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Cryptosporidium parvum In Diarrheic Children In AL-Muthanna Province– Iraq

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Abstract

The aim of this study was to make comparison for prevalence of *Cryptosporidium parvum* among children by traditional microscopic examination techniques (Direct,Flotation,Sedimentation and Ziehl-Neelsen stain) as well as RT-PCR . This study was carried out in AL-Muthanna province during the period from October 2013 to May 2014 in the Educational -AL- Hussein hospital in AL-Samawa, General AL-Rumaiytha hospital, Feminine and Children's hospital in AL-Samawa and AL-Rumaiytha health centers. A total of 100 stool samples were taken from children aged 1 month to less than 10 years who suffering from acute or persistent diarrhea and it examined by Direct, Flotation and Sedimentation methods and Ziehl-Neelsen stain and RT- PCR assay. The present study recorded the prevalence percent of *Cryptosporidium parvum* that detected in 100 fecal samples examined by direct , flotation and sedimentation methods were 30%, 36 and 40% respectively. While in this study , the fecal samples of children were examined by Ziehl-Neelsen, out of which 21 fecal samples had suspected *Cryptosporidium parvum* with percent 21%. The results showed oocysts stained purple bodies against a dark blue back ground, with a clear halo around the oocyst. Alsoin this study , the fecal samples of children were examined by real time PCR out of which 18 fecal samples had found *Cryptosporidium parvum* with percent 18%. The present study showed that high percentage of positive cases was recorded in microscopical examination while 18% of positive cases were detected by molecular assay.

Keyword: ***Cryptosporidium parvum*, Children, Stool, RT-PCR, Iraq. Corresponding author: Gmail: nouranraad @gmail.com.**

1-Introduction:

Cryptosporidium is a consider the major cause of diarrhea in children, *Cryptosporidium* is a protozoan parasites that a wide range of vertebrates are infecting including humans and animals, and results in significant morbidity and mortality in both the developed and developing countires (Fayer, 2004). Cryptosporidiosis is transmitted via fecal–oral route, or by ingestion of food and water contaminated with *Cryptosporidium* oocysts (Meinhardt *et al.*,1996).

The protozoan parasite *Cryptosporidium* is important cause of gastrointestinal disease that gives rise to chronic life threatening condition in immune compromised individual and to acute gastroenteritis and diarrhea in healthy people (Cook, 1987). Sturdee *et al.*, (1999), they found that this parasite invades epithelial cells of the intestinal tract and respiratory tree of vertebrate hosts.

The prevalence of *Cryptosporidium* oocysts in children with diarrhea in the neighboring countries, such as, In Turkey, Akyon *et al*., (1999) detected that Cryptosporidiosis prevalence under the age of 12 years and showed that *Cryptosporidium* oocysts in seven of the cases were (3.5%), this result showed that *Cryptosporidium* could be a causative agent of diarrhea in children. While in Iran, stool samples collected include 104 children and adult patients with gastroenteritis, the study reported that *Cryptosporidium* infected (2.9%) of the patients (Nahrevanian *et al.*, 2007). In Saudi Arabia, Al- Braiken *et al*., (2003) revealed the highest prevalence of *Cryptosporidium* infection among children with diarrhea who were presenting to pediatric outpatient clinics was (32%). In Kuwait, Iqbal *et al*., (2001) collected 3549 stool samples, 509 children had diarrhea during the period from September 1995 to August 1997, *Cryptosporidium* oocysts were detected in 51(10%) children with diarrhea.

While in Iraq , there are many studies such as (Al-Shahery and Manal, 2007) They found the percentage of infection in calves with this parasite in Mosul city of Iraq was 36.6% , other study includes the test of 115 samples of faces taken from children in Ramadi province, results show a total infection percentage was 39.13% including which 26.08% in rural area and 13.4% in the city (Mohanad, 2008), and (33.83 %) in Kut city of Iraq (Abdulsadah *et al*., 2013).

The most used technique for the diagnosis of *Cryptosporidium* is the detection of oocysts in a fecal smear, there are varieties of techniques, including microscopy, immunological and molecular techniques , for the detection of oocysts of *Cryptosporidium*, microscopic examination include concentration methods and staining methods of fecal smears (Casimiro *et al*., 2009).

Recently, developed PCR protocols have proven to be very specific and highly sensitive (Sulaiman *et al*., 2005). Also there is no studies on this parasite in Al-Muthanna province so that we needs for undertaking the study.

2-Material and Methods.

2-1.Patients:

This study was carried out in AL-Muthanna province during the period from October 2013 to May 2014 in the educational AL-Hussein hospital in Samawa, general AL-Rumaiytha hospital, Feminine and children's hospital in AL-Samawa and Rumaiytha health centers. A total of 100 stool samples were taken from children aged 1 month to less than 10 years who suffering from the acute or persistent diarrhea and it examined by direct, flotation , sedimentation methods and Ziehl-Nelseen stain and RT-PCR assay.

2-2.Fecal samples collection:

Fresh fecal samples were collected by using a sterile containers and transported in to a cooled box (temperature approximately 10°C). Then, the samples were transported to the laboratory at College of Science- AL-Muthanna University, at the laboratory the fecal samples were divided into two portions , one portion was for the microscopic examination of parasites while the other portion was stored immediately at-20°C for molecular analysis (RT-PCR).

2-2-1.Laboratory Tests:

2-2-1.1.Direct Smear By Using Lugol's Iodine:

According to Coles (1986), the method was done as follows:

1-A drop of lugol's iodine solution was placed on a glass slide.

2-Small amount of fecal sample of human (1gm) was put on lugol's iodine drop and mixed thoroughly using wooden stick.

3- Cover slip was applied with forceps or fingers.

4-Examination of slide under (40X) and (100X) powers.

2-2-1.2.Concentration Techniques:

Flotation Method By Sheather's Sugar Solution : (Henrikson and Pohlen , 1981).

(5gm) of feces were diluted in (10ml) of tap water, filtered using gauze and centrifuged at (2500 rpm) for (10 min), then the supernatant was poured and the sediment was mixed in Sheather's solution in a (15ml) plastic tube , this suspension was centrifuged at (2500 rpm) for (10 min) , after that placed the cover slip was touched the surface of the solution for (10-15min), then the cover slip was examined on a glass slide using microscope at 40x then 100x magnification .

Oocysts were counted, if more than 4 oocysts were observed, the same (5gm) sample was re centrifuged and oocysts were counted on a second and sometimes a third cover slip (Gondim *et al.*, 2002). The oocysts of feces were measured with a calibrated ocular micrometer by using bright field microscopy, the oocysts with a diameter of (4-6µm) considered to be positive for *Cryptosporidium Spp*. (Hammond and Long, 1973; Schares *etal.*, 2005).

2-2-1.2.1.Formal-Ether Concentration Technique:

This technique is recommended as the best overall techniques for the concentration of parasites in feces (Al-Harthi, 2004).

Procedure:

1. Emulsify 1gm of feces in about 10 ml of 10% formal saline.
2. By using glass beads, were mixed well by shaking for about 20 seconds.
3. Sieves the emulsified feces collecting the sieved suspension in beaker.
4. Transfer the suspension in a test tube and was added about 3ml of ether.
5. Stopper the tube and was mixed well for 1 minute.
6. Centrifuge immediately at approx 3,000rpm for 15 minutes .
7. After centrifugation was used a stick to remove the fecal debris from the side of the tube and decants the supernatant layer.
8. The sediment will remain.
9. Washed for three times.
10. Examined microscopically using 40x then 100x to identify the parasite.

2-2-1.2.2.Modified Acid-Fast Staining Technique (Z-N Technique): Procedure: (Baxby *etal*., 1984).

1. Sediment from concentration method was mixed, and then smear 10 µL on slide within etched circle by use transfer loop (10 µL).
2. Air-dry for at least 1 hour.
3. Fixed in Methanol for 3 to 5 minutes.
4. Stained with carbon fuchsin for 20 minutes.

5. Rinsed with tap-water for 4 minutes.
6. Decolorized with acid alcohol (HCl with 95% alcohol).
7. Rinsed with tap-water for 2 minutes.
8. Counter stain with 3% methylene blue for 30 seconds or malachite green.
9. Rinsed with tap water for 2 minutes.
10. Air dry.
11. Examined by standard light microscopy.
12. By Z-N method, *Cryptosporidium spp*. oocyst stain red against a blue-green Background. *Cryptosporidium* is round and approximately (4-6 µm) in diameter.

2-3.Calibration By Ocular Micrometer:

Using of ocular micrometer for determination of length and width of oocysts according to method described by (Benson, 2002).

2-4.Primers:

The Real-Time PCR primers and probe that used in this study were design by Stephen *et al*., (2011) and provided by (Bioneer company, Korea) for detection of *Cryptosporidium parvum* as following:

Table (2-1): Primers of *Cryptosporidium parvum*

Primer	Sequence	
SSU rRNA	F	TCCTTGAAATGAATATTTGTGACTCG
	R	TTAATGTGGTAGTTGCGGTTGAAC
SSU rRNA Probe	VIC-TATCTCTTCGTAGCGGCGTA MGB-NFQ	

2-5.Genomic DNA Extraction:

Genomic DNA of supernatant sporulated oocyst was extracted by using AccuPrep®Genomic DNA extraction kit (Bioneer, Korea) and done according to company instruction as following steps:

1-A 200µl of stool suspention was transferred to sterile 1.5ml microcentrifuge tube, and then added 20µl of proteinase K and mixed by vortex.

2-After that, 200µl of Binding buffer was added to each tube and mixed by vortex to achieve maximum lysis efficiency, and then all tubes were incubated at 60℃ for 10 minutes.

3-A 100µl of isopropanol was added to mixture and mixed well by pipetting , and then briefly spin down to get the drops clinging under the lid. The lysate was carefully transferred into GD Binding filter column that fitted in a 2 ml collection tube, and then closed the tubes and centrifuged at 8000 rpm for 1 minute.

4-Through out lysate was discarded in disposal bottle, and then 500µl Washing buffer 1 (W1) was added to each Binding filter column, and centrifuged at 8000 rpm for 1 minute.

5-Throughout Washing buffer 1 was discarded in disposal bottle, and then 500µl Washing buffer 2 (W2) was added to each Binding filter column, and centrifuged at 8000 rpm for 1 minute.

6-Throughout Washing buffer 2 was discarded in disposal bottle, and then the tubes were centrifuged once more at 12000 rpm for 1 minute to completely remove ethanol.

7-After that, GD Binding filter column that containing genomic DNA was transferred to sterile 1.5ml microcentrifuge tube, and then added 50µl of Elution buffer and left stand the tubes for 5 minutes at room temperature until the buffer is completely absorbed into the glass filter of Binding column tube.

8-Finally, all tubes were centrifuged at 8000 rpm for 1 minute to elute DNA, and storge at-20℃ freezer.

2-6.Genomic DNA Profile:

The extracted genomic DNA was checked by using Nanodrop spectrophotometer (THERMO, USA), that check and measurement the purity of DNA through reading the absorbance in at (260/280 nm) as following steps:

1-After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).

2-A dry Chem-wipe was taken and cleaned the measurement pedestals several times. Then carefully pipet 2µl of ddH2O onto the surface of the lower measurement pedestal.

3-The sampling arm was lowered and clicking OK to blank the Nanodrop, then cleaning off the pedestals.

4-After that, the pedestals are cleaned and pipet 1µl of DNA sample for measurement

2-7.Real-Time PCR Master Mix Preparation:

Real-Time PCR master mix was prepared for specific primer by using AccuPower® DualStarTM qPCR PreMix kit (Bioneer, Korea), and done according to company instructions as following table.

Table (3-2): Real-Time PCR Master Mix Preparation

qPCR master mix	Volume
Genomic DNAQ2QY6,T 6R	5µL
TaqMan probe (10pmol)	2
Forward primers (10pmol)	1µL
Reverse primers (10pmol)	1µL
PCR water	11 µL
Total	25µL

These qPCR master mix reaction components that mentioned above were added into TaqMan probe qPCR master mix reaction . Then all tubes vortex for mixed the components and centrifuge for 3000rpm for 3 minutes in Exispin centrifuge, after that transferred into Miniopticon Real-Time PCR thermocycler.

2-8.Real-Time PCR Thermocycler Conditions:

Real-Time PCR thermocycler conditions was done according to primer annealing temperature and qPCR Syber green kit instructions as following tube.

Table (3-3): Real-Time PCR Thermocycler Conditions

Step	Condition	Cycle
Pre-Denaturation	**95 °C 1 min**	**1**
Denaturation	**95 °C6RRRRR 15 sec**	**45**
Annealing/Extension	**60 °C 30 min**	
Detection (Scan)		

2-9.Real-Time PCR Data Analysis:

qPCR data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification gene in Real-time cycle number.

2-10.Statistical Analysis

The Chi-square test was used to analysis the overall prevalence data , and differences were considered significant at level ($P< 0.05$) (Sorlie, 1995).

3-Results and Discussion:

3-1.Microscopic Examination:

3-1-1.Traditional Examination By Direct Smear, Flotaion, Sedimentation and Ziehl-Neelsen staining Methods:

In the present study, a total of 100 fecal samples of children were examined by direct, flotation and sedimentation methods, out of which 30, 36 and 40 respectively of fecal samples had *Cryptosporidium parvum* with percent 30%, 36% and 40% respectively. Table (3-1),Figure (3-2,3-3,3-4).

In Ziehl-Neelsen stain, The results showed the total percent of infection was 21% and these oocysts are smooth, thick walled, colorless, have spherical or slightly ovoid bodies containing, that fully developed (sporulated), four elongated, naked (i.e. not within a sporocyst) sporozoites and a cytoplasmic residual body,Table (3-1), Figure.(3-5). In figure (3-1), the mean measurements of spherical oocysts of *C. parvum* from fecal sample was (5-6×4.5-5.4) µm in range (4–6)x(3-5) µm, this results in agreement with (AL-Ta'ey,1997; Sears and Kirkpatrick, 2001; AL-Gelany, 2003; Fayer, 2008) and this morphometric variations may due to the different species belong to *Cryptosporidium* (AL-Warid, 2010). The results showed oocysts stained red bodies against a dark blue back ground, with a clear halo around the oocyst, these results agreement with (Baxby *et al*., 1984; John and Petri, 2006) they confirmed that oocysts of *Cryptosporidium parvum* were visualized as red spots on blue background.

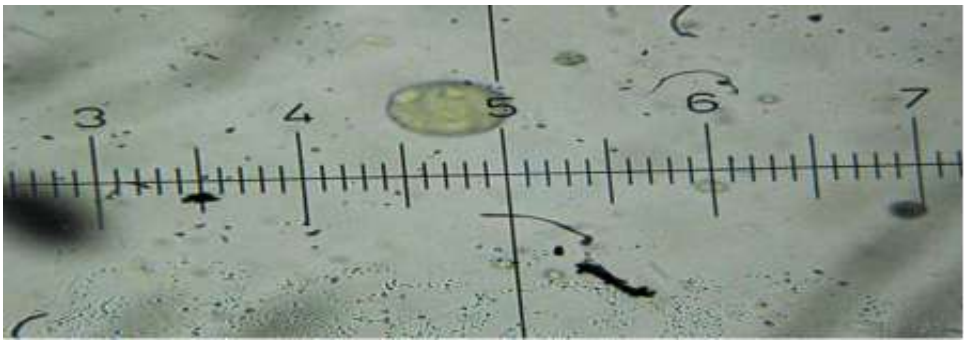

Figure (3-1): Measurment of *C.parvum* oocyst by Ocular Micromete(100x)

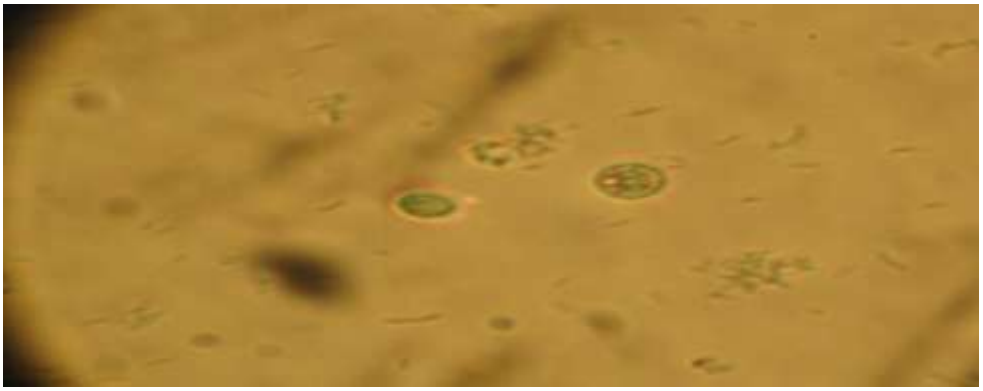

Figure (3-2): Oocyst of *C.parvum* by Direct method by using Lugol's Iodine (40x)

Figure (3-3): Oocyst of *C.parvum* by Flotation method by using Sheather's Sugar Solution (100x)

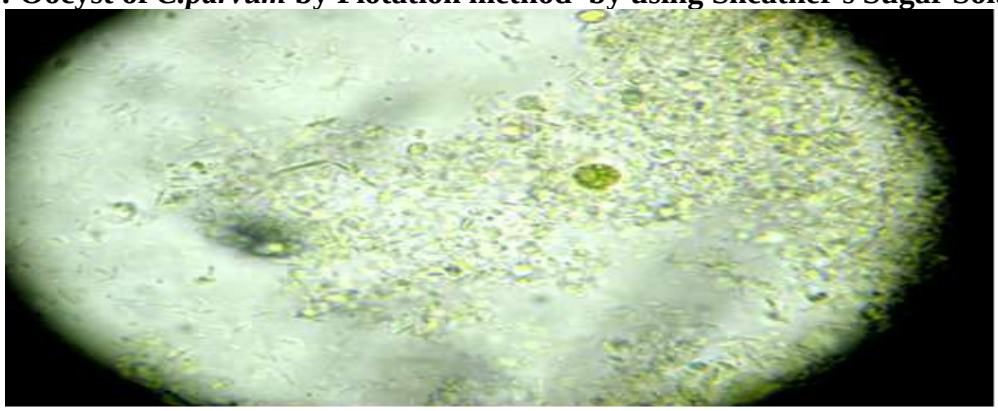

Figure (3-4): Oocyst of *C.parvum* by Sedimentation method by using Formal–Ether solution (40x)

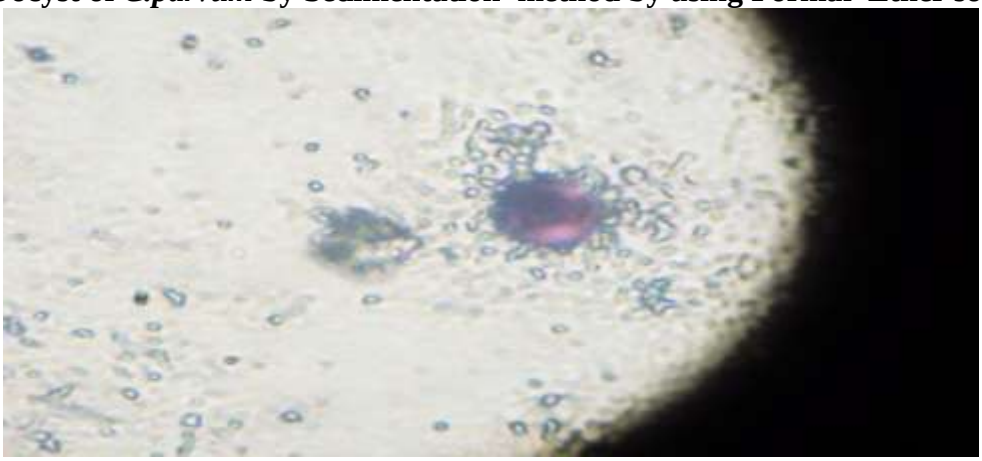

Figure (3-5) : Oocyst of *C.parvum* by Ziehl–Nelseen stain (100x)

Table (3-1) : Comparison between All Methods of Examination .

Methods of Examination	No. of samples	+ve	%	-ve	%	Chi-value	P-value
Direct smear	100	30	%30	70	%70	*17.26	0.05
Flotation test		36	%36	64	%64		
Sedimentation test		40	%40	60	%60		
Ziehl-Neelsen		21	%21	79	%79		
Real-Time PCR		18	%18	82	%82		

*** Significant differences at (P <0.05).**

Cryptosporidium spp. for taxonomical research, although there is potential for diagnostic use (Caccio *et al.*, 2005).

The present study recorded the total infection of *Cryptosporidium* by molecular assay diagnosed positive for *Cryptosporidium parvum* were genotyped on the basis of the 18S rRNA gene was 18%, this result is agreement with Akiyoshi *et al*., (2006) and the results higher than koffie *et al*., (2014) in Africa (36.93) this may be due to large number of patient samples.

3-2.Molecular Assay:

Polymerase Chain Reaction (Real-Time PCR Assay):

In this study, a total of 100 fecal samples of children were examined by real time PCR out of which 18 fecal samples had found *Cryptosporidium parvum* with percent 18% Table (3-1), Fig. (3-6). PCR based techniques can detect a single cyst and also distinguish between different species and strains of parasites (Roberts and Janovy, 2000). PCR has primary been used for identification of different species and genotypes of

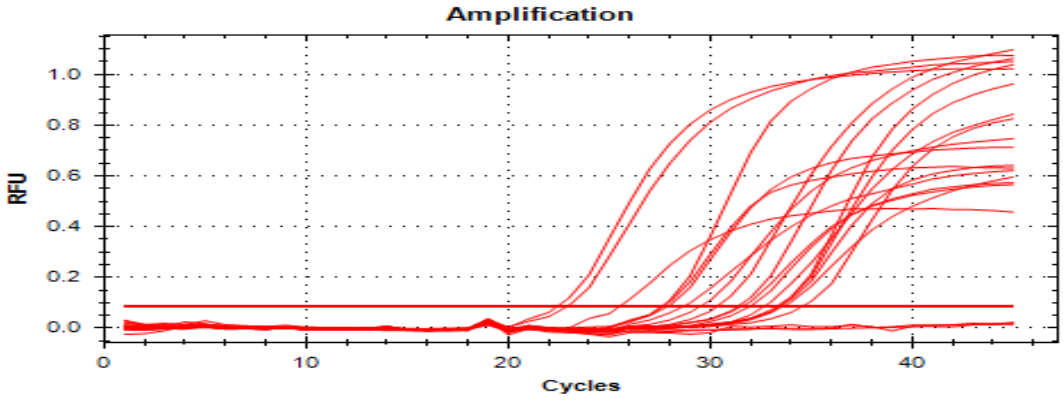

Figure (3-6): Real-Time PCR amplification plot of ssurRNA gene of Cryptosporidium parvum in positive samples

3-3.Comparison Between Infection Percentage Of *C.parvum* With Methods Of Examination:

In table (3-1), the results shows the comparison between microscopic examination and molecular assay in the diagnosis of Cryptosporidium parvum, high percentage of positive cases was recorded in microscopical examinations while 18% of positive cases were detected by molecular assay . The present study was carried out to asses prevalence and comparison between microscopic examination and molecular assay for diagnosis of *C.*

parvum . In this study showed significant differences at level (P<0.05) between molecular and microscopic methods , the molecular assay was high sensitive than the microscopic methods for the detection of *Cryptosporidium* table (3-1), this result is agreement with (Harrington and Kassa, 2002).

That the Compared between the microscopic examination and molecular assay, microscopic examination of oocysts is not a suitable diagnostic method to be carried out on humans due to small number, isolation of organism from other tissues is also a difficult and time consuming procedure, therefore the molecular assay appear to be the method of choice (Coupe *et al*., 2005). The molecular assay had the advantage of being less time consuming and simpler to carry out , and did not require specialized equipment (Trisha *et al.*, 2012). Molecular method is possible to diagnose the factors quickly , reliably to identify subtypes and importantly to determine the source of and risk factors for an epidemic (Ramirez *et al*., 2004) . The sensitivity of the RT-PCR method is about 20 oocysts in 1 mL of stool sample (Lindergard *et al*., 2003).

As a result, after application of molecular assay, *C. parvum* oocysts were determined in 18 samples of the 100 feces samples (18%), microscopic examination is less sensitive and less specific than PCR analysis, this result is agreement with (Morgan *et al*., 1997; Morgan *et al.*, 1998; Rasha *et al.*, 2009; Amar *et al*., 2004; Quah *et al*., 2011). The explanation for these results is the fact that there are very few oocysts in the stool or oocysts may have been damaged before DNA extraction, and this could account for differences between PCR results and microscopic evaluation.

The results showed that the total infectivity rate of *Cryptosporidium parvum* by using microscopic examination (direct method, flotation method, sedimentation method and Ziehl-Neelsen) was (30%,36%,40%and 21%) respectively, these results are agreement with Abdulsadah *et al*., (2013) who recorded the high percentage of positive cases (100%) was in microscopic examination, and it was greatly different from the results of AL-Ta'ey, (1997) who got total infectivity rate 2.7% in Diala.

The differences in infectivity rate of *Cryptosporidium parvum* may be due to the following factors: the possible explanations for the discrepancy between the present and previous study finding might be the result of variation in sampling techniques used, variation in the environmental condition of the different study localities and different methods used for detection of Cryptosporidiosis.

CONCLUSIONS

The first study for diagnosis of *Cryptosporidium parvum* in children in Iraq by using Real–Time PCR, and molecular tool (RT-PCR) is a perfect method for detecting *C. parvum*.

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REFERENCES

1-Abdulsadah, A.R.; Magda, A.A. and Al-Charrakh, A.H.(2013).Prevalence of *Cryptosporidium parvum* among Children in Iraq. Am. J. Life Sci.,1(6): 256-260.

2-Akiyoshi, D.E.;Tumwine,J.K.; Bakeera-Kitaka, S. and Tzipori , S.(2006). Subtype analysis of *Cryptosporidium* isolates from children in Uganda.J.Parasitol., 92:1097-1100.

3-Akyon,Y.;Erguven,S.;Arikan,S.; Yurdakok, K. and Gunalp,A.(1999). *Cryptosporidium parvum* prevalence in a group of Turkish children. Turk. J. Pediatr., 41:189-196.

4-AL-Braiken, F.A.; Amin , A.; Beaching, N.J.; Hommel, M. and Hart, C.A. (2003). Detection of *Cryptosporidium* among diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia. An. Trop. Med. Parasitol., 97(5).

5-AL-Harthi , S.A. (2004) . Prevalence of intestinal parasites in schoolchildren in Makkah , Saudi Arabia. The New Egypt. J. Med., 31: 37-43.

6-AL-Gelany, B. A. (2003). An Epidemiological and Diagnostic Study of *Cryptosporidium* in the Man and Animal in AL-Thahab AL-Abiydh Village. Ph.D thesis, University of Baghdad. pp:192.

7-AL-Ta'ey,M.H.(1997).Study in the Epidemiology of Cryptosporidiosis in Diala Province. M.Sc. thesis,University of Baghdad. pp:67.

8-AL-Warid, AL-Saqur and Mahmood. (2010). Occurrence of *Cryptosporidium spp.* among People Live in North of Baghdad. Eu. J. Sci. Res., 78(4): 539-545.

9-Amar, C.F.L.;Dear, P.H.;McLauchlin, J. (2004).Detection and identification by real time PCR/RFLP analyses of *Cryptosporidium* species from human feces.Let.Appl.Microbiol.,38:217-22.

10-Baxby, D.; Blundell, N. and Hart, C.A. (1984). The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in feces. J.Hyg.,92:317-323.

11-Benson,J.H.(2002).Microbiological Application . 8th ed . MC. Graw. Hall., pp: 22-24.

12-Caccio, S.M.; Lalle, M.; Jimenez-Cardosa, E. and Pozio, E. (2005). Genotyping of Giardia duodenalis from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay. J. Parasitol., 91: 203-205.

13-Casimiro, A.M.; Carvalho, T.T.R. and Kanamura, H.Y. (2009). Serological evidence of *Cryptosporidium* infections in a group of pregnant women attended by the prenatal routine care at a public hospital in Sao Paulo (SP), Brazil. Rev. Panam. Infect., 11(2): 38-43.

14-Coles, E.H.(1986). Veterinary Clinical Pathology.4th ed.,W.B.Saunders Company, Philadelphia. Pp: 375-379.

15-Cook,G.C.(1987).Opportunistic parasitic infections associated with the acquired immune deficiency syndrome (AIDS): parasitology, clinical presentation , diagnosis , and management. Q. J. Med., 65: 967-983.

16-Coupe S,Sarfati C, Hamane S, and Derouin F.(2005).Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. J. Clin. Microbiol.,43 (3):1017-1023.

17-Fayer,R.(2004).*Cryptosporidium*:a water-borne zoonotic parasite.Vet. Parasitol.,126:37–56.

18-Fayer, R. (2008). General biology in: Fayer, R. and Xiao, L. editors. *Cryptosporidium* and Cryptosporidiosis. 2ed edition. Taylors and Francis Groups. USA. pp:1-42.

19-Gondim, L.F.; Gao, L. and McAllister, M.M. (2002). Improved production *of Neospora caninum* oocysts, cyclical oral transmission between dogs and cattle, and in vitro isolation from oocysts. J. Parasitol., 88: 1159-1163.

20-Hammond ,D. M. and Long, P.L. (1973). The Coccidia .1st ed. University Park press London.

21-Harrington BJ, and Kassa H. (2002).A comparison of an immunoassay with acid-fast staining to detect *Cryptosporidium* .Lab. Med.,6:451-454.

22-Henrikson,S.A.and Pholen, J. F. L. (1981).Staining of Cryptosporidia by amodified Ziehl– Neelsen Technique. Act. Vet. Scand., 22: 594– 596 .

23-Iqbal,J.; Hra, P. R.; Al-Ali, F. and Philip, R. (2001). Cryptosporidiosis in Kuwaiti children: seasonality and endemicity. Clin. Microbial. Infect.,7: 261-266 .

24-John , D.T. and Petri. (2006) . Medical Parasitology 9th edition. Elsevier. Inc., USA. pp: 463.

25-Koffi,N'Djeti,Konan and Djè. (2014). Molecular characterization of intestinal protozoan parasites from children facing diarrheal disease and associated risk factors in Yamoussoukro. Afr. J. Environ. Sci. Tech., 8(3): 178-184. 26-Lindergard, G.; Nydam, D.V.; Wade, S.E.; Schaaf, S.L.; Mohammed, H.O. (2003). A novel multiplex polymerase chain reaction approach for detection of four human infective *Cryptosporidium* isolates: *Cryptosporidium parvum*, types H and C, *Cryptosporidium* canis, and *Cryptosporidium* felis in fecal and soil samples .J.Vet. Diagn. Invest., 15: 262-67.

27-Meinhardt,P.L.;Casemore, D.P. and Miller, K.B.(1996).Epidemiologic aspects of human Cryptosporidiosis and the role of waterborne transmission. Epidem. Rev., 18: 118–136.

28-Mohammed N. Al-Shahery and Manal H.Hassan.(2007).immunological and experimental study for cryptosporidiosis. J. Iraq. Vet. Sci.,21(1):(140-129).

29-Mohanad M.M.(2008).The study of *Cryptosporidium parvum* dispersal in children less than five years old in Ramadi province. J. Al-Anbar Uni. Sci., 2(2).

30-Morgan,U.M.C.,Constantine,C.; Forbes, D. A. and Thompson, R. C. A. (1997). Differentiation between human and animal isolates of *Cryptosporidium parvum* using rDNA sequencing and direct PCR analysis.J.Parasitol.,83:825–830.

31-Morgan,U.M.;Pallant, L.; Dwyer, B.W.; Forbes, D. A.; Rich, G. and Thompson, R.C. (1998).Comparison of PCR and microscopy for detection of *Cryptosporidium parvum* in human fecal specimens: clinical trial. J. Clin. Micro., 36: 995–998.

32-Nahrevanian,H.;Assmar,M. and Samin, M. G. h.(2007) .Cryptosporidiosis among immunocompetent patients gastroenteritis in Iran: a comparison with other enteropathogenic parasites. J. Micro. Immun. Infect.,40:154-156.

33-Quah,J.X.;Ambu,S.;Lim,Y.A.L.; Mahdy, M. A. K. and Mak, J.W.(2011). Comparison study of Real-Time PCR and microscopy for detection of *Cryptosporidium parvum* in Diarrheic children in Al-Muthanna province. J., 138: 573–577.

34-Ramirez,N.E.;Ward,L.A.and Sreevatsan, S.(2004).A review of the biology and epidemiology of Cryptosporidiosis in humans and animals. Micro.Infect.,6(8): 5-773.

35-Rasha,H.Soliman and Ahmad, A. Othman.(2009).Evaluation of DNA Melting Curve Analysis Real-Time PCR for Detection and Differentiation of *Cryptosporidium* Species.P.U.J.,2(1):47–54.

36-Roberts, L. and Janovy, J.(2000). Gerald,D. Schmidt. and Larry, S. Roberts' Foundations of Parasitology,6th ed. McGraw.Hill,Massachusetts,USA.pp: 701.
37-Schares,G.;Pantchev,N.;Barutzki,D.; Heydorn,A.O.;Bauer,C.andConraths, F.J. (2005). Oocysts of *Neospora caninum*, *Hammondia heydorni*, *Toxoplasma gondii* and *Hammondia hammondi* in faeces collected from dogs in Germany.Int.J. Parasitol.,35: 1525–1537.
38-Sears, C.L. and Kirkpatrick,B.D.(2001). Cryptosporidiosis and Isosporiasis. in:Gillespie, S.and Pearson ,R.D.editors. Jhon Wiley and Sons L.t.d., New York. Princ. Prac. Clin. Parasitol., pp:139-164.
39-Stephen , J. Hadfield ; Guy Robinson; Kristin Elwin and Rachel, M.Chalmers. (2011). Detection and Differentiation of *Cryptosporidium spp.* in Human Clinical Samples by Use of Real-Time PCR. J. Clin. Microbiol., 49(3): 918-924.
40-Sturde, A.P.; Chalmers, R.M. and Bull, S.A. (1999).Detection of *Cryptosporidium* oocysts in wild mammals of mainland Britain. Vet. Parasito., 80: 273-280.
41-Sorlie,D.E.(1995).Medical biostatistics and epidemiology:Examination and Board review.1st ed.Norwalk,Connecticut, Appleton and Lange.pp: 47-88.
42-Sulaiman,I.M.; Hira ,P.R.; Zhou,I.; Al-Ali,F.M.;Shelahi,F.A.;Shweiki,H.M.; Iqbal,J.*et al*.(2005).Unique endemicity of Cryptosporidiosis in children in Kuwait .J. Clin.Microbiol.,43:2805-9.
Cryptosporidium parvum: Structural Components of the Oocyst Wall . J. Robin Harris and Franz Petry.J. Parasito.,85(5): 839-849.
43-Trisha, J. R.; Elizabeth, A. C.;Charlott, T. and Kirk, E.S.(2012).Evaluation of the positive predictive value of rapid assays used by clinical laboratories in Minnesota for the diagnosis of Cryptosporidiosis. Oxf. J. ,50:1-.

دراسة مقارنة بين تقنية تفاعل سلسلة البوليمر الحقيقي والفحوصات المجهرية لتشخيص طفيلي *(Cryptosporidium parvum)* في الاطفال المصابين بالإسهال في محافظة المثنى-العراق

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الخلاصة

هدف الدراسة الحالية لعمل مقارنة لانتشار طفيلي *Cryptosporidium parvum* بين الاطفال بواسطة الفحوصات المجهرية التقلدية والمتضمنة (الطريقة المباشرة، التطويف، الترسيب وصبغة الزل نلسن اضافة الى تقنية تفاعل سلسلة البوليمر الحقيقي RT-PCR. الدراسة اجريت في محافظة المثنى للفترة من تشرين الاول 2013 الى مايس 2014 في مستشفى الحسين التعليمي ومستشفى الرميثة العام ومستشفى الولادة والاطفال في مدنية السماوة والمراكز الصحية في مدنية الرميثة. من مجموع 100 عينة براز اخذت من الاطفال بعمر 1 شهر– اقل من 10 سنوات الذين يعانون من الاسهال الحاد والمستمر والتي تم فحصها بواسطة الفص المباشر وطرقة التطويف والترسيب وصبغة الزل نلسن اضافة الى فحص سلسلة البوليمر الحقيقي RT-PCR. الدراسة الحالية سجلت نسب الاصابة بطفيلي *Cryptosporidium parvum* في 100عينة براز والمفحوصة بطريقة الفص المباشر وطرقة التطويف والترسيب وبنسبة 30% ،36% ،40% على التوالي. بينما عينات البراز المفحوصة بصبغة الزل نلسن ظهرت نسب الاصابة 21%، حيث ظهرت اكياس البيض المفحوصة بالصبغة على شكل اجسام ارجونية ذات خلفية زرقاء مع هالة واضحة حول الكيس. بينما عندما تم فحص العينات بواسطة فحص سلسلة البوليمر الحقيقي RT-PCR وجدت نسب الاصابة 18%. الدراسة الحالية اظهرت نسب عالية من الاصابة بالطفيلي عندما تم فحص العينات بالفحوصات المجهرية بينما بفحص سلسلة البوليمر الحقيقي RT-PCR وجد نسب الاصابة 18%.