

Early Genetic Detection of *Cyclospora Cayetanensis* that Causes Diarrhea in Children at the Children's and Gynecology Hospital in Ramadi – Iraq

Noor Mohammed Hussein, Thaer Abdulqader Salih

Biology Department – college of Education for Pure Sciences – University Of Anbar

Sc.thaerparasit@uoanbar.edu.iq

Summery

This study was conducted to find out the prevalence of Cyclosporiasis in children arriving at the Children's and Gynecology Hospital in Ramadi – Iraq , Because this parasite has a significant impact on causing dehydration and the loss of large amounts of body fluids, and consequently the confirmed death of the infected person if not treated within the specified period, as well as the identification of the means of transmission to humans and our results showed its transmission through water and food contaminated with *C. cayetanensis*Oocysts , and it was used. Simple and modern methods of diagnosing infect .

This study included examining (250) samples of faeces from children under the age of 15 years from the period 22-7-2020 to 28-2-2021 and those suffering from diarrhea who visited Children's and Gynecology Hospital since July of 2020.

The results showed that the percentage of infection prevalence in children for all pathological parasites is (23.6%) and the number reached 59 infected samples, while the percentage of infection with the *Cyclospora* was 6.4% (according to microscopic diagnosis using modified Ziehl–Neelsen stain, sedimentation and sedimentation methods, and direct method. , Where the infection rate of *Entamoeba histolytica* (10.4), *Giardialambelia* (1.60), *Cryptosporidiumparvum*(3.20), *Cyclosporacayetanensis* (6.4), *Trichomonas hominis* (0.4) and *Entamoeba coli* (1.60), and the prevalence of infection in children according to the genetic diagnosis of the *Cyclospora* sample were (4.0%), and this was the first study in Anbar Governorate to prove the presence of this parasite.

Key words: Cyclosporiasis , PCR , Diarrhea , Ziehl–Neelsen modified stain.

Introduction

Cyclospora cayetanensis is one of the globular microorganisms that cause cyclosporiasis, which is considered an acute intestinal disease especially for patients with immunodeficiency, children and the elderly (Hadjilouka and Tsaltas, 2020) . This parasite belongs to the eukaryote protozoa and belongs to the subfamily Apicomplexa and under the class coccidiasian and family Emeridae (Ortega and Sanchez, 2010).

Epidemiological studies that were conducted on the parasite *C. cayetanensis* revealed the role of water and food, especially fresh vegetables and fruits, in the spread of the disease (Robertson and

Gjerde, 2000; Lopez et al ,2003), and one of the most dangerous cases is the parasite's severe resistance to disinfectants and sterilizers, especially chlorine and its natural rate used in water. Drinking (between 0.2 to 0.5 milligrams / liter). The Oocysts also remain attached to the vegetable leaves even after washing them frequently (Ortega and Sanchez, 2010).

The typical symptoms of annular infection are watery diarrhea, abdominal cramps, vomiting, loss of appetite, weight loss, severe fatigue, and in a few cases, the patient has flu-like symptoms (Marques et al, 2017; Giangaspero and Gasser, 2019).

The clinical symptoms of cyclosporiasis are also related to the age, immune status of the host, and endemicity in certain areas, and these symptoms begin to appear about a week after the ingestion of mature Oocysts . The incubation period for parasite infection ranges from 2-11 days and the average incubation period is about 7 days (Almeria et al, 2019) , Clinical symptoms may disappear with treatment and with the use of certain drugs, however infection persists in untreated patients, which may last from a few days to months or even longer (Thapa and Basnyat, 2017).

There is no vaccine available against cyclosporiasis (Giangaspero and Gasser, 2019), but chemical treatments have proven effective against the disease, including trimethoprim by 160 mg with 800 mg of sulfamethotazole (Tmp-smx) also known as (co-trimoxazole), where it is prescribed. By doctor and given twice daily for 7 days (Hoge *et al*, 1995; Escobedo et al, 2009).

Based on the above, the spread of this parasite, its danger and its epidemic, is of utmost importance and due to the lack of a previous study in Anbar Governorate in diagnosing the presence and spread of this parasite and because of its lack of diagnosis in hospitals and health centers and because infection with it leads to severe diarrhea that may lead to dehydration in children with Weak immunity and because its treatments differ from traditional treatments for other parasites that cause diarrhea, so we decided to do this study that aims to:

- 1- Laboratory investigation of the parasite by means of dyes in children with diarrhea arriving to the Children's and Gynecology Hospital in Ramadi
- 2- Genetically diagnosing the parasite to confirm the infection.
- 3- Recommending the Ministry of Health to come out with the parasitic types that are widespread in our environment and that cause diarrhea to take the necessary measures and provide the appropriate treatment for this case, especially as it has similar symptoms with other parasites.

Methods

Samples were collected from patients arriving at the Children's and Gynecology Hospital and those suffering from diarrhea randomly under the age of 15 years and from both sexes using sterile plastic boxes. A questionnaire form was made that includes the name, age, gender, residence, living condition and the consistency of the faeces whether (solid, semi-solid, or soft or hard). Watery) and the color of faeces (brown or yellow or white or green or with blood) and the treatments used , The investigation stage for the parasite begins by using a number of primary diagnostic methods that search for a macroscopic examination that depends on the texture, color and smell of the examination by sedimentation using a centrifuge or by sedimentation using the dyes used in the iodine dye. Using an eyepiece to present to confirm parasite size and then using

molecular detection or diagnosis, by molecular detection of *Cyclospora* samples, using PCR and primers.

SAD For 5-G CAGTCACAGGAGGCATATATCC-3

SAD Rev 5-ATGAGAGACCTCACAGCCAAAC-3

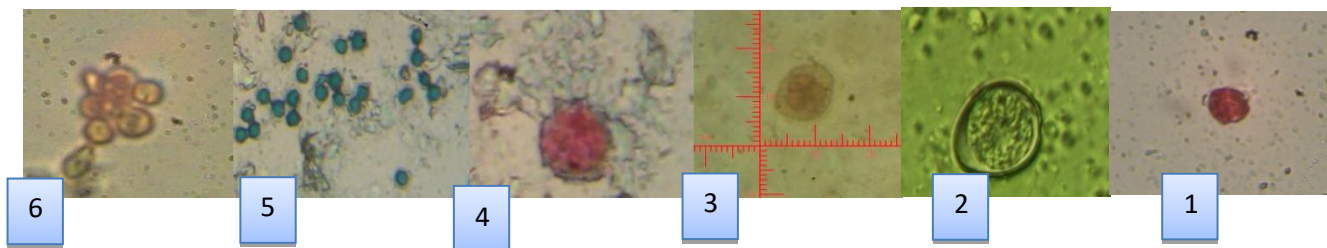
After concentrating the Oocysts and extracting DNA from the samples.

Results and discussion

The results obtained through the collection of 250 samples from the arriving children of the Children's and Gynecology Hospital since July 2020 indicated that 16 children were infected with cyclosporiasis according to the microscopic diagnosis using the modified Ziehl–Neelsen stain, as well as sedimentation and staining methods, direct diagnosis and iodine staining and upon confirmation of the diagnosis using the Genetic diagnosis: The number of infections was 10 children only, as shown in Table (1) and Picture (1).

Table (1) the results of micro diagnosis and genetic diagnosis

Total samples	type of parasite that causes diarrhea	Cases of diarrhea for those infected with parasites	%	Cases of diarrhea for those infected without parasites	%	Screening method for detecting the Cyclospora	number of positive cases	%
250	<i>Entamoeba histolytica</i>	26	10.4	191	76.4	Direct method	8	3.2
	<i>Giardia lamblia</i>	4	1.6			Sedimentation	8	3.2
	<i>Cryptosporidium parvium</i>	8	3.2			Flotation with Shethers solution	10	4
	<i>Cyclospora cayetanensis</i>	16	6.4			Iodine	10	4
	<i>Trichomonas hominis</i>	1	0.4			Ziehl–Neelsen Modified stian	16	6.4
	<i>Entamoeba coli</i>	4	1.6			Genetic method	10	4
	Total	59	23.6			Total	10.34	4.134

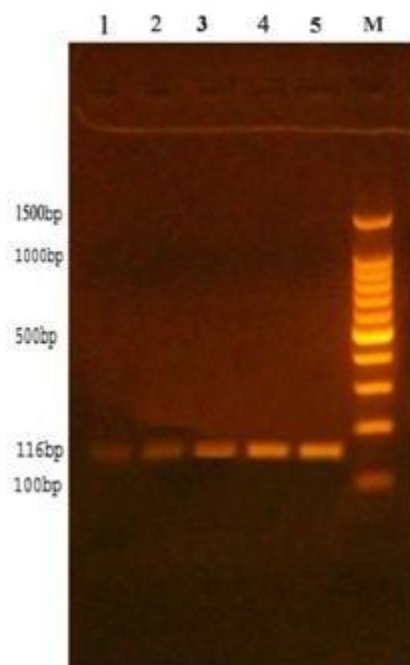


Giardia -6 . *Entamoeba histolytica* -5 . *Entamoeba coli* -4 . *Cryptosporidium* -3 . *Trichomonas* -2 . *Cyclospora* - 1 . *lambellia*

Picture (1): The parasites that cause diarrhea and are excreted in the stool of patients (by microscopic examination) show the strength is 40x.

The diagnosis of *Cyclospora cayetanensis* was confirmed by genetic method and gene design, which showed positively only ten samples of the total samples accompanying the cases of diarrhea. and as shown in the picture (2).

Through the results of the current study, the results confirmed that there is a difference in the accuracy of the diagnostic methods, as the molecular diagnosis is more accurate than the microscopic diagnosis, as the similarity in the form of the parasite with other parasites as well as the staining of fungi and food remains with the same pigment make the microscopic diagnosis less accurate, and through the study The number of cases of diarrhea in children coming to the hospital was found to be very small compared to previous years, due to the increase in health awareness and the continuous sterilization of water, vegetables and fruits due to the spread of the Corona epidemic, which led to the emergence of small percentages in the infection of cyclosporaisis .



Picture (2) illustrates the diagnosis of *C. cayetanensis* by PCR methods and according to the small piece 116 pb.

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