosing Cryptosporidium due to several factors such as ease of application, and low cost [16]. Although the modified acidic stain method was widely used, the immunological and molecular methods were widely used to detect due to its high sensitivity and accuracy in diagnosis and speed in carrying out the test, as it can detect 100 egg sacs in a sample size of 1 ml of stool. [17] requires diagnosing the infection in general and identifying the infectious stage of the parasite, which is egg sacs, which they are usually 4-6 µm in size. The modified acid stain technique is used to detect egg sacs that are of small size [18].

Egg cysts are more difficult to detect in asymptomatic patients or patients with even mild symptoms using modified acid staining techniques [19]. The molecular assay is the optimum test for the detection of Cryptosporidium [20]. The molecular assay is less time consuming and easier to implement [21]. Comparing microscopy and molecular examination, because oocyte microscopy is not a suitable diagnostic method for human samples due to the small number of oocytes and because isolating the organism from other tissues is a difficult and time-consuming procedure, the molecular method allows us to diagnose the factors quickly and with high accuracy, allowing us to identify the subspecies and, more importantly, the source and factors that contribute to infection [22].

The sensitivity of the molecular test is about 20 egg sacs in 1 ml of stool sample [23]. Using the modified acidic dye technique, the present study found that men had the greatest rate of Cryptosporidium parasite infection in Karbala governorate, at 10%, while females had the lowest prevalence, at 6.4 percent (1999), Maaleh (2008), Hussein (2011) Oleiwi (2015). [24] also found that the infection rate among males was 2.6% and 1.1% in females, and a study was conducted in Palestine that found that the infection rate among males was 11.3%. and 9.7% in females [25]. But it differed with studies. [26], and the study of Saneian et al. (2010) [27], in the city of Isfahan / Iran that the percentages of infection with *Cryptosporidium* were 2% and 2.6% in males and females, respectively. In Al-Qadisiyah Governorate, Mohammad (2018) [28] found that the infection rate among females was 56.6% higher than that of males, 43.3%.

The reason for the difference in the rate of infection between males and females may be due to several factors, including the difference in the quality of nutrition, as well as males being more active than females, and because they are in contact with the external environment factors that play a role in the transmission of parasite egg sacs and their being in a working group in societies, which makes them more Vulnerable to infection and exposure of males to pollution more than females because of their consumption Moreover, PCR is the best diagnosis of Cryptosporidium because the microscopy is

ineffective especially if there are few egg sacs in the stool sample but the patient is still infected. PCR as a molecular method allows for differentiation between species, although it is a fast, reliable and sensitive method, it has some limitations that lead to false positive results due to laboratory contamination [29].

The results of the current study confirmed that the Cryptosporidium parasite infects all age groups at different rates and from both sexes. The study showed that the highest rate of infection among age groups was in children aged 1-10 years, when the rate was 114%, and the lowest rate of infection was among the age groups 21- 30 years, when it reached .61%. The reason for the high rate of infection in children is due to lack of health awareness, lack of interest in hygiene, presence in contaminated places, failure to wash hands before eating and playing with tools contaminated with egg sacks, as well as the incompleteness of their immune system, and infection can also occur Once swallowing a small dose of egg sacs, all these factors helped spread the parasite in children [30].

This study agreed with many studies of researchers who confirmed the spread of the parasite in children under the age of ten, including [31], if a high rate of infection was found in children aged 1-8 years, as well as the studies of many researchers who confirmed on the high incidence of *Cryptosporidium* in children Das *et al*. (2006) ; Palit *et al*. (2005) ; Othman (2000) and also agreed with Akyon *et al*. (1999) [32] in Turkey, as they indicated that the highest percentage of cryptosporidiosis was in the age group less than 12 years old, and in Diyala Governorate, such as the study of Waldron et al. (2011) [33] in Australia that cryptosporidiosis was highest in children aged 1-4 years, and previous studies conducted in different tropical countries showed that children under the age of two years are most likely to be infected with *Cryptosporidium*, with reported percentages ranging from than 18.9% in southern India [34]. Moreover, the results of the current study were inconsistent with those of Mbanugom & Agu (2006) [35] who reported that the incidence was 14.0% among children aged 3 months to 15 years in Nigeria as well as the findings of [36] who found that C. cryptosporidiosis predominantly infects children under five years of age in Diyala Governorate.

Another study in Kuwaiti hospitals by Iqbal *et al*. (2011) who found that the incidence of cryptosporidiosis was highest in children aged 4-8 years was 41%, while a study in Iran that included stool samples collected from 104 children and adults with gastroenteritis, the study reported that The infection rate of Cryptosporidium was 12% (Nahrevanian *et al*., 2010) and other studies such as Abdel-Messih (2007) [37] in Cairo / Egypt showed that the highest infection rate was 88.8% for the age group between one and two years. In Palestine, the Abu-Alrub (2008) [38] study found that the highest prevalence of *Cryptosporidium* was in children between one day and five years (14.4%), the study of Kia *et al*. (2008) [39], who noted that the parasite infects both sexes and at different ages. As for the age groups (50-41), the results of the study coincided with the study of a number of researchers, including Baqai et al. (2005) [40]. Moreover, microscopy and PCR may give different results because some egg sacs may be damaged before DNA extraction during the latter method [41]. In addition, the nested PCR technique has been shown to be four to five times more sensitive than the simple PCR technique [42].

The molecular method for the detection of Cryptosporidium using a series of polymerase chain reaction (PCR) technology is more accurate compared to other laboratory methods, microscopic and immunological, because it can distinguish between the types of this genus and discover different genes using primers dedicated to each gene to be searched for, while other methods of diagnosis cannot Distinguishing between species belonging to this genus [43].

In addition to the fact that there are different rates of parasite infection diagnosed by PCR method, including the study of Al-Shabani (2014) [44] with a percentage of 72.64% in Al-Diwaniyah Governorate, and the study of El-Settawy and Fathy (2012) [45] with a percentage of 24.4% in Egypt, as well as Lim *et al*. (2011) [46] by 64% in Malaysia. The reason for the difference in ratios is due to several factors, including the methods of preserving and storing samples, as well as the number of samples and the method used to extract DNA, as well as the accuracy of microscopic diagnosis and the extent of distinction between parasite egg sacs and other parasite egg sac

CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

From the results of the current study, the following was concluded:

1- One of the most effective methods for parasite diagnosis is the nested polymerase chain reaction.
2- *Despite its modest prevalence, Cryptosporidium is one of the most common parasites that cause diarrhea in the holy city of Karbala.*
3- The presence of the accompanying parasites has a significant impact on the rates of infection with the Cryptosporidium parasite.
4- The influence of some epidemiological factors such as gender, age, housing and drinking water source on the parasite presence.
5- Cryptosporidium infects both sexes and all ages, and children are more susceptible to infection with the parasite.
6- The infection rate could not be accurately calculated using the modified acid stain approach or immunological assays.

RECOMMENDATIONS

1- Extensive immunological and molecular research of the Cryptosporidium parasite to determine the parasite's genetic sequence, as well as diagnostic studies.
2- Because the parasite affects both people and animals, researchers are studying it in animals.
3- Conducting research on the link between this parasite and other causes of diarrhea, including parasites, viruses, and bacteria.
4- Ascertain that this parasite is examined as part of the normal exams for instances of diarrhea that are available in hospitals and health facilities.
5- Because of the threat these parasites provide to human life, health institutions should set up introductory and instructional courses for all health professionals on parasites that cause diarrhea in humans.
6- The use of precise identification of the causes of diarrhea, particularly in children, in order to prescribe appropriate treatment and remove it and therefore minimize the number of children who die.
7- Paying attention to public cleanliness and raising health awareness among city and village people, as well as committing to keeping one's house, food, and drink clean.

REFERENCES

1. Al-Ezzy, Ali Ibrahim Ali, Abeer Thair Khadim, and Raad Hamudi Hassun. "A comprehensive Evaluation of Transmission Methods for Cryptosporidium species with special emphasis to Cryptosporidium Parvum." *Research Journal Of Pharmaceutical Biological And Chemical Sciences* 8.5 (2017): 555-570.
2. Clark, Douglas P. "New insights into human cryptosporidiosis." Clinical Microbiology Reviews 12.4 (1999): 554-563.
3. Cole, D. J., et al. "Detection of Cryptosporidium parvum in horses: thresholds of acid-fast stain, immunofluorescence assay, and flow cytometry." *Journal of clinical microbiology* 37.2 (1999): 457-460.
4. Costa, Damien, et al. "Epidemiology of Cryptosporidiosis in France from 2017 to 2019." *Microorganisms* 8.9 (2020): 1358.
5. Coupe, Stephane, et al. "Detection of Cryptosporidium and identification to the species level by nested PCR and restriction fragment length polymorphism." *Journal of Clinical Microbiology* 43.3 (2005): 1017-1023.
6. Fayer, Ronald, Jitender P. Dubey, and David S. Lindsay. "Zoonotic protozoa: from land to sea." *Trends in parasitology* 20.11 (2004): 531-536.
7. Al-Ezzy, Ali Ibrahim Ali, Abeer Thair Khadim, and Raad Hamudi Hassun. "A comprehensive Evaluation of Transmission Methods for Cryptosporidium species with special emphasis to Cryptosporidium Parvum." *Research Journal Of Pharmaceutical Biological And Chemical Sciences* 8.5 (2017): 555-570.
8. Li, Na, et al. "Development and evaluation of three real-time PCR assays for genotyping and source tracking Cryptosporidium spp. in water." *Applied and Environmental Microbiology* 81.17 (2015): 5845-5854.
9. Mohammad, Fatima Ibrahim. "Detecting of virulence factors COWP gene and CP15 gene for Cryptosporidium parvum by polymerase chain reaction (PCR)." Al-Qadisiyah Journal of Pure Science 23.2 (2018): 39-47.
10. Carreno, Ramon A., Donald S. Matrin, and John R. Barta. "Cryptosporidium is more closely related to the gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using small-subunit ribosomal RNA gene sequences." *Parasitology Research* 85.11 (1999): 899-904.
11. Nahrevanian, Hossein, et al. "Current situation of Cryptosporidium and other enteroparasites among patients with gastroenteritis from western cities of Mazandaran province, Iran, during 2007-2008." *Gastroenterology and Hepatology from Bed to Bench* 3.3 (2010).
12. Oleiwi, M.K., prevalence study for main protozoa diarrheal among patients by using microscopically and molecular methods in Babylon province, Thesis of master College of Science for Women, Babylon university, ((2015) :23-25pp.
13. Razakandrainibe, Romy, et al. "Common occurrence of Cryptosporidium hominis in asymptomatic and symptomatic calves in France." *PLoS neglected tropical diseases* 12.3 (2018): e0006355.
14. Ryan, U. N. A., Ronald Fayer, and Lihua Xiao. "Cryptosporidium species in humans and animals: current understanding and research needs." *Parasitology* 141.13 (2014): 1667-1685.
15. Sponseller, Jerlyn K., Jeffrey K. Griffiths, and Saul Tzipori. "The evolution of respiratory cryptosporidiosis: evidence for transmission by inhalation." *Clinical microbiology reviews* 27.3 (2014): 575-586.
16. Sunnotel, O., et al. "Photocatalytic inactivation of Cryptosporidium parvum on nanostructured titanium dioxide films." Journal of water and health 8.1 (2010): 83-91.
17. Fayer, Ronald, and Lihua Xiao, eds. *Cryptosporidium and cryptosporidiosis*. CRC press, 2007.
18. Yacoub, Maisaa Mohammad Mahmoud. *Intestinal Protozoa and Cryptosporidium genotypes in North of West Bank/Palestine*. Diss. Faculty of Graduate Studies Intestinal Protozoa and Cryptosporidium genotypes in North of West Bank/Palestine By Maisaa Mohammad Mahmoud Yacoub Supervisor Dr. Ayman Hussein This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Life Sciences (Biology), Faculty of Graduate Studies, An-Najah National University, 2014.
19. Patient, U. K. "From Wikipedia, the free encyclopedia." epidemiology 4: 11.
20. Costa, Damien, et al. "Epidemiology of Cryptosporidiosis in France from 2017 to 2019." *Microorganisms* 8.9 (2020): 1358.
21. Fayer, Ronald, Jitender P. Dubey, and David S. Lindsay. "Zoonotic protozoa: from land to sea." *Trends in parasitology* 20.11 (2004): 531-536.
22. Al-Ezzy, Ali Ibrahim Ali, Abeer Thair Khadim, and Raad Hamudi Hassun. "A comprehensive Evaluation of Transmission Methods for Cryptosporidium species with special emphasis to Cryptosporidium Parvum." *Research Journal Of Pharmaceutical Biological And Chemical Sciences* 8.5 (2017): 555-570.
23. Mohammad, Fatima Ibrahim. "Detecting of virulence factors COWP gene and CP15 gene for Cryptosporidium parvum by polymerase chain reaction (PCR)." *Al-Qadisiyah Journal of Pure Science* 23.2 (2018): 39-47.
24. Nahrevanian, Hossein, et al. "Current situation of Cryptosporidium and other enteroparasites among patients with gastroenteritis from western cities of Mazandaran province, Iran, during 2007-2008." *Gastroenterology and Hepatology from Bed to Bench* 3.3 (2010).
25. Ramirez, Norma E., Lucy A. Ward, and Srinand Sreevatsan. "A review of the biology and epidemiology of cryptosporidiosis in humans and animals." *Microbes and infection* 6.8 (2004): 773-785.
26. Razakandrainibe, Romy, et al. "Common occurrence of Cryptosporidium hominis in asymptomatic and symptomatic calves in France." *PLoS neglected tropical diseases* 12.3 (2018): e0006355.
27. Ryan, U. N. A., Ronald Fayer, and Lihua Xiao. "Cryptosporidium species in humans and animals: current understanding and research needs." *Parasitology* 141.13 (2014): 1667-1685.
28. Sponseller, Jerlyn K., Jeffrey K. Griffiths, and Saul Tzipori. "The evolution of respiratory cryptosporidiosis: evidence for transmission by inhalation." *Clinical microbiology reviews* 27.3 (2014): 575-586.
29. Squire, Sylvia Afriyie, and Una Ryan. "Cryptosporidium and Giardia in Africa: current and future challenges." *Parasites & vectors* 10.1 (2017): 1-32.
30. Squire, Sylvia Afriyie, and Una Ryan. "Cryptosporidium and Giardia in Africa: current and future challenges." *Parasites & vectors* 10.1 (2017): 1-32.

31. Sunnotel, O., et al. "Photocatalytic inactivation of Cryptosporidium parvum on nanostructured titanium dioxide films." *Journal of water and health* 8.1 (2010): 83-91.
32. Fayer, Ronald, and Lihua Xiao, eds. *Cryptosporidium and cryptosporidiosis*. CRC press, 2007.
33. Xiao, Lihua, and Vitaliano A. Cama. "Cryptosporidium and cryptosporidiosis." *Foodborne parasites*. Springer, Cham, 2018. 73-117.
34. Xiao, Lihua, and Vitaliano A. Cama. "Cryptosporidium and cryptosporidiosis." *Foodborne parasites*. Springer, Cham, 2018. 73-117.
35. Al-Hindi, Adnan Ibrahim, Abdelraouf A. Elmanama, and Kamal Jad Allah Elnabris. "Cryptosporidiosis among children attending Al-Nasser pediatric hospital, Gaza, Palestine." *Turkish Journal of Medical Sciences* 37.6 (2007): 367-372.
36. Al-Warid, H. S., S. H. Mahmood, and I. M. Al-Saqur. *Study in epidemiology and PCR detection of cryptosporidiosis in north of Baghdad*. Diss. Ph. D. Thesis. College of Science, University of Baghdad, Iraq, 2010.
37. Patient, U. K. "From Wikipedia, the free encyclopedia." *epidemiology* 4: 11.
38. Clark, Douglas P. "New insights into human cryptosporidiosis." *Clinical Microbiology Reviews* 12.4 (1999): 554-563.
39. Cole, D. J., et al. "Detection of Cryptosporidium parvum in horses: thresholds of acid-fast stain, immunofluorescence assay, and flow cytometry." *Journal of clinical microbiology* 37.2 (1999): 457-460.
40. Costa, Damien, et al. "Epidemiology of Cryptosporidiosis in France from 2017 to 2019." *Microorganisms* 8.9 (2020): 1358.
41. Costa, D., et al. "French National Network on Surveillance of Human Cryptosporidiosis, Dalle F, Favennec L. 2020." *Epidemiology of Cryptosporidiosis in France from* (2017).
42. Coupe, Stephane, et al. "Detection of Cryptosporidium and identification to the species level by nested PCR and restriction fragment length polymorphism." *Journal of Clinical Microbiology* 43.3 (2005): 1017-1023.
43. Huang, Yan, et al. "Isolation and identification of sporozoite membrane protein of Cryptosporidium parvum and evaluation of calmodulin-like protein immune protection." *Parasite Immunology*: e12937.
44. Dhal, Ajit Kumar, et al. "An update on Cryptosporidium biology and therapeutic avenues." *Journal of Parasitic Diseases* (2022): 1-17.
45. Abd El-Hamed, Wafaa Fayez, et al. "Anticryptosporidium Efficacy of Olea europaea and Ficus carica Leaves Extract in Immunocompromised Mice Associated with Biochemical Characters and Antioxidative System." *Cells* 10.9 (2021): 2419.
46. Dyab, Ahmed K., et al. "Cryptosporidiosis in immunocompromised children." *Age* 59.28 (2018): 48-40.