# Prevalence of *Cryptosporidium* Oocysts In Different Types of Water in Al-Mansoria Diala Province

Ahmed J. Al-Baytee<sup>\*</sup>, Shatha Q. Jawad<sup>\*\*</sup> and Hadi S. Mehdi<sup>\*\*\*</sup>

\*Department of Pathology, College Medicine, University of Diala.

\*\*Department of Basic Science, College of Dentistry, University of Baghdad.

\*\*\* Department of Public Health, College of V. Medicine, University of Diala.

#### Abstract

The aim of the present study was to evaluate the prevalence of *Cryptosporidium* oocysts, and the importance of the water source in the prevalence of *Cryptosporidium* spp. Four types of water samples (400 L.s.) .100 Ls .tap water supplies, 100 L.s. house tank water, 100 L.s. Stagnant water, and 100 L.s septating water were collected from November 2008 to December 2009. In Al-Mansoria city, Diala Province. Cryptosporidium Oocysts were found in 125 of 400 liters (31.25 %) (table 1), which divided in to 22 liters (22%) of tap water supplies that appear at spring only, This parasite oocysts found in 32 liters (32%) of house tank water which range from 72% in spring to 16% in summer, and they found in 35 liters (35%) and 36 liters (36%) of septating and stagnant water respectively, the highest rates 48% ,of septating water and 60% of stagnant water were shown in spring but the lowerst 24% and 20% in summer respectively (table 2). The prevalence rate varied according to the seasons over the year, this indicates that the seasonal factors may effect on the presence of oocysts (fig.1), with a significant differences (p<0.05).

Keywords: Cryptosporidium, oocysts, water, prevalence.

### Introduction

*Cryptosporidiosis* Water borne now recognized as a significant cause of threating ubiquitous human health due to the distribution of Cryptosporidium spp. In human, Animals, and water, the oocvsts have resistance to harsh environmental conditions, various disinfectants and some treatment practices.<sup>(1)</sup>

Many *Cryptosporidium* species and genotypes have been found in domestic and wild animals, but only five spp . are major human pathogens C *parvum, C. hominis*, *C. meleagridis, C. canis, and C. felis.*<sup>(2 3)</sup> all *Cryptosporidium* spp. Oocysts have the potential to be present in water and (most of them are morphologically similar), sensitive detection of them with the correct diagnosis of species and genotypes of Cryptosporidium oocysts are essential for the source water management and risk assessment.<sup>(4, 5)</sup>

Transmission of *C. parvum* occurs by the fecal-oral rout and has been shown to involve drinking water, vecreational water, and food borne, pcrson-to-person, and animals to-person exposures or contacts, and some studies have suggested that the contribution of sexual

practices may be important among persons with human immunodeficiency virus HIV<sup>(6)</sup>, but the majority of these cases are left unexplained.<sup>(7)</sup>

For compare the concentration of *Cryptosporidium* oocysts between them, and to evaluate the distribution or the infection source in Al-Mansoria from Diala city. Water is perhaps the major route for massive outbreaks of the infection, as Result of contamination of either raw or treated water.<sup>(8)</sup>

### Method Experimental Work

Water samples were collected from November 2008 to December 2009 from Al-Mansoria city, Diala province. A total of 400 liters of water samples were collected: 100 liters from drinking water supplies, 100 liters from tanke water, 100 liters stagnant water, and 100 liters septating water (2 liters for every sample, every week). Then put in a sterile bottle (disposable, collapsible), which delivered to the laboratory of pathology at the college of medicine in the university of Diala.

In the laboratory, samples were seeded, filtered, eluted, (according to type of water sample), filtered through an envirochek HV  $(0.02-0.45 \ \mu m)$  filter using procedures

described by Musial (1987)<sup>(9)</sup>. Several filters were sometimes required to filter each sample. These filters were processed separately, labeled, depending on the number of filters used, Material on the filter was eluted, and then take the supernant after centrifuged (1000 for 10-15 min), after washing and repeat this step for three times, finally *Cryptosporidium* oocysts examined by taken a drop on the slide, fixed and stained (zeal nealson stain) for Microscope identification the stained oocysts by many criteria:s ,as size, shape, surface feature, color stain.<sup>(10)</sup>

The statistical analysis were performed to study the association between the presence of oocysts and seasons, and between the presence of oocysts with the type of water sample  $(X^2 \text{ test})$ .<sup>(11)</sup>

## Results

*Cryptosporidium* oocysts were found in 125 of 400 liters of water samples with a total prevalence 31.25%. This rate varied according to the source of the water, with a significant differences (P>0.05) (Table (1)).

*Cryptosporidium* oocysts appear in 22% of tape water supplies, this rate appear in the Spring only, while there was sharply decrease in the oocysts appearance in Summer, *Cryptosporidium* oocysts were found in 32% of houses tank water ,this rate of prevalence varied from 72% in spring to 16% in summer, but this oocysts appear in 35%, 36% of septating, stagnant water samples respectively, which ranging from 48% and 60% in spring to 24% and 20% in summer of septating and stagnant water samples respectively (Table (2)).

A significant differences (P<0.05) was recorded between the season of the year and the rate of the prevalence in the different type at water samples (Fig.(1)). Also, it appear sharp differences in the size and shape between all the sampling water along the period of the study. This is the first report of the finding *Cryptosporidium* oocyst in the drinking water supplies in Al-Mansoria city, Diala province.

## Discussion

*Cryptosporidiosis* is one of the major diarrheal diseases caused by protozoan parasites and poses a significant public health world wide <sup>(1, 12)</sup>, but the epidemiological studies for this parasite has been poorly understood in Iraq.

study, this we found In that *Cryptosporidium* parvum oocysts were prevalent especially in areas of Al-Mansoria. These regions are relatively under develop and have many livestock farming industries and some of these areas as will as the water have been thought to be a source of zoonotic infections.

The role of water and food in the spreading of this disease is now well recognized, water is perhaps the major route for massive outbreaks of the infection, as a result of contamination of either raw or treated water.<sup>(13)</sup>

*Cryptosporidium* hosts excrete large numbers of infective transmissive stages (oocysts) in feces. A part from the difficulties of isolation, detection of this parasite from environmental samples by currently available methods remains difficult and costly, has and Rose stated that an out break would probably occur if the tap water contained more, 10 to 30 *Cryptosporidium* oocysts /100 L.<sup>(14)</sup>

The number of *Cryptosporidium* oocysts for surface water has been detected in different countries of the world varied from 0.005 to 252.7 oocysts/L.<sup>(15)</sup>

*Cryptosporidium* is a prime candidate for worrisome levels of endemic transmission, because it is ubiquitous in surface waters and is extremely resistant to various environmental pressures, including some chemical disinfectant, and its passage exist in water supplies, none of the barriers, including filtration, can be considered fail- safe.<sup>(16)</sup>

In recent years, there has been a dramatic increase in the incidence of water borne disease outbreaks caused by the protozoan parasite *C. parvum*. Regulatory agencies are concerned that source and finished water is screened for these organisms, but major obstacle is the lack of reliable methodologies and baseline information on oocysts prevalence in various water sources  $^{(17)}$ .

Areliable, reproducible, and simple detection method with a short assay time to enumerate viable infectious oocysts would be the most ideal tool to prevent drinking water-related *Cryplosporidiosis*.

Table (1)Prevalence of Cryptosporidium oocysts in different water sources in Al-Mansoria city, Diala<br/>province.

	Source of water	Source of water No. examined (liter) Oocyst positive				
				Oocyst positive %		
	Tap water supplies	100	22	22%		
	Tank / houses	100	32	32%		
	Septating water	100	35	35%		
	Stagnant water	100	36	36%		
	Total	400	125	31.25%		
	$X^2 = 2$	.135 P=0.045	P<0.05 (Signi	ficant)		
Prevalence	(1 a) water) (1 a) (1	— Seasons	House tar 60 50 40 20 10 0 Wintar Spring St	Seasons		
Prevalence	$\begin{array}{c} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{array}$ $(Septating water)$ $\begin{array}{c} 0 \\ 0 \\ 0 \\ 0 \end{array}$		$\begin{array}{c} 80 \\ 70 \\ 60 \\ 80 \\ 60 \\ 50 \\ 40 \\ 30 \\ 20 \\ 10 \\ 10 \\ \end{array}$ (Segnant )	water)		
(	) Wintar Spring Summar Outum	——Seasons	0 Wintar Spring Sum	mar Outum		

Fig. (1) The relation ship between season and the prevalence rate of Cryptosporidium oocyst from November 2008 to December 2009.

#### *Table* (2)

The prevalence rate of Cryptosporidium oocysts in the different seasons along the period of study from November 2008 to December 2009.

Seasons	Tap water supplies (+ve/%)	House tank water (+ve/%)	Septating water (+ve/%)	Stagnant water (+ve/%)	Total	Oocysts positive
Winter	25 (0/0%)	25 (0/0%)	25 (6/ 24%)	25 (5/ 20%)	100	11(11%)
Spring	25 (22/ 88%)	25(18/72%)	25 (12/48%)	25(15/60%)	100	67 (67%)
Summer	25 (0/0%)	25(4/ 16%)	25 (6/ 24%)	25 (7/ 28%)	100	17 (17%)
Outum	25 (0/0%)	25 (10/ 40%)	25(11/44%)	25 (9/ 36%)	100	30 (30%)
Total	100 (22/ 22%)	100(32/ 32%)	100(35/35%)	100(36/36%)	400	125(31.25%)

 $X^2 = 2.135$  P = 0.045

P<0.05 (Significant)

#### References

- [1] Johason, D.W; Pieniuazek, N.J.; Griffin, D.W.; Mispner, L. and Rose, J.B. "Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocysts in water samples". App. Environ. Microbiol. 61; 3849\_3855.1995.
- [2] Lake, L.; Pearce, J. and Savill, M. "The seasonality of human *Cryplosporidiosis* in New Zealand". Epidemiol. Infect. 136:1 383\_1387. 2008.
- [3] Xiao, L ;Fayer, R.; Ryan ,U.; Upton, \_ S. 'Cryptosporidium taxonomy ; recent advance and implications for public health". Clin. Microbiol. Rev. 17:72-97. 2004.
- [4] U.S. Agency. "National primary drinking water -regulations long term 2 enhanced surface water treatment. Rule". Fed. Regist.71:654-786.2006.
- [5] Gostin, L. O.; Lazzarini, Z.; Neslund, V. S. and Osterholm, M.T. "Water quality laws and water borne diseases- *Cryptosporidium* and other emerging pathogens". Am. J. Public health. 90:847-853. 2000.
- [6] Meinhardt, P.L.; Casemore, D.P.; Miller, K.B. "Epidemiologic aspects of human *cryptosporidiosis* and the role of water borne transmission". Epidemiol Rev. 18:18-136. 1996.
- [7] Donnely, J.K. and Stentiford, E.I. "The *Cryptosporidium* problem in water and food supplies". Food. Sci. Technol. 30: 1 11-120. 1997.

[8] Eisenberg, J.N.S.; Seto, E.Y.W.; Colfold, J.M.; Olivieri, and Sperar, R.C. "An analysis of the Milwaukee *cryptosporidiosis* outbreak based on dynamic model of the infection process". Epidemiol. 9:255-263.

- [9] Musial, C. E.; Arrowood, M.I.; Sterling, C. R. and Grerba, C.P. "Detection of *Cryptosporidium* in water by using polypropylene cartridge filters". Am. Soci. Microb 53 (4): 687-692. 1987.
- [10] Xiao,L.; Alderisio, K. and Jiang, J. "Detection of *Cryptosporidium* oocysts in water. Eeffect of the number of samples and analytic replicates on test results". Am. Soci. Microb.72 (9): 5942-5947. 2006.
- [11] Sorlie, D.E. "Medical biostatistics and epidemiology". Postgraduate doctor- Meddle East. 9 (7): 412-418. 1995.
- [12] Kostrznsla, M; Sankey, M.; IIaac k,E.; Power, J.E.; Aldom, J.; Chag la, A.; Unger, S.; Palmateer, G.; Lee, H.; Trevors. J. and De Grandis, S. "Three sample preparation protocols For polymerase chain reaction based detection of *Cryptosporidium parvum* in Environmental samples". J. Microbiol. Medods. 35:65-71. 1999.
- [13] Hvnter, P.R. and Thompson, R.C. "The zoonotic transmission of Giardia & *Cryptosporidium*". Int. J. Parasitol. 35: 1181-1190. 2005.
- [14] Haas, C.N. and Rose, J.B. "Developing an action level for *Cryptosporidium*". J. Am. Water. Works. Assoc. 87: 81-84. 1995.

- [15] Stezenbach, L.D.; Arrowood, M.J.; Mershell, M. M. and Sterling, G.R. "Monoclonal Antibody based immunofluoresent assay for Giardia and *Cryptosporidium* Detection in water samples". Water Scoi. Technol. 20: 193-198. 1988.
- [16] Sorvillo, F. J.; Lieb, L.E.; Kerndt, P.R. "Epidemiology of *cryptosporidiosis* among persons with acquired immunodeficiency syndrome in losangeles". Am. J. Trop. Med. Hyg. 51: 326-331-. 2006.
- [17] Hansen, J.S. and Ongerth, J. E. "Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts river water". Am. Soci, Microb 57(10): 2790-2795. 1991.

الخلاصة

هدف الدراسة الحالية الى معرفة مدى انتشار اكياس

بيض طفيلي الأبواغ الخبيئة cryptosporidium واهمية مصادر المياه في انتشارها في منطقة المنصورية في محافظة ديالي. تم فحص 400 لتر من المياه موزعة على اربعة نماذج، 100 لتر جمعت من احواض التعقيم لمصدر مياه الشرب مباشرة، 100 لتر من حاويات المياه المنزلية (تانكر ات). 100 لتر من مياه البرك والمستنقعات و 100 لتر من مباه المجاري للمدة من تشربن الثاني 2008 الى كانون الأول 2009. عثر على اكياس بيض طفيلي الابواغ الخبيئة في 125 لتر وبنسبة انتشار كلية بلغت 31.25%. توزعت بواقع 22 لتر من اللعينات التي جمعت من احواض التعقيم لمياه الشرب والتي ظهرت في موسم الربيع فقط و 32% في مياه حاويات المياه المنزلية، كانت هذه النسبة موزعة بنسبة 72% و 16% في فصل الربيع والصيف على التوالي. وقد وجدت اكياس بيض الطفيلي في 35% من العينات التي جمعت من المياه البرك و المستنقعات طيلة فصول االسنة تراوحت بين 84% خلال فصل الربيع و 24% خلال فصل الصيف، بينما عثر على اكياس بيض. الطفيلي في 36% من مياه المجاري طلية فصول السنة وكانت نسب الانتشار بين 60% و 20% في فصلى الربيع والصيف على التوالي. لقد بينت الدراسة الحالية وجود اختلاف في نسب انتشار اكياس بيض طفيلي الابواغ الخبيئة (التلوث) خلال فصول السنة المختلفة مما يدل على وجود عوامل فصول

السنة والتي ربما لها علاقة او تاثير على الانتشار.