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Detection of *Cryptosporidium parvum* in human by Conventional methods in AL-Qadisiyah Province

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

مَا كَانَ لِبَشَرٍ أَنْ يُؤْتِيَهُ اللَّهُ الْكِتَابَ وَالْحِكْمَ وَالنَّبُوءَةَ
ثُمَّ يَقُولَ لِلنَّاسِ كُونُوا عِبَادًا لِي مِنْ دُونِ اللَّهِ
وَلَكِنْ كُونُوا رَبَّانِيِّينَ بِمَا كُنْتُمْ تُعَلِّمُونَ الْكِتَابَ وَبِمَا
كُنْتُمْ تَدْرُسُونَ

صدق الله العظيم

سورة آل عمران (٧٩)

DEDICATION

To ... the Prophet Mohammed and

The family of the Prophet (Ahleelbait)

The Word the piety and cresset true religion

To My beloved Father

To The flow of affectionateness My Mother

To My Brothers and Sister

To All my friends

I Present My Modest effort

.....Haider and Yasser

ACKNOWLEDGMENT

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Haider and Yasser



SUMMARY

Summary

The present study was conducted during the period from September 2016 until February 2017 , 50 fecal samples was collected from human in different age and from both sexes, the sample collected from different regions in AL-Diwianyah teaching hospital, AL-Belad medical lab, Baghdad medical lab and AL-Zawraa clinic . The study designed to evaluate the microscopic features of the parasite by used acid fast stain .

The results of microscopic examination showed that the oocyte of parasite appeared oval-shaped or spherical objects with a color dark pink, or red on a blue ground and the results of the current study recorded the percent of infection to the *Cryptosporidium* is(22%) 10 positive samples out of 50 samples also the results showed that the highest rate of infections (29.41 %) was observed in the ages (1-5year) and highest rate of infection in male (22%) while the female show lowest infection (15.78%). Also the result depend of months of the year are ranged between (0%-27 %).

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CHAPTER ONE
INTRODUCTION

Introduction

1-1 Introduction

Cryptosporidiosis represents the public health concern of water utilities in developed country (Fayer *et al.*, 2000). Transmission of *Cryptosporidiosis* is through ingestion of oocysts from the infected individuals by contaminated food, water and pasture (Fayer *et al.*, 2000; Rose *et al.*, 2002). Currently there are more than 50 genotypes of *cryptosporidium* (Ryan *et al.*, 2005; Santin *et al.*, 2007).

Cryptosporidiosis is a major cause of diarrhea in developing countries and generally causes self-limited watery diarrhea in immunocompetent patients or chronic severe diarrhea in immunocompromised individuals (Iqbal *et al.*, 1999, 2001). Previous studies in various tropical countries have shown that children of 2 years of age are the most susceptible to *Cryptosporidium* infection, with the reported incidence ranging from 1.1 to 18.9% (Ajjampur *et al.*, 2007, 2010; Xiao *et al.*, 2001).

The protozoan parasite protozoa from a single organism cell Unicellular ,Eukaryotic cell, one of the pathogens that are not less important than the etiology biogenic other bacteria, fungi, viruses, and includes protozoa numerous types of organisms, as he found about 70,000 species of primary parasites that infect humans and animals in various ways such as drink and food containing the infectious parasite Infective stage (Dalmaso *et al.*, 2011; Ayeh-Kumi *et al.*, 2009).

Cryptosporidium spread all the world, especially in poor areas and third world countries, especially among children (Feng & Xiao, 2011; Stanley, 2003), *Cryptosporidium* causing infection to human and case health problems, including anemia , tissue damage, diarrhea and general weakness (Al-Mohammed *et al.*, 2010).

C.parvum small-sized parasites is slightly smaller than a red blood cell (4-5) milli microns and has a wide range of affects humans and animals so it is one of the causes of common diseases Zoonotic disease,

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(Schmidt *et al.*, 2000; Jones *et al.*, 1997) . *C.parvum* life cycle in the small intestine and causes Cryptosporidiosis who became difficult health problems all over the world as causes inflammation of the stomach and intestines ,they infect disease and this has recently attracted much attention from researchers because of the wide spread easily and move to through drink and food contaminated as well as its transmission mediated ,insects and rodents to humans (El-Hamshary *et al.*, 2008; Noordeen *et al.*, 2002; Guerrant, 1997).

The parasite causes diarrhea specially with , Rota Virus Escherichia coli (Udaya & Prakash, 1997), are contracting this parasite accompanied by inflammation of the intestines as it results in watery diarrhea and pain in the abdominal area and high temperature, nausea or vomiting , Cryptosporidiosis in its resistance to many medicines and sterilizers and disinfectants (Lowery *et al.*, 2000).

1-2 Aim of the study

- 1- study the prevalence of *Cryptosporidium parvum* in human by using conventional method in AlQadisiyah province .
- 2- Studied the effect of some factors like age , sex and months on infection rate of the parasite .



CHAPTER TWO
REVIEW OF LITERATURES

Review

2.1 Historical Background:

Cryptosporidium was described at late of ninety century as one of causative agent of diarrhea , Clark in (1895) was first noted description of *cryptosporidium* in gastric mucosa of mice as motile merozoite (swarm spores) for *C.muris* . After 12 years Tyzzer in 1907 describe the life cycle of protozoa parasite and found it in fecal and gastric gland in mice .

In 1910 Tyzzer noted great details in shape of *C. muris* and in 1912 he describe development stage in small intestine and shedding of oocyst smaller than oocyst of *C. muris* which is later known as *C. parvum* as a new species (Tyzzer ,1912). In 1955 new species was reported it as *C. melegratas* in turkey and in 1971 considered as reason of bovine diarrhea (Panciera *et al* .,1971). In 1974 Barker and Carbonen discovered first case in sheep .In 1982 the *cryptosporidium* considered the causative agent of severe diarrhea and have a risk for life especially people who have immune depressed, it increase important when AIDS distribution , and it is increasingly important when it is named (Traveler's diarrhea) because it is transport in word by people (Shinta *et al* ., 1994). *Cryptosporidium* is considered more important when it considered the cause of gastritis and intestinalis company with diarrhea in all animal and human (Pettollo-mantovani *et al* .,1995) *Cryptosporidiosis* was first described in lambs with diarrhea in Australia, but no causative role could be ascribed to the organism because of the coincidental infections with pathogenic bacteria. Its role as a primary etiological agent of diarrhea in lambs was confirmed in the early 1980 in the studies on natural and experimental infections (Angus, K.W *et al* .,1982; Snodgrass, D.R *et al* ., 1984).

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Many researchers appear parasite's ability to cause disease in a human being, causing satisfactory to the animal only until 1976 when it recorded its first case in the girl 3.5 years in the state of Tennessee suffer from acute intestinal, also got an increase and wide in the incidence of illness Cryptosporidiosis when the spread of acquired immune deficiency syndrome (AIDS) has become a disease epidemic was a close correlation between the disease Cryptosporidiosis status of immune infected in early 1980 a large impact in the parasite transfer to the forefront as one of the pathogenic to humans factors (Anon, 1984 Fayeretal., 2000; Naghibi & Vaheid, 2002;; Leav et al., 2003).

2.2 Classification :

The genus of cryptosporidium has been classified as shown in (Finch and Belosevic,2002 ; Gabriel, 2010).

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Apicomplexa

Class: Sporozoa

Subclass: Coccidiorida

Order: Eucoccidiorida

Suborder: Eimeriorina

Family: Cryptosporidiidae

Genus: *Cryptosporidium*

Species: *Parvum*

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2.3 Species and host range :

One major reason for the long disputes in *Cryptosporidium* taxonomy is the difficulty in fulfilling the definition of biological species. The classical definition of species as groups of interbreeding natural populations reproductively isolated from other groups is difficult to apply to many organisms like *Cryptosporidium*, because it is very difficult to conduct genetic crossing studies with many *Cryptosporidium* spp. Even though *Cryptosporidium* has a sexual stage and intra-species sexual recombination has been demonstrated in *C. parvum* (Feng *et al.* ,2002, Mallon *et al.* ,2003), the huge reproductive potential of the parasite results in vast numbers of genetically similar parasites in localized areas. Therefore, mating in *Cryptosporidium* normally occurs between siblings. As a result, *Cryptosporidium* has a large bias toward a clonal population structure, as demonstrated by multilocus analysis (El-Kariem, 1999).

Currently, morphology, especially oocyst measurements, represents the cornerstone of apicomplexan taxonomy. Measurements allow microscopists to identify large numbers of genera and morphologically distinct species, and the importance of a good morphologic description cannot be understated. Therefore, oocyst structure is usually one of the requirements for establishing a new species. However, for *Cryptosporidium*, morphology is not adequate by itself and should not be the sole criterion for naming a new species (Fayer , 2010).

Oocysts of many species are virtually identical in size, and similarities in oocyst structure have even caused confusion about the historical validity of several *Cryptosporidium* spp. (Xiao *et al.* ,2004).

More than twenty species of *Cryptosporidium* have been described from a variety of vertebrates (the parasite has been identified in greater than 150

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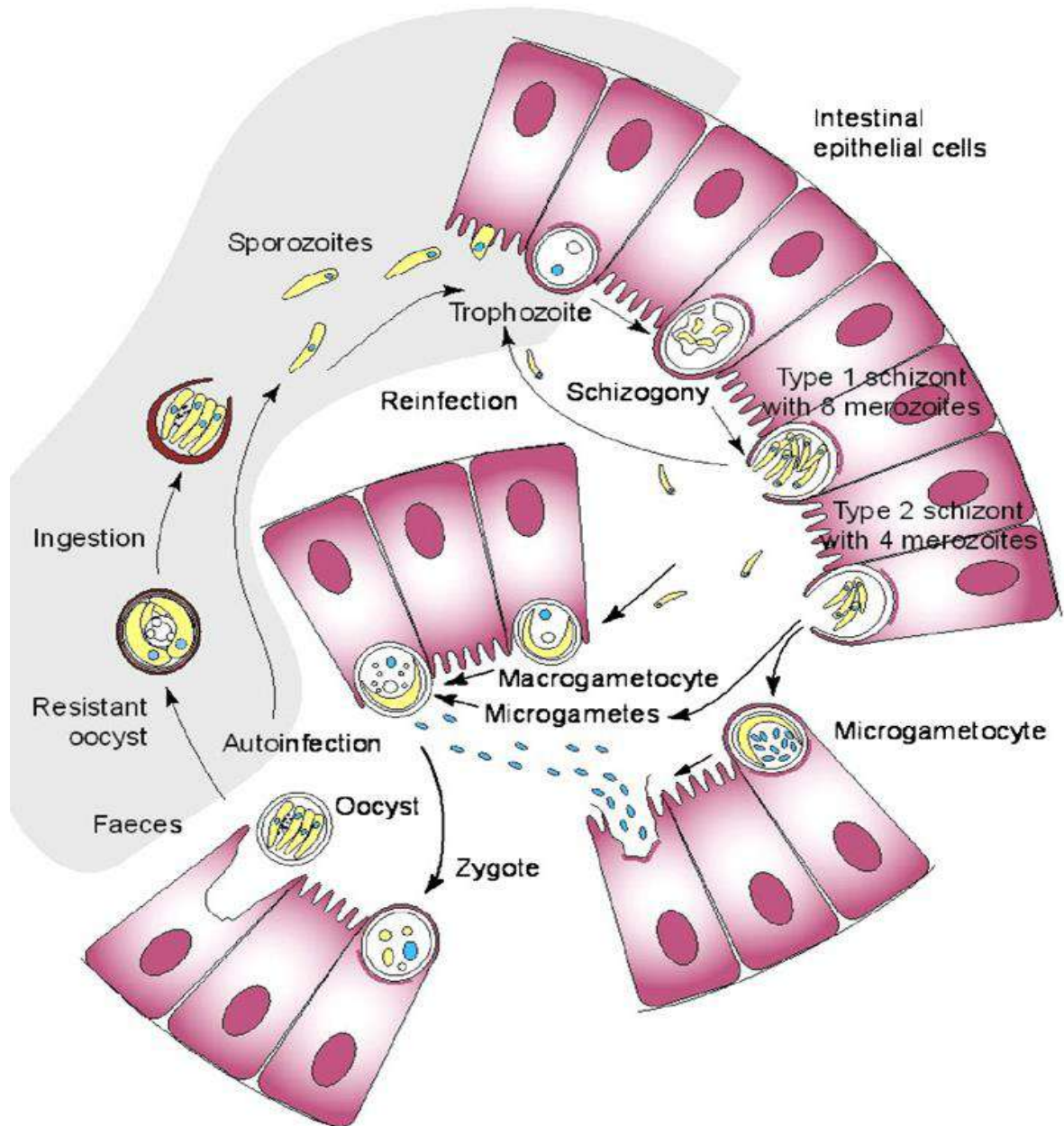
vertebrate hosts) including mammals, birds, reptiles and fish (John & Petri, 2006; Bowman & Forester, 2010; Feng, 2010; Power, 2010).

2.4 Life cycle :

Cryptosporidium life cycle is complex and has sexual and asexual reproductive stages, it occurs completely in the gastrointestinal tract of the host, it is a direct, monoxenic life cycle, the start of infection happens by ingestion of infective thick-walled oocysts, the oocyst is transmitted by fecal-oral route and by direct contact (host-to-host) or indirectly (contaminated water and food). Oocysts have resistance to disinfectants used in treated water such as chlorine, the water represents an important transmission route to all types of animals (Fayer, 2004). The life cycle takes 24-72 hours to complete, the pre-patent period from (3-10) days depends on several factors some depend on the host, individual resistance, age, sex, strain and immunity or depend on the parasite like virulence of strain and site of infection (Hornok *et al.*, 1999; Casemore, 2000). The epithelial cells of the distal jejunum, caecum, colon and ileum are invaded by sporozoites, within the epithelial cell each sporozoite transforms into a trophozoite retained within the membrane called parasitophorous vacuole just below the cellular membrane, the parasite remains extra-cytoplasmic. Trophozoites go to asexual cycle (schizogony) and develop into type I meronts, then release merozoites into the intestinal lumen to infect new epithelial cells. There are two ways to a new asexual cycle or into type II meronts and go to sexual cycle (Fayer *et al.*, 2005). Meront type II contains four merozoites after release and infection of new epithelial cells they develop to male microgamete or female macrogamete, the microgamete releases microgametes that fertilize the macrogamete that produces zygotes which develop into oocysts, the oocyst is released from the epithelial cell, almost 80% of oocysts have thick

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wall and exit from the host by faeces , the oocyst with thin wall can turn and exist in the same host and cause auto-infection (Feltus *et al.* , 2006).



(*Cryptosporidium* Life cycle Smith *et al.* ,2007)

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2.5 Pathogenesis :

Cryptosporidium infection different in severity in intestinal canal for many factors which are ferocity of strain and susceptibility of parasite to invasion, its ability to invasion, secretion of enterotoxin, this factor contact with another factor which is age of host ,individual variation and natural resistant and others like environment and management (Muir *et al* .,2000). Studies proved present of parasite on the epithelial cell make change in it shape and position of it nucleus (Tzipori ,1988) .Lead to death of epithelial cell (Agnew *et al* ., 1998) .

The area of strong contact between the parasite and the infected cell cytoplasm has effect to prevent drift the parasite with intestinal fluids (Soave and Armstrong , 1986) . That lead to damage of mucous layer and villi atrophy lead to malabsorbtion (Mahdi *et al* ., 1996) .

Tzipori (1988) and Gerba *et al* (1996) explain the effect of the infection on metabolism by effect on the lactose that secreting from intestinal cell that responsible for conversion disaccharide to monosaccharide that lead to absorption the water from the blood and tissue to ward intestinal lumen causing diarrhea .

2.6 Clinical sign :

This parasite known from veterinarians as the most important case of diarrhea in many kind of mammalian and small animal (Tzipori,1983) Symptoms deferent from patient to anther depend on the immune state of host and severity of infection and its impact on the epithelial cell in mucosa layer , the diarrhea is most impotent symptoms that noted on host (Davies and Chalmers ,2009)

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2.7 Epidemiology:

Cryptosporidium disease with high epidemiology is taken for several reasons :

- 1-lack of species specificity (Chalmers *et al* .,2009) .
- 2- ability to consummation entire it life cycle in single host (Fayer *et al* .,2000).
- 3- not specific for tissue or organ (Meinhard *et al* ., 1996).
- 4-mode of transmission (direct –faecal oral),(person –to- person) (Muraleedharan ,2009) and other method for transmission including sputum and vomits (Dupont *et al* .,1996) and indirect environmental reservoirs lead to wide range of suffusion (Meinharder *et al* ., 1996).
- 5- the dose size of 10-100 oocyst (Percival *et al* ., 2004) .
- 6- lack of specific completely effective treatment (Sockdale *et al*.,2008).
- 7- oocyst can resiste harsh condition for 3 month and resistant to disinfectants and resistance to chlorine which use to sterilization of water (Fayer ,2004) . Oocyst maintains vitality for 60 days in water and for 4-6 month in 4c° in 2.5% in solution of chromate potassium (Dworkin *et al* .,1996; Radostits *et al* .,2007) .Otherwise oocyst affected on freezing and drying ,it is perish in less than 0c° and more than 65c° (Manjini *et al* ., 1992) . Oocyst when exposed to uv light for 150 minute not be infected to laboratory animal (mice) (Lorenzo – lorenzo *et al* ., 1993).
- 8- ability of oocyst infection immediately after it exit with faces infected patient (Casemor ,2000) .

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2.8 Transmission

Studies have shown that contaminated water with high degree of seriousness of risk for *cryptosporidiosis* (Castro –Hermida *et al* .,2009 ;WHO,2009). Drinking water even from treated water can case sickness when it contain enough number of oocyst to *cryptosporidium spp* ,which it can survive for up to 1 year at 4c° in artificial sea water (Tamburrini and Pozio ,1999) .

Radostits *et al* in 2007 proved role of mice and rat for contamination food by it faces. The dog can put away oocyst for 40 days and flies and beetles important role in transmission of oocyst (mechanical transmission) and found the oocyst in the digestive system of bugs (Graczyk *et al* ., 1999). Fayer *et al* in 1998 were proved that oocyst was found in water sea and snail which is consider as mechanical transmission to parasite.

The unpasteurized milk consider source of infection to human (Herper *et al* .,2002) . The people who breeding the animal is more rate to infection (Ungar ,2000) .the veteran was infected during the treating the animal and deal with it, or the researcher during the conduct scientific experiment (Nouri and Toraghi ,1991) .

2.9 Diagnosis:

1- Clinical Sign

The main clinical sign is watery diarrhea which will be green to yellow with amount of mucous (Radostitis *et al* .,2007) but there is a lot of causative agent which is case diarrhea so diarrhea not consider the final diagnosis (Anderson and Bulgin ,1981; Nath *et al* ., 1999).

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2- Laboratory diagnosis

a-Gross examination :- which depends on notice the odor, color and consistence of feces

b- microscopic method

1-direct method :- conducting examination of feces not enough to diagnosis because smaller oocyst and it similar to yeast so for that is used in other methods (Crawford and Vermund, 1988).

2- staining method :- attend the swabs light and permanent pigmentation one of the following :-

a- modified Ziehl Nelsen stain (Current and Garcia, 1991).

b- Giemsa stain (Ogatto *et al.* 2009, Garcia *et al.* , 1983).

c- Auramine and Rhodamine stain (Goddard *et al.* ,2000).

d- Gram stain (Ogatta *et al.* ,2009).

e- Trichrome stain (Ignatius *et al.* , 1997).

f- Acridin orange (Ignatius *et al.* , 1997).

g- Safranin – methylene blue (Baxby *et al.* ,1984).

3- Concentration

A- Flotation

1- Sheather's sugar solution mostly important method use for flotation (Current and Garcia, 1991)

2- NaCl flotation solution make by smelting 360 g of sodium chloride in 640 ml of distill water (Kuczynska and Shelton, 1999)

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3- Zinc – sulfate flotation make by smelting 703 g of zinc sulfate in 297 ml of distill water (Ma and Soave , 1983).

B –Sedimentation

1-the best method use it by ether and water that material not affection on vitality of the parasite (Casemore , 1991).

2- formalin 10% with ether , centrifuge at 1000 r / min for 10 minute (Markell *et al .*, 1999).

3- formalin 10% with potassium dichromate 2.5%,centerfuge 500 r/min for 10 minute (Garcia *et al .*, 1983).

4- Immunological methods

a- Latex agglutination test :- by investigating antibodies Igm, IgG,IgA (Casemore , 1991)

b- Direct immunofluorescence test :- this method depends on using oocyst antigen in fecal sample , this test gives adjusted results (Xiao and Herd ,1993).

c- Enzyme linked Immunosorbent Assay (ELISA) antigen detection Elisa test for diagnosis the infection for detection of *cryptosporidium* antigens in feces , used commercial kits contain ant-*cryptosporidium* antibodies (Smith,2008)several serological surveys had report that more than 50% of person without any infection but have anti-*cryptosporidium* IgG, suggesting recent exposure to parasite (Ungar *et al.*, 1994; Koch *et al .*,1995).

5-Histological Examination

Gastrointestinal biopsy had taken from death of infection animal , the section prepare and stain by Hematoxylin and Eosin stain than the

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development stage of parasite in the villi of gastrointestinal under light and electron microscopic this technique used for research purpose only (Sherwood *et al.*,1982;Casemore,1989)

6-Histologica culture

This method use in vitro , it describes the detect drug effect by culture the parasite in dog kidney media (Gud,1991) or pig kidney media (Rosalas *et al.* ,1993)

7- Molecular diagnosis.

Polymerase Chain Reaction (PCR)

It is a dependable scientific technique used in molecular biology to amplify a single or few copies of a DNA fragment across several orders of magnitude generating thousand to millions of copies of a certain DNA sequence. This technique has been developed since 1984 by the American biochemist, Kary Mullis (Bartlett and Stirling, 2003)

2.10 Treatment:

Immunocompetent individuals typically recover without intervention (Chalmers & Davies, 2010). Treatment of cryptosporidiosis may involve oral or intravenous liquid to replace water and electrolytes if patient are suffering from dehydration (Fayer & Ungar ,1986 ;Rossignol ,2010). Re-establishing a healthy immune system is the most effective method for combating chronic cryptosporidiosis in immunocompromised patients (Chalmers & Davies, 2010).

However , this may be difficult or impossible for severely immunocompromised individuals. Antiretroviral therapies for AIDS patients , if successful in restoring the immune system (increase CD4

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counts) , can led to recovery from cryptosporidiosis (Rider Jr. & Zhu, 2008) . Nitazoxanide (NTZ) is currently the only drug available in the United State market for Cryptosporidiosis, and this drug belongs to the thiazole family and is reported to display broad spectrum activity against helminthes, several anaerobic microorganisms, and some viruses. NTZ rarely causes any sever side effect patients. However, it could have negative effects on commensal gut microbes. NTZ is believed to target pyruvate-ferrodoxine oxidoreductase in some organisms, but may have additional mechanisms of action in others.

Paromomycine has been used experimentally to treat cryptosporidiosis in AIDS patients with several trial given different results.

Other drugs , including some antiretroviral agents , have been tested against cryptosporidiosis with some promising results .however NTZ and paromycine remains as the two most well recognized treatment option. Additionally, anticoccidia drugs have been tested or used in animals, but their utility remains unclear (Rider Jr. & Zhu, 2008).



CHAPTER THREE
MATERIALS AND METHODS

Materials and Methods

3. Materials and Methods

3.1 Materials

3.1.1- Chemical materials

Table (3-1) chemicals with their companies and origin :-

No.	Chemical	Company and Origin
3	Carbol fuchsin	Syrbio (Syria)
7	Methanol 100%	BDH (England)
8	Methylene blue	Syrbio (Syria)
9	Normal Salin	Haidylena (Egypt)
10	Oil immersion	BDH (Englan
13	Hydrochloric Acid 100%	BDH (England)
15	Xylol	BDH (England)

3.1.2-Instuments and Equipment:-

Table (3-2) Instruments and equipment with their companies and countries of origin

No.	Equipment & instrument	Company
2	Compound light microscope	Olympus (Japan)
3	Digital camera	Samsung/ china
4	Disposable syringe 10 ml, 5ml and 3ml	Sterile EO. / China
13	Plastic Containers	Kaeaho(Russian)
16	Slides and cover slipes	Superestar(India)
17	Sterile test tube	Superestar/ India
21	Wach timer	Japan

Materials and Methods

3.1.3-Solution

Acidic Alcohol

This solution was prepared by adding 3 ml of concentrated HCL to 97 ml of ethanol 95 % (Coles, 1986).

3.1.4-Stain

a- Modified Ziel -Neelsen stain

It was prepared according to Beaver and Jung (1985).

1-Basic carbol fuchsin	4gm
2-phenol	8 ml
3-Ethanol	20 ml
4- distal water	100 ml

Preparation

it was prepared by

prepare solution A

4 gram of basic carbol fuchsin was dissolved in 20 ml of ethanol 95%

prepare solution B

dissolve 8g from phenol in 100 ml of distal water with continuous movement until it completely dissolving than add solution B to solution A than mix it to gather until the stain be ready

b-Methylene Blue :- The stain was prepared by dissolved 1g of the stain in 100 ml of distal water (Levine ,1961).

Materials and Methods

3.2-Method

3.2.1-Sample collection and experimental design

a- Sample collection

Stool samples were collected randomly from 50 of human, in different age, from both sexes during the period from the beginning from September to the end of February, the study involved different regions in province of AL-Qadisiyah, Diwaniyah Teaching Hospital, AL-Belad medical lab, Baghdad medical lab, Al zawraa clinic/Al jazaer.

All samples were collected in clean plastic containers (100 ml) and tightly closed and labeled it with writing the number, age, region and sex with taking of protective measure such as wearing disposable gloves and change in each sample to avoid contamination, the samples were transported in refrigerated bag to the laboratory which belongs to college of medicine- university of AL-Qadisiyah, then kept cool until the sample examined.

3.2.2-Laboratory test

3.2.2.1-Microscopically examination

The oocyst detected by examination each sample by pigmented fecal sample by (MZN stain)

(A) preparation of fecal smears and staining by MZN

a- a small amount of feces was mixed in tube with distilled water than filter it with 4 layer of gauze than collection the leaky in test tube, then use centrifuge for 1000 rpm for 5 min then spread over all the slide and let it to dry for 10 minutes (label the slide with sample number)

b- the smear was fixed in concentrated methanol 100% for 5 minutes to let it dry

c- the slide were staining with red strong carbol fuchsin for 3 minutes

d- the slide washed by tap water

e- used acid alcohol for decolorized red color for 30 seconds and then the slide washed in tap water

Materials and Methods

f- methylene blue was used as a counterstain for 2 min than rinsed in tap water and dry

g-the slide examined to find the oocyst by scanning use microscopic in 40X objective lens of microscopic than in oil immersion objective lens 100X (Bearever and Jung ,1985).

3.2.3 statistical analysis:

The results of the present study were analyzed by SPSS program (version) software 2010, using Chi-square test(X^2) and P values of $p \leq 0.05$ were considered to record statistical significance (Leech *et al.*, 2011).



CHAPTER FOUR
RESULTS

Results

4- Results

4-1 Results of Microscopically Examination

Diagnostic characterization of *cryptosporidium parvum*.

Cryptosporidium parasite was seen in human and when they are examined under high oil emersion (100×) lens of light microscopic as in figure(4-1)

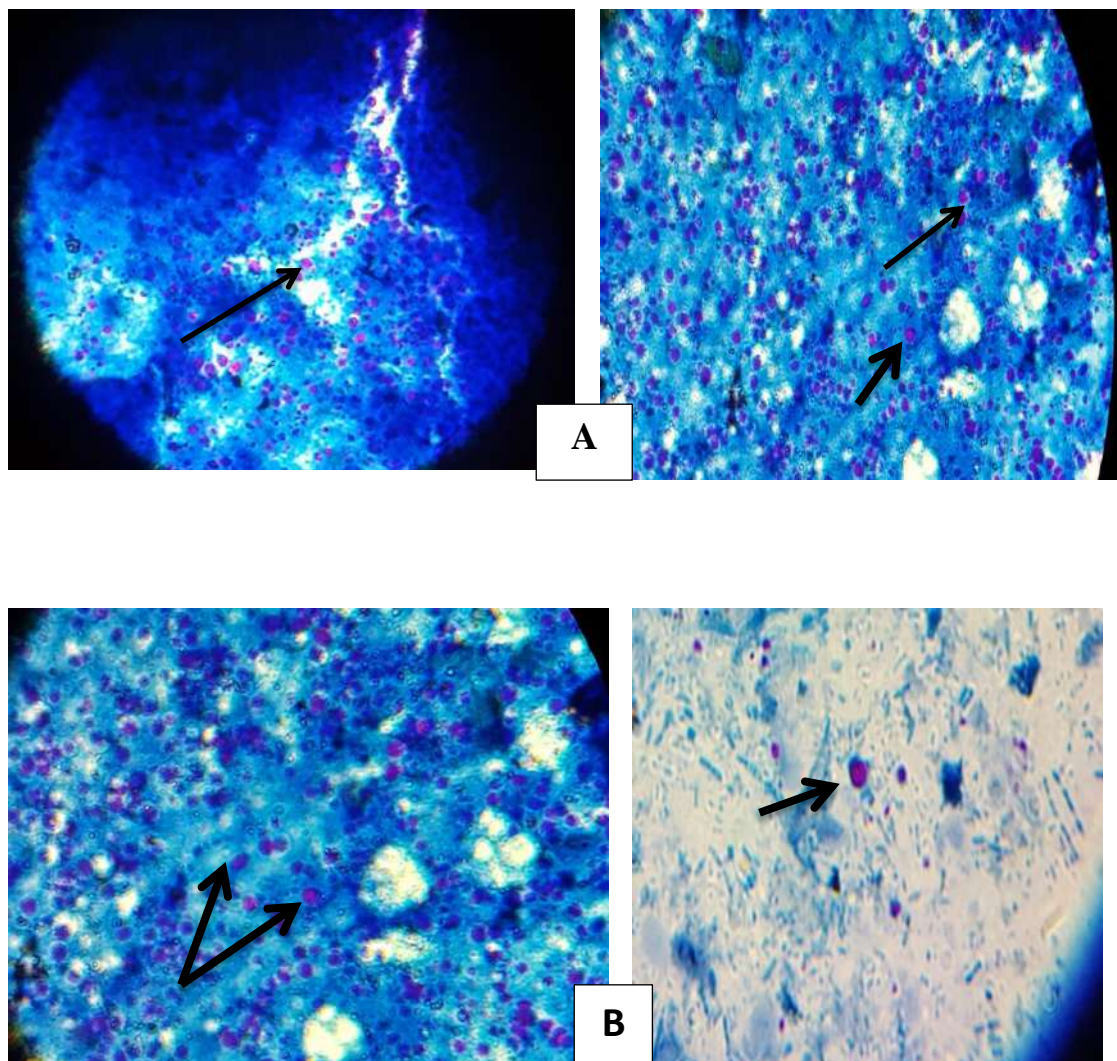


Figure (4-1)A and B show *cryptosporidium parvum* oocyst stained with M Z N

Results

4-2 Prevalence of *Cryptosporidium* in human according to the age:

The ages of human were divided into four groups which were, from (1month-1year), from (1-5) years, from (6-10) years and up (>10), years. The results showed that the highest rate of infections (29.41 %) was observed in the ages (1-5year).

Table (4-1) Prevalence of *Cryptosporidium* according to age

Ages	No.	Positive	Percentage
1month-1year	16	4	(25 %) ^b
1-5 year	17	5	(29.41 %) ^a
6-10 year	6	1	(16.67 %) ^c
>10 year	11	0	(0 %) ^d
Total	50	10	20%

Differences in small letters, vertically, referred to significant differences at level of $P \leq 0.05$

4-3 Prevalence of *Cryptosporidium* in human according to the sex:

Our results showed that the highest rate of infection in male (22%) while the female show lowest infection (15.78%)

Table (4-2) Prevalence of *Cryptosporidium* according to sex

Sex	No.	Positive	Percentage
Male	31	7	(22.58 %) ^a
Female	19	3	(15.79 %) ^b
Total	50	10	20%

Differences in small letters, vertically, referred to significant differences at level of $P \leq 0.05$

4-4 Prevalence of *Cryptosporidium* in human according to type of stool

Our results show highest infection in diarrhea stool which is (22.22%) and the lowest infection in solid stool .

Table (4-3) Prevalence of *Cryptosporidium* according to type of stool

Stool	No.	Positive	Percentage
Solid	23	4	(17.39 %) ^b
Diarrhea	27	6	(22.22 %) ^a
Total	50	10	20%

Differences in small letters, vertically, referred to significant differences at level of $P \leq 0.05$

Results

4-5 Prevalence of *Cryptosporidium* in human according to study's area:

The highest rates of infection were (60%) that observed in Women's and pediatric teaching hospital while have no positive collected from Baghdad medical lab

Table (4-4) Prevalence of *Cryptosporidium* according to study's area

Regions	No.	Positives	Percentage
Al- Diwaniyah teaching hospital	10	2	(20 %) ^b
Women's and pediatric teaching hospital	10	6	(60 %) ^a
Al-Belad medical lab.	10	1	(10 %) ^c
Al-Zawraa clinic	10	1	(10 %) ^c
Baghdad medical lab.	10	--	(0 %) ^d
Total	50	10	20%

Differences in small letters, vertically, referred to significant differences at level of $P \leq 0.05$

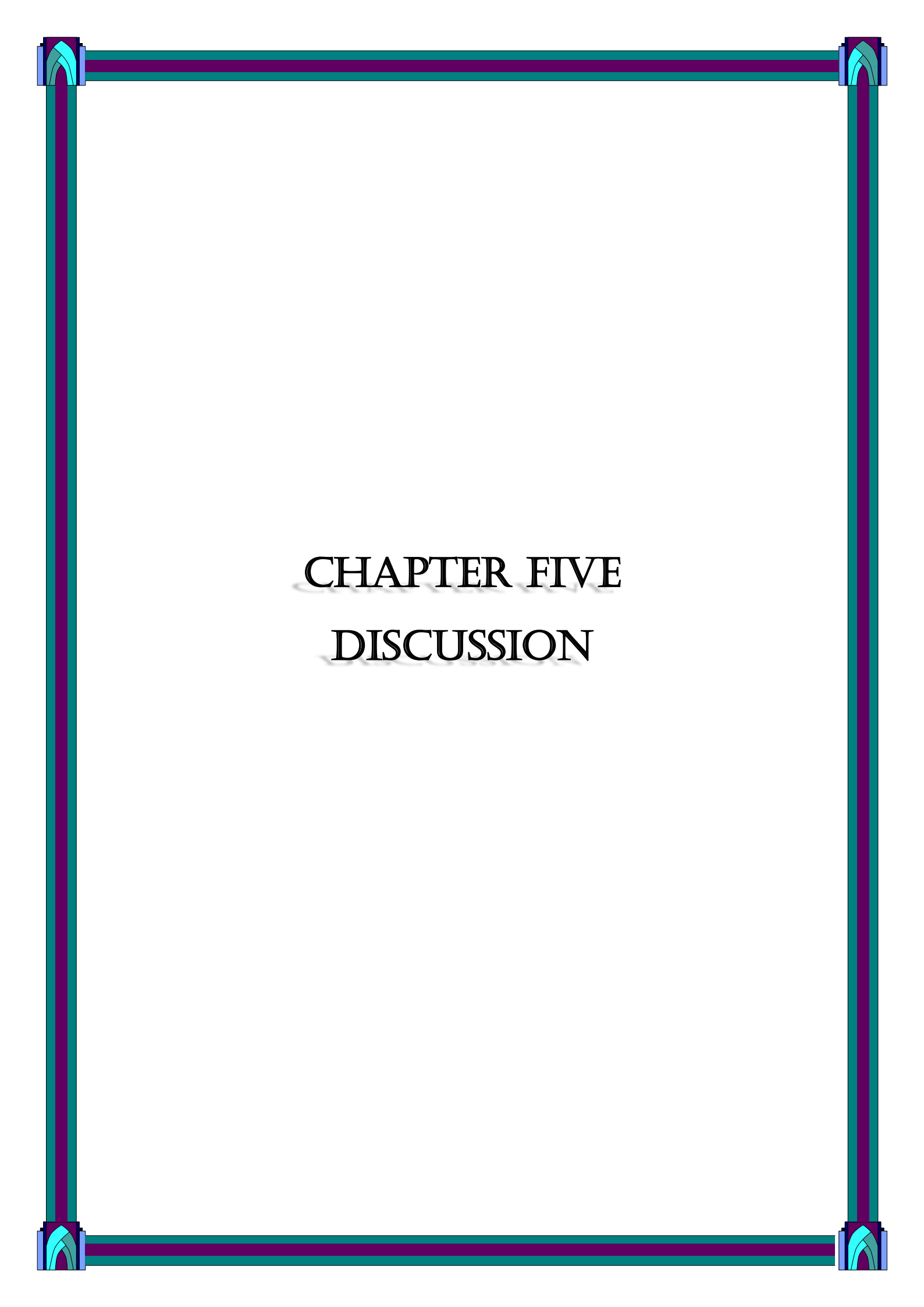
4-6 Prevalence of *Cryptosporidium* according to the month of study:

According to the results, it was observed that the rates of infection in a different study months were relatively different, and ranged between (0%-27 %), and the months from September toward February showed different results

Table (4-5) Prevalence of *Cryptosporidium* according to the month of study

Month	No.	Positives	Percentage
September	2	-	(0 %) ^d
October	5	1	(20 %) ^b
November	10	1	(10 %) ^c
December	10	2	(20 %) ^b
January	11	3	(27 %) ^a
February	12	3	(25 %) ^a
Total	50	10	22%

Differences in small letters, vertically, referred to significant differences at level of $P \leq 0.05$



CHAPTER FIVE
DISCUSSION

5-1 Discussion

The results of the current study recorded the percent of infection to the *Cryptosporidium* is 22% of 10 positive samples from total 50 samples examined microscopically using pigment ZN that this percentage is a health risk in the province has been attributed to several reasons, including poor health awareness among children and their families as well as the lack of services municipal and health in the province, which which accordance with the results of Bakr (2005) who recorded the percent of 17.0% after examining 470 sample for children aged less than one year to seven years in the province of Nineveh, and also agreement with Khalil & Dawood (2007) who reported the percent of infection 15.23% after examining 302 samples faces of children in the province of Mosul, also Al-Alousi & Mahmood (2012) who recorded the 18.9% after examining 92 sample of children suffer from diarrhea ages (one month - 12 years) in Mosul, and in conformity with the findings of the al-Bayati (2013) in his study which included physiological changes in children with certain parasites intestinal in Diwaniyah province where the percentage of infection parasite *Cryptosporidium* 17.9%, also came identical to the ratio of 18.6% reported by El-Settawy & Fathy (2012) in this study, which included a comparison of some of the diagnostic methods in the investigation of the parasite *Cryptosporidium* in Egypt.

The current study also recorded a higher percentage infection which recorded Kaabi (2006), as recorded infection rate of 6.6% through the study of the causes of diarrhea in children in the province of Diwaniyah. as well as higher than his record Al-Rikabi (2012) in Dhi -Qar (9%) after examined 200 samples of faeces of children under 5 years.

The Abash (2004) recorded 35.20% the highest recorded rate in the current study in the city of Mosul. also Mekhleif (2008) recorded 39.13% in the city of Ramadi and the surrounding area is higher than the current study.

The high infection rate in the current study for several reasons, foremost among which poor social and economic conditions as well as malnutrition and lack of attention to personal hygiene and surrounding, the environment ignorance and poor health culture.

Discussion

The results of the current study parasite cryptosporidium For months the study, as the highest percentage found the parasite in the winter, specifically in the month (1) amounting to 27% while the lowest rate of infection to the parasite in the summer months, especially in the month of September, amounting to 0%. This study disagreement with (Amin, 2000, Mahgoub *et al.*, 2004; Hlavsa *et al.*, 2005; Kaabi, 2006; Amin, 2008; Al-Warid, 2012). But agreement with other studies showed an increase in the incidence of the parasite during the winter (Al-Rikabi,2012; Gatei *et al.*,2006 Sulaiman *et al.*,2005; Iqbal *et al.*,2001).

The results of the present study was that the parasite *Cryptosporidium* affects all age groups studied, as it recorded the highest rate of infected parasite within the age group(1-5year), amounting to 29.41%, while the lowest rate of infection recorded in the age group(>10) years and amounted to 0% . Attributed infection concentration in children have one year of age for non-completion of the immune system to have as the low-dose parasite oocytes enough to cause infection as well as leaving breastfeeding to artificial feeding and the ignorance of many mothers things sterilization and cleanliness of the water and bottles of milk so suffer more than others (Abbassa, 1994; Areeshi *et al.*, 2007).

The results of the present study also recorded high percent in male 22.58% compare with 15.78% in female , this agreement with (Ali,1998 ; Al-Gelany,1998), the researchers attributed the reason for this is due to the fact that males more active than females in terms of playing outside with the animals, in soil which leads to transmission of the parasite oocytes to them (Park *et al.*, 2006).



CONCLUSIONS AND RECOMMENDATIONS

Conclusions and Recommendations

Conclusions

- 1- *Cryptosporidium parvum* are endemic in AL-Qadisiyah province
2. Factors studied, such as age, sex, and months, and clinical symptoms influential in the development of parasite *C.parvum*.
- 3- *Cryptosporidium parvum* parasite infection that may lead to the emergence of signs of diarrhea, but the absence of diarrhea not mean absence the presence of the parasite in children, especially at the beginning of the infection.

Conclusions and Recommendations

Recommendations:

- 1- New techniques as polymerase chain reaction(PCR) can be used to get accurate result about *Cryptosporidium parvum* .
2. Emphasis on the use of acid fast stain in hospitals and laboratories to diagnose the parasite, which is one of the pathogens that cause health problems.
3. Start implementing a health education program through health education to people in areas infected with the parasite programs about the symptoms of the disease and the mode of transmission
4. Conducting epidemiological surveys between the duration and the other to get to know the real reasons that contribute to the spread of the parasite for the purpose of identifying infected areas.



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الخلاصة

أجريت هذه الدراسة خلال الفترة من ايلول ٢٠١٦ حتى شباط ٢٠١٧، تم جمع ٥٠ عينة من الغائط من الإنسان في اعمار مختلفة ومن كلا الجنسين، والعينة التي تم جمعها من مناطق مختلفة في مستشفى الديوانية التعليمي، ومختبر البلاد ومختبر بغداد ، ومركز الزوراء الطبي. وقد صممت الدراسة لتقييم الملامح المجهرية للطفيل باستخدام صبغة زيل نيلسن

أظهرت نتائج الفحص المجهرى أن بويضة الطفيلي ظهرت بيوض بيضوية أو كروية ذات لون وردي غامق أو أحمر على أرضية زرقاء.

نتائج الدراسة الحالية سجلت نسبة العدوى إلى *Cryptosporidium* (٢٢٪)، ١٠ عينات إيجابية من أصل ٥٠ عينة أظهرت النتائج أيضا أن أعلى نسبة إصابة (٢٩,٤١٪) لوحظت في أعمار (١-٥ سنوات) وأعلى معدل إصابة لدى الذكور (٢٢٪) في حين أن الإناث تظهر أدنى اصابه (١٥,٧٨٪). كما أن النتيجة التي تعتمد على أشهر السنة تراوحت بين (٠٪-٢٧٪).



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الانسان باستخدام الطرق التقليدية في محافظة القادسية.

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