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Incidence of Cryptosporidiosis among children at Ramadi city

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حدوث الإصابة بمرض Cryptosporidiosis في أطفال مدينة الرمادي

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الخلاصة

يعد طفيلي *Cryptosporidium* من الطفيليات الابتدائية المهمة مرضيا" للأنسان والمضائف الفقرية الاخرى وينتقل هذا الطفيلي مباشرة عن طريق تلوث الطعام والماء بالبراز الحاوي على لاطوار المعدية للطفيلي.

تم فحص 441 عينة براز من الذين يعانون من الاسهال الشديد و٣١٥ عينة براز للسيطرة. أجريت الدراسة للمدة من ايلول عام ١٩٩٥ لغاية اذار من عام ١٩٩٦ في مدينة الرمادي في كلية الطب.

تم استخدام طريقة مشتركة من التركيز بمحلول الفورمالين– إيثر ثم الصبغ بصبغة القصر بالحامض المطورةللعينات كافة.

تم تشخيص وعزل ٩ حالات موجبة للطفيلي من مجموع ٤٤١عينة مرضية حيث كانت نسبة الاصابة للطور السبوري للطفيلي ٢ %، بينما لم تذكر اي حالة اصابة في عينات المقارنة . وأوضحت الدراسة

ان اعلى نسبة إصابة بين الاطفال بعمر(١-١٢) شهرا ، وكانت اصابة الذكور بنسبة اعلى من اصابة الاناث.

كما اشارت الدراسة الى وجود علاقة بين كل من منطقة سكن الاشخاص المصابين ومصدرمياه الشرب لهم مع نسبة انتشار الطفيلي . كانت اعلى نسبة اصابة في شهر ايلول من عام ١٩٩٥ (٥.٣٥%)، بينما ادنى نسبة اصابة سجلت في كانون الثاني من عام ١٩٩٦(٠.٠) حيث لم تذكراي حالة اصابة. تبين ان استخدام التقنية المشتركة للتركيز والقصر بالحامض المطورة في هذه الدراسة كانت ذات دقة ونوعية عاليتين ، لذا نقترح استخدامها في العمل المختبري اليومي لاسيما مرضى الاسهال .

Abstract

Cryptosporidium is a protozoan parasite and important pathogen of human and other vertebrate hosts. Transmission of parasite is direct,by either the fecal- oral route or the contamination of water supplies with the resistant infective oocyst stage (oocyst).

Examinations were performed on ٤٤١ stool specimens refered for screening on the basis of diarrhoea and **315** stool specimens as controls. The

study was carried out during the period from **September,1995** to **March, 1996** in **Ramadi** city.

Combination of Formalin-Ether concentration method with modified Acid-Fast stain were used for all specimens . From **9 (2.2%)** of **441**specimens obtained from patients , ***Cryptosporidium*** Oocysts were isolated, but no parasite isolated from **315** stool specimens of control groups **(0.00)**.

High rate infection was among (1-١٢**)** months age groups and males recorded the highest**(5 / 226) (2.2 %)**than females **(4/215) (1.9%).** The study includes two types of residency, then water source plays a role in the transmission of the infection .The majority of ***Cryptosporidium*** Oocysts were recovered during **September (5.35 %)** while the minority during **January(0.00).**

1-Introduction :-

Cryptosporidiosis, long considered to be a veterinary disease, has emerged as a serious human health problem , being the most frequent secondary diagnosis in people with AIDS and significantly contributing to their mortality(DuPont, *et al*., 1995). Human *Cryptosporidium parvum* – associated disease is the result of zoonotic or anthro-ponotic transmission of the parasite's infectious stages, the oocysts. The parasite is transmitted via a fecal-oral route and very frequently via contaminated water and food (Graczyk *et al*., 2001; De Graaf *et al*., 1999).

Cryptosporidium is a tiny protozoan: a single-cell parasite that usually lives In the intestines of animals , wild and livestock . However , it can also infect humans and their pets(Fayer *et al*.,2000).

It enters its hosts as **oocysts: tiny, protective capsules, similar to eggs, but only micron in diameter.** Each oocyst contains one to four "sporozoites" that can develop into adults . The oocysts break open inside the host's intestines , allowing the parasite to grow and spread (through both sexual and asexual reproduction).

During their spread , they irritate the surfaces of the small intestine, which cause diarrhoea . Eventually the form of oocysts which are transmitted through feces into the water and to other hosts . *Cryptosporidium* can also be passed from human - to - human , or human - to - pet orally ; through contaminated lakes and swimming pools (McCole *et al*., 2000; Hoogenboezem *et al.*, 2001)

Although first described in 1907, it wasn't until 1976 that the first case in a

human was identified.In 1981, the first case in AIDS patient was described. Most of the fatalities due to *Cryptosporidium* have been in AIDS victims who suffer from compromised immune systems and are not able to recover from the parasite.

Outbreaks of the parasite have been relatively rare until the last decade, but may have been diagnosed as other illnesses or infections.Outbreaks have occurred in water systems ranging from simple chlorination to full filtration and ozonation.

2-Literatures review :-

Cryptosporidium is a coccidian protozoan parasite that has gained much attention in the last 20 years as a clinically important human pathogen . The discovery of **Cryptosporidium** is usually associated with E.E. Tyzzer , who , in 1907, described a cell- associated organism in the gastric mucosa of mice (Korich, *et al*., 1990). For several decades , ***Cryptosporidium*** was thought to be a rare, opportunistic animal pathogen , but the first case of human Cryptosporidiosis in 1976 involved a (3)-years- old girl from rural Tennessee who suffered severe gastroenteritis for two weeks (Toyoguchi *et al*., 2001) . Electron microscopic examination of the intestinal mucosa led to the discovery that **Cryptosporidium parvum** was the infectious species in humans . In the early 1980s , the strongassociation between cases of Cryptosporidiosis and immunodeficient individuals (such as those with AIDS -- acquired immunodeficiency syndrome) brought ***Cryptosporidium*** tothe forefront as a ubiquitous human pathogen.

2-1 Life Cycle:

Cryptosporidium is taxonomically classified as a Sporozoa,since its oocyst releases four sporozoites (its motile infectious agents) upon excystation. However, it differs from related parasites such as *Toxoplasma* by its monoxenous life cycle- completing its entire cycle within a single host (Flanigan and Soave, 1993).

The life cycle is complex; there are both sexual and asexual cycles,and there are five distinct developmental stages (Korich *et al*., 1990):

1. Excystation of the orally ingested oocyst in the small bowel with release of the four sporozoites.

2. Invastion of intestinal epithelial cells via the differentiated apical end of the sporozoite within a vacuole formed of both host and parasite membranes and the initiation of the asexual intracellular multiplication stage.
3. Differentiation of microgametes and macrogametes.
4. Fertilization that initiating sexual replication.
5. Development of oocysts,The formation of new, infectious sporozoites within the oocyst, which is then excreted in the stool .

2-2 Clinical manifestations:

The various symptoms of Cryptosporidiosis differ greatly between immunocompetent and immunocompromised individuals . In immunocompetent
patients, cryptosporidiosis is an acute, yet self-limiting diarrheal illness (1-2 weekduration), and symptoms include :-Watery
diarrhea,nausea,vomiting,abdominal
cramps and low- grade fever(Juranek,1995).

For immunocompromised persons , the illness is much more severe (Casemore *et al*., 1994):-
Debilitating , cholera - like diarrhea (up to 20 liters/day) , severe abdominal
cramps,malaise,low-grade fever,weight loss and anorexia.

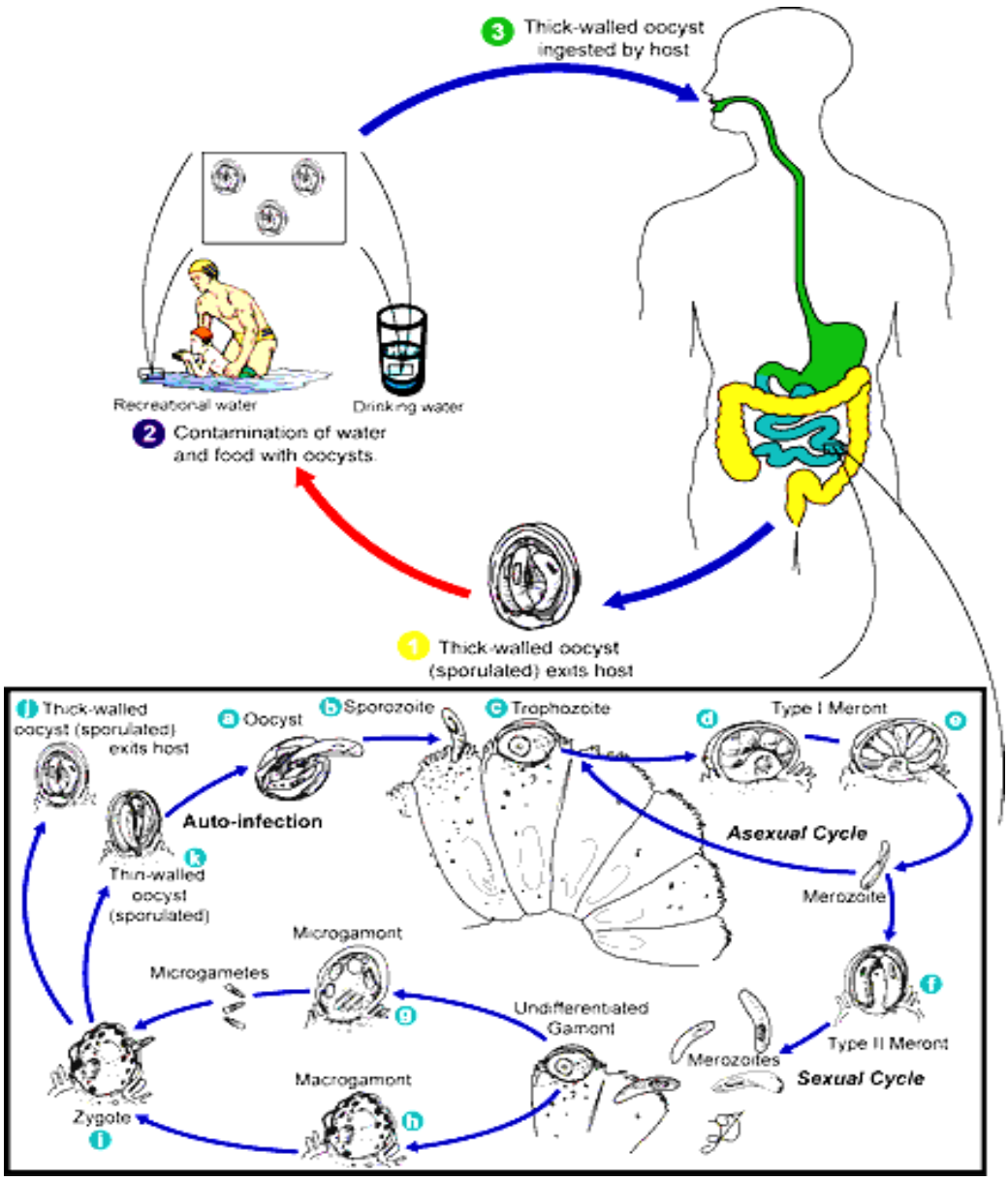

Figure 1 (Life cycle of Cryptosporidium(Heyworth, 1992)

2-3 Pathogenisis :-

Cryptosporidium parvum , a widespread enteric pathogen , causes severe, watery diarrhea in both humans & calves. This pathogen acts in a myriad of ways to infect the intestine and cause damage. As of yet, no effective vaccine or antibiotics exists, which has led, in part, to the search for the most efficacious treatment regime (Atherton et al., 1995; Laurent et al.,1999).

Upon oocyst excystation , four sporozoites are released which adhere their apical ends to the surface of the intestinal mucosa (Korich et al.,1990). Below is a phase contrast photograph of sporozoite release from the **Cryptosporidium** oocyst (Goodgame,1996; Josephs et al., 1999)(Figure 2).

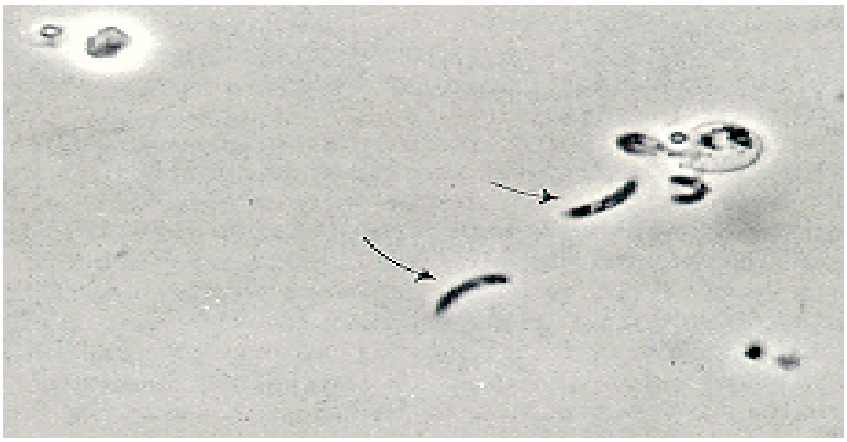

Figure 2 (Sporozoites of *Cryptosporidium*)

Consequently, epithelial cells are damaged by one of two models:
1-Cell death is a direct result of parasite invasion, multiplication, and extrusion.

or

2-Cell damage could occur through T cell-mediated inflammation, producing villus atrophy and crypt hyperplasia(Goodgame, 1996).

3-Material and Methods:

During the priod September 1995 to March 1996,a total of 760 stool samples from patients of acute diarrhoea were received for routine bacteriological and parasitological studies . All the samples were screened for various parasites including *Cryptosporidium* oocysts.faecal samples from 315 patients

who did not have diarrhoea or other gastrointestinal symptoms were examine as controls.

Stool samples were processed within 2 hours of collection.direct wet mounts ,concentration by formal-ether technique and acid fast staining were used for the detection of *Cryptosporidium* oocysts.

Concentration of the stool samples were carried out as follows : a heavy suspension of faeces was made in saline and strained through Four layers of gauze into centrifuge tube . Equal volume of Formal- Ether solution was added and gently mixed . A slide was placed on the surface of the suspension and left undisturbed for (25-30) minutes . The slides were then examined by wet mount under high power.

Staining of the direct faecal smears and the concentrates were carried out both by modified Ziehl - Neelsen technique (Garcia *et al*., 1983).The modified Ziehl-Neelsen technique was performed as follows :

After heat fixation ,the slide was placed on astaining rack and flooded with carbol fuchsin . The slide was heated to steaming and allowed to stain for 5 minutes . If the slide begin to dry , more stain was added without additional heating.The slide was rinsed with tap or distilled water and decolourized with10 %sulphuric acid for 2 minutes (thick smears required alonger time).The slide was rinsed again and counterstained with Loefflers methylene blue for 1 minute .The stained smears were examined under high power and oil immersion.

Cryptosporidium oocysts were seen as bright red rounded bodies(4--6 Mm) against ablue back ground . All the stool specimens positive for *Cryptospoidium*by the modified acid fast staining were reexamined and confirmed Giemsa staining method (Mata *et al*., 1984). Oocysts appeared as faintly blue with reddish or purple cells.

4- RESULTS and DISCUSSION

4-1 RESULTS:

The patient and control groups were within an age range (1 month- 6 years).

Males accounted for 0.02 % (5/ 226) in patient groups and 100 % (165 /165) in control groups , also females accounted for 0.019 % (4/ 215) in patient groups and 100 % (150 /150) in control groups .

Tap water source and river accounted for 0.01% (3/235)& 0.03 % (6 /206) respectively in patient groups , while in control groups 100 % (170 /170) ,100 % (145 /145) respectively.

4-1-1 Prevalence of Cryptosporidiosis in relate to age:

The total number of children involved in this study was divided into six age groups (table 4-١).

Cryptosporidium infection found among children (1-12)months age of the Patient groups (0.053%). This rate is higher than the other age groups which was decreased reaching to(0.00) in (4-5) years of age groups ,while in control groups there was no infection recorded (0.00) in all age groups (Table 4-1).

Table (4--1) Prevalence of Cryptosporidium infection in relation to age

Age Group Years	Patient No.	+Ve	%	Control No.	+Ve	%	Total No.	+Ve	%
>١	56	٣	٠.٠٥٣	٣٦	٠	٠.٠٠	٩٢	3	٠.٠٢
٢-1	٦٤	١	٠.٠١٥	٤٤	٠	٠.٠٠	١٠٨	١	٠.٠٠٩
٣-٢	٩٥	٢	٠.٠٢	٧٥	٠	٠.٠٠	١٧٠	2	٠.٠١٨
٤-٣	٨٧	٠	٠.٠٠	٥٥	٠	٠.٠٠	١٤٢	٠	٠.٠٠
٥-٤	٨٤	٢	٠.٠٢	٦٤	٠	٠.٠٠	١٤٨	٢	٠.٠١٤
٦-٥	٥٥	١	٠.٠١٨	٤١	٠	٠.٠٠	٩٦	١	٠.٠١
Total	٤٤١	٩	٠.٠٢	٣١٥	٠	٠.٠٠	٧٥٦	٩	٠.٠١

4-1-2 Prevalence of Cryptosporidiosis in relate to sex:

Males were shown with higher infection (0.02%) (5/ 226) and females (0.019%) (4/215) in patient groups, while no infection for both sex in control groups (table 4-٢).

4-1-3 Prevalence of Cryptosporidiosis in relate to water source:

The infection was higher in river consumed water (0.03 %) than tap water (٠.٠١%)among patients ,while no infection appeared in control groups (table 4-٣).

Table(4-2) Prevalence of Cryptosporidium infection in relation to sex

		Infection	+Ve	-Ve	Total
Patients	Male	No.	٥	٢٢١	٢٢٦
		%	٠.٠٢	٠.٩٨	١٠٠.٠٠
	Female	No.	٤	٢١١	٢١٥
		%	٠.٠١٩	٠.٩٨	١٠٠.٠٠
	Total		٩	٤٣٢	٤٤١
Controls	Male	No.	٠	١٦٥	١٦٥
		%	٠.٠٠	١٠٠.٠٠	١٠٠.٠٠
	Female	No.	٠	١٥٠	١٥٠
		%	٠.٠٠	١٠٠.٠٠	١٠٠.٠٠
	Total		٠	٣١٥	٣١٥

Table(4-3)Prevalence of Cryptosporidium infection in relation to water source

		Infection	+Ve	-Ve	Total
Patients	Tap	No.	٣	٢٣٢	٢٣٥
		%	٠.٠١	٠.٩٩	١٠٠.٠٠
	River	No.	٦	٢٠٠	٢٠٦
		%	٠.٠٣	٠.٩٧	١٠٠.٠٠
	Total		٩	٤٣٢	٤٤١
Controls	Tap	No.	٠	١٧٠	١٧٠
		%	٠.٠٠	١٠٠.٠٠	١٠٠.٠٠
	River	No.	٠	١٤٥	١٤٥
		%	٠.٠٠	١٠٠.٠٠	١٠٠.٠٠
	Total		٠	٣١٥	٣١٥

4-1-4 Prevalence of Cryptosporidiosis in relate to residence:

The higher infection appeared in rural (0.03%)than in urban (0.01%) among patients, while no infection in control groups (table 4-٤).

Table(4-4) Prevalence of Cryptosporidium infection in relation to residence

Infection			+ Ve	- Ve	Total
Patients	Urban	No.	٣	٢٣٢	٢٣٥
		%	٠.٠١	٠.٩٩	١٠٠.٠٠
	Rural	No.	٦	٢٠٠	٢٠٦
		%	٠.٠٣	٠.٩٧	١٠٠.٠٠
	Total		٩	٤٣٢	٤٤١
Controls	Urban	No.	٠	١٧٠	١٧٠
		%	٠.٠٠	١٠٠.٠٠	١٠٠.٠٠
	Rural	No.	٠	١٤٥	١٤٥
		%	٠.٠٠	١٠٠.٠٠	١٠٠.٠٠
	Total		٠	٣١٥	٣١٥

1-Between Urban & Rural of patients : X = 0.0009 P < 0.05 DF =1 Significant

2-Between Urban &Rural of controls : X = 0 P < 0.05 DF = 0 Significant

4-1-5 Monthly diagnosis rates of *Cryptosporidium* Oocysts :

During the period of the study (September,1995 to March ,1996) monthly isolation of oocysts among 756 stool specimens was shown (figure 4-5).

The highest appearance was recorded during (September,1995) in which there were 3 cases out 56 cases (5.357 %), while the lowest appeared during (January, 1996)(0.00).

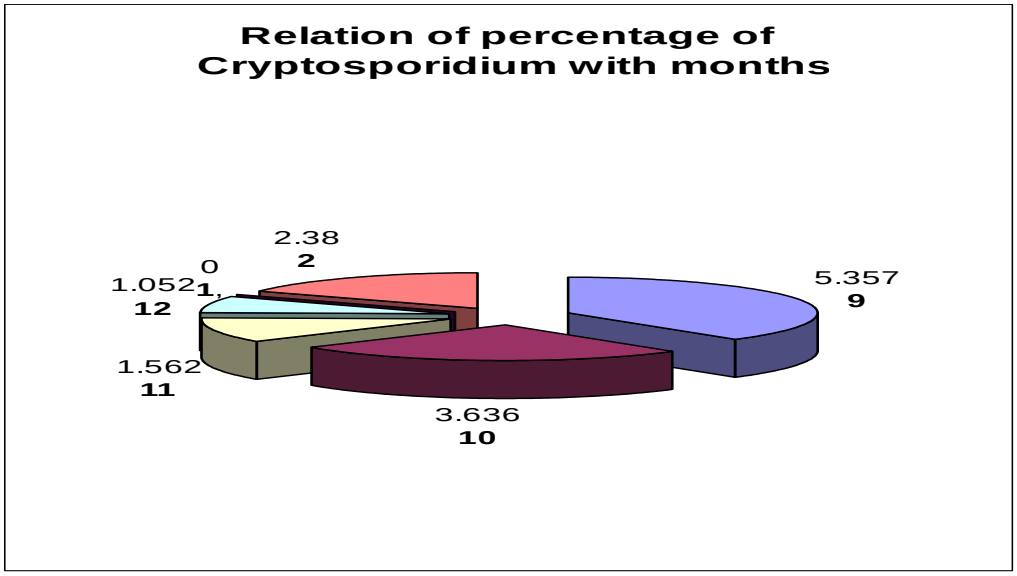

Figure (4-5) Monthly distribution of *Cryptosporidium* infection

4-2 Discussion:

Among total human patients ,Oocysts were isolated from (0.02 %).This result was almost comparable with those reported in different contries such as in Wales (U. K) recorded 2 % (P.H.L.S.S.G.,1990), 2.8 % in immunocompetent patients of Massachusetts (U.S.A)(Wolfson *et al*.,1995), and 5% in an urban community of Bristol (England)(Hunt *et al*.,1984), while the parasite was not isolated from control groups .

4-2-1 Prevalence of Cryptosporidiosis in relate to age :

The patients of this study divided into six age groups .The highest rate of Cryptosporidiosis was found among childrens(1-12) months age of the patient groups (0.035)and began to decreased reaching to (0.00)among (4-5) years age .

This relation pattern is in agreement with many other studies (Soave ,1996 ; Wolfson *et al*.,19^5; Tziporis *et al*.,1987). The increase of infection among (1-12)months may due to immune system was not well developed while in (4-5) yearsage it was developed, in addition to the continuous exposure to low level ofparasite so the infection decreased (Al-Kassar, 2005).

4-2-2 Prevalence of Cryptosporidiosis in relate to sex:

Males in patient groups were more infected (0.02 %)(5/226) than females (0.019%)(4/ 215).This is in agreement with finding reported(Wolfson *et al*.,1985; Molbk *et al*.,1994; Hira *et al*., 1989).This can be related to the fact; that males are more active,mobile, and involved among agricultural community.

4-2-3 Prevalence of Cryptosporidiosis in relate to water source:

The infection was higher among river consumed water (0.03%) than tap water (0.01%) among patient groups .This result might lead to fact ; the river water was contaminated with human and animals faeces also the efficiency of water teatment was decreased (Jokipii and Jakopii, 1985).

4-2-4 Prevalence of Cryptosporidiosis in relate to residence :

The higher infection was seen among rural population (0.03 %) than in urban (0.01%) because of using river water in drinking ,their habitat and low hygiene level (Mahdi *et al*.,1994).

4-2-5 Seasonal distribution of the disease:

The highest isolation rate of Cryptosporidium Oocysts were appeared during September 1995 (5.35%). This result is in agreement with many other studies (Wolfson et al.,1985 ; Tziporis et al.,1987 ; Cruz et al.,1988).
In general,evolution of seasonal distribution is unreliable and need study of four season period.therefore,the evolution in this study was on monthly basis.

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