

## Isolation and Identification of *Cryptosporidium* sp. by Reverse Osmosis System of Tap water in Baghdad

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### Abstract:

A total of 60 samples of drinking water filtrated by Reverser Osmosis Filtration System from April to October 2012, from different houses in Baghdad – Al Resafa, so as to identify the eggs and cysts of protozoa. Two methods applied direct smear and staining technique with zeal nelson stain, which appeared Tape warm eggs, *Ascaris lumbricoides* eggs and oocyst of *Cryptosporidium* sp.

This study revealed that total contamination rate with intestinal parasites in tap water were 96.6% this high rate, refers to filtrate tap water by reverse osmosis system was useful to prevent or reduce the contamination of drinking water, in order to reduce risks to public health; So recommended to apply this method at water purification stations. Distribution of *Cryptosporidium* sp. as study months of year appeared that Spring (April & May ) were recorded the highest rate of contamination in filtrate tap water samples, that due to potential temperatures degrees for this protozoa as (20-30c°). When the drinking water was better as environmental maintained decreased the infection with these parasites.

**Key wards:** *Cryptosporidium*, tap water, osmosis reverse, *Ascaris*, intestinal parasites

### Introduction:

*Cryptosporidium* is a pathogenic protozoan parasite that causes gastroenteritis in humans. It may be asymptomatic and are usually self-limiting with clearance in 2–4 weeks, but chronic infections also occur. Individuals with a healthy immune response recover from *Cryptosporidium* infections in 1 or 2 weeks, but infections may be severe and life threatening in immune compromised persons [1]. Many outbreaks of cryptosporidiosis due to consumption of contaminated drinking water or consumption of raw contaminated surface water have been reported throughout the years [2,3], some cases *Cryptosporidium* oocysts could be detected in the filter In previous study in Iraq about Cryptosporidiosis was found in 6.85% of children below 5 years with

increased frequency among malnourished children with diarrhea 14.89% compared to 11% in malnourished children without diarrhea[5]. The infection rate of cryptosporidiosis in children (Iraq & Jordan) was ranged from 2.7% to 37.3%, in Iraq, source of drinking water is an important risk factor for transmission of infection as fellow: Niniva (North) 14.3%, Baghdad (Middle) 14.6%, Diala (Middle) 2.7%, Babylon (Middle) 11.0%, Basra (South) 8.6% [6].

The aim of this study is to investigate about pathogenic parasites in filtrate drinking water by reverse osmosis system.

### Materials and Methods:

A total of 60 samples of drinking water filtrated by reverse osmosis system from April to October 2012,

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from different houses in Baghdad – Al Resafa, which used for this study to detected the eggs and cysts of protozoa in it. For this purpose two methods applied:

1-Wet mount: direct smear from all the samples of water and tested slides under the compound microscope power 10x, 40x.

2-Staining technique: stain the slides with zeal nelson stain, to identify the oocysts of protozoa and tested under the power 100x [7].

Color Photographs of eggs and oocysts were taken after Ocular micrometer calibration [8] after diagnosis by using references key [8,9,10,11].

**Results:**

Tape worm eggs as 6(10%) and one species of nematode (*Ascaris lumbriciodes*) 11(18.3%) were identified in this study from tap water filtrated by reverse osmosis system as shown in table 1 and fig. (1, 2) by using direct technique, while staining technique showed two species of protozoa oocyst, *Cryptosporidium parvum* as 25(41%) and *Cryptosporidium muris* 10(16%) as shown in table 2 and fig.(3).

*Cryptosporidium parvum*: Oocyst is spherical shape, red color, 4 μ in diameter

contain one dark spot to be sporozoites. *Cryptosporidium muris*: oocyst slightly elliptical shape, colored with violet 5μ to 7 μ, contain four dark spots to be sporozoites.

April was recorded the highest rate 75% of *C. parvum* and May was recorded the highest rate 40% of *C. muris* as distributed by months of study, table 3. This study revealed also that total contamination rate with intestinal parasites in filtered tap water by reverse osmosis system was 96.6% in table 4 and fig.(4).

**Table 1: Parasites are identified by using direct test of filter water samples.**

Species of parasites	class	No. of positive samples	% of total
Tape worm egg	Cestode	6	10
<i>Ascaris lumbriciodes</i>	Nematode	11	18.3

**Table 2: *Cryptosporidium* sp. are identified by stained with zeal nelson stain.**

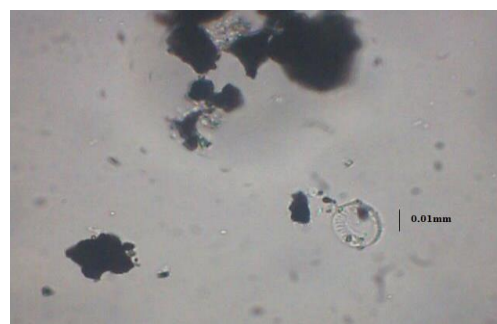
Species of parasites	Class	No. of positive samples	% of total
<i>C. Parvum</i> cyst	Protozoa	25	41.6
<i>C. Muris</i> cyst	Protozoa	10	16.6

**Table 3: Distribution of *Cryptosporidium* sp. by months.**

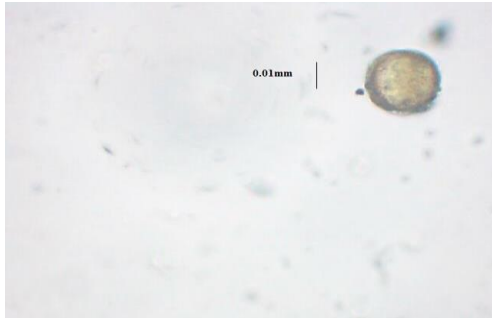
months	No. samples	<i>C. parvum</i>	% of total	<i>C. muris</i>	% of total
April	10	7	75	2	20
May	10	7	70	4	40
June	10	6	60	2	20
July	5	0	0	0	0
August	5	0	0	0	0
September	10	2	20	1	10
October	10	3	30	1	10
Total	60	25	41.6	10	16.6

**Table 4: Total positive samples of filtrated tap water with parasites.**

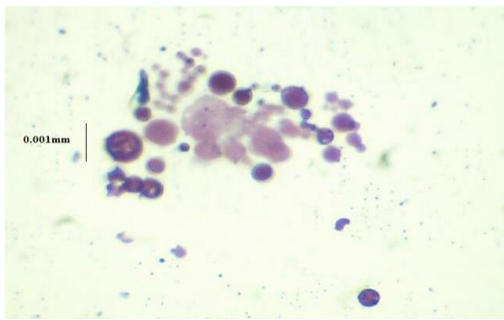
No. of filtered water samples	No. of positive samples	% of total
60	58	96.6



**Fig.1: Tape worm egg presented in filtered tap water 40x.**



**Fig.2: *Ascaris lumbricoides* egg presented in filtrate tap water 40x.**



**Fig.3: *Cryptosporidium* sp. presented in filtered tap water 100x.**



**Fig.4: Reverse Osmosis system for drinking water used in current study.**

### Discussion:

*Cryptosporidium* parasites get into surface water sources, such as rivers and lakes, from the stool (feces) of infected animals or people. Public water systems that get their water from these surface water sources can contain *Cryptosporidium* oocysts [12]. Consumption of viable oocysts and cysts of the protozoan parasites or eggs of the helminthes in drinking water can result in large numbers of consumers

being infected[13]. Even though no approach will guarantee 100 % protection to the drinking water all of the time, it has been demonstrated that the most effective way to manage drinking water systems is to implement a multi-barrier approach. The multi-barrier approach is an integrated system that prevents or reduces the contamination of drinking water, from source to tap, in order to reduce risks to public health, so filtration by Reverse Osmosis System were applied in current study that appeared contamination of drinking water with eggs of tape worm and *Ascaris lumbricoides*, that similar to previous survey of Baghdad[14] , reported a big problem that contamination of tap water with sewage water in collected regions, and appeared that drinking water polluted with *Ascaris lumbricoides* 2.2%, in addition to[15] which recorded 2.8% infection rate between the children in Baghdad Al-Rusafa. On the other hand, these eggs were surrounded by thick shell that product them from abnormal conditions and his life cycle was direct and doesn't need intermediate host. [16] .There is lack of information about contamination of drinking water in Iraq; We don't have specific laboratory to detect waterborne parasites and protozoa [17], we need more surveys for other regions with serology tests like ELIZA enzyme-linked immunosorbent assay[18].

Combined sewer overflows which can overflow during storm events and discharge raw sewage from sewer pipes into waterways, are common contributors to high fecal pollution levels in urban areas[19]. This is a chronic problem in the study area. Highest protozoal infection rate was recorded for *Cryptosporidium parvum* 25(41%) and *Cryptosporidium muris* 10(16.6% ), the prevalence of these parasites in Baghdad in human:

*Cryptosporidium sp.* 15.15%, in rats: *Crypto. sp.* 50%, in cats & dogs: *Crypto. Sp.* 20%, in vegetables: *Crypto. Sp.* 7.40% [20].

Current method for detection of *Cryptosporidium sp.* oocysts in water is filtration of tap water by reverse osmosis system. The method is time consuming, laborious and particularly not-specific. It cannot determine the infectivity of detected oocysts in water samples. Water sample concentrates were spiked with *Cryptosporidium* oocysts. Oocyst numbers in filtered water samples will vary between different contamination incidents and not all *Cryptosporidium* oocysts considered viable and possibly infectious. Detailed data on oocyst infectivity in tap water are, however, lacking. Furthermore the method could not distinguish between species of *Cryptosporidium* oocysts and this is important because not all species of *Cryptosporidium* are infectious [21].

Distribution of *Cryptosporidium sp.* as study months of year appeared that Spring (April & May ) were recorded the highest rate of contamination in filtrate tap water samples, that due to potential temperatures degrees for this protozoa as (20-30c°) [22].

When the drinking water was better as environmental maintained decreased the infection with these parasites.

Parasites in water bodies can indicate the presence of fecal contamination and related disease causing microorganisms in a body of water. This study revealed that total contamination rate with intestinal parasites in tap water were 96.6% this high rate, refers to filtrate tap water by reverse osmosis system was useful to prevent or reduce the contamination of drinking water, in order to reduce risks to public health; So recommended to apply this method at water purification stations.

## References:

- 1-Arrowood M. J. 1997. Diagnosis. In: *Cryptosporidium and Cryptosporidiosis*, ed. Fayer, R. CRC Press, Boca Raton, Florida.
- 2-Furtado C., Adak G. K., Stuart J. M., Walu P. G., Evans H. S. & Casemore D. P. 1998 .Outbreaks of waterborne infectious intestinal disease in England and Wales. *Epidemiol. Infect.* 121: 109–119.
- 3-Fayer R., Morgan U. & Upton S. J. 2000. Epidemiology of *Cryptosporidium*: transmission, detection and identification. *Int. J. Parasitol.* 30: 1305–1322.
- 4-McAnulty J. M., Fleming D. W. & Gonzalez A. H. 1994. A community-wide outbreak of Cryptosporidiosis associated with swimming at a wave pool. *J. Am. Med. Assoc.* 272(20): 1597–1600.
- 5-Raghad M. J., Nadham K. M., & Hassan M. K. 2005. Intestinal Parasitic Infections Including Cryptosporidiosis And Immunological Aspects Among Malnourished Children. *Journal of the Bahrain Medical Society.* 17( 1): 43-48.
- 6-Baha L. 2011. Cryptosporidiosis Food & Water Borne Parasitic Disease. Faculty of Medicine University Teknologi MARA Shah Alam, Selangor, MALAYSIA  
[http://jknkelantan.moh.gov.my/v3/uploads/files/pdfs/khc2011/OP\\_08.pdf](http://jknkelantan.moh.gov.my/v3/uploads/files/pdfs/khc2011/OP_08.pdf)
- 7-Causape A., Quilez J. & Sanchez-Acedo C. 1996. Prevalence of intestinal parasites, including *Cryptosporidium parvum*, in dogs in Zaragoza city Spain. *Veterinary Parasitology.* 7: 161-167.
- 8-Thienpont E., Rochette F. & Vanparijs O. F. 1986. Diagnosing helminthiasis by coprological examination. Turnhoutsebaan 30, 2340 Beerse, Belgium. Available from: Vet lab Services, UNIT 11. Station Road, South water, Sussex RH 13 7HQ.

- 9-Edward K. M. & Marietta V. 1959. Diagnostic Medical parasitology. W. B. SAUNDERS COMPANY .philadelphia and London. Library of Congress Catalog Card No .58-7955 :276p.
- 10- Al- Joobori T.I. 2002 .Medical parasitology laboratory manual medical helminthology. Department of medical microbiology .med .coll. Al-Nahreen Univ.:159 p.
- 11-World Health Organization 2004. Integrated Guide to Sanitary Parasitology. Regional office for the Eastern Mediterranean Amman – Jordan:120 p.
- 12-VDH- Virginia Department of Health, 2013. Cryptosporidiosis and Drinking Water. <http://www.cdc.gov/parasites/crypto/>
- 13-Meena P., Peter H. , Lucy Allen M. J. & Juliet Ch. S.2010. Clearing the Waters: A focus on water quality solutions. Pacific Institute 654 13th Street Preservation Park Oakland, CA 94612 :91p.
- 14- Hadi, A. M.; Faraj, A. A. 2008. Distribution of intestinal parasites in drinking water in some regions in Baghdad. Al – Qadisiya J. Vet. Med. Sci. 17(12):62-64.
- 15- Hadi A.M. 2005. Prevalence of intestinal parasites in children in Baghdad Al- Rusafa. MSc. Thesis. Vet. Med. Coll. Baghdad Univ. 91p.
- 16-Jones G.T., Hunt R. C. & King N.W. 1997. Veterinary pathology. 6<sup>th</sup>(ed) .William and wilkins. Baltimore: 550p.
- 17-Abaas A. Kh., Hamza S. E., Jassim S. A., Fakhri S. S. & Abid Al-Hameed F.F. 2012. Investigation of *Balantidium coli* in bottles water. Inter. J. for Sci. and Tech. 7 ( 3): 128-134.
- 18- Al-Saeed A.T. & S.H. Issa.2010. Detection of *Giardia lamblia* antigen in stool specimens using enzyme-linked immunosorbent assay. Eastern Mediterranean Health Journal EMHJ. 16 ( 4 ):362-364.
- 19- Hussein T. Kh. 2010. Prevalence and related risk factors for *Giardia lamblia* infection among children with acute diarrhea in Thi- Qar southern Iraq. Thi-Qar Med. J. (TQMJ): 4 (4): 68-74.
- 20- Al- Akeeli S. G. 2007. A study of epidemiology of *Cyclospora cayetanensis* and some intestinal protozoa for human, animal and vegetables in Baghdad /Al- Karkh. MSc. Thesis. Vet. Med. Coll. Baghdad Univ. 135p.
- 21-LeChevallier M.W., Di Giovanni G.D.,Clancy J.L., Bukhari Z., Bukhari S. & Rosen J.S. 2003. Comparison of Method1623 and Cell Culture-PCR for Detection of *Cryptosporidium* spp. in Source Waters. Appl. Envir. Microbiol. 69: 971-9.
- 22-Peter C., Merran G., Mark A., Daniel D. & Christobel F. 2005. Concentrations of pathogens and Indicators in animal feces in the Sydney watershed. Appl. Environ. Microbiol. 71(10 ): 5929-5934.

## عزل وتشخيص طفيلي الكربتوسبورديوم بالترشيح الازموزي لماء الشرب في بغداد

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### الخلاصة:

جمعت 60 عينة من مياه الشرب المرشح اوزموزيا من منازل مختلفة في بغداد، ابتداء من شهر نيسان الى شهر تشرين الاول 2012. فحصت العينات مجهريا بالطريقة المباشرة ثم بتقنية صبغة الزيل نلسن للبحث عن الطفيليات المعوية المنتشرة فيها وقد سجلت نسبة التلوث الكلية بالطفيليات لمياه الشرب 96.6% وهي: بيوض الديدان الشريطية، وبيوض ديدان الاسكارس، واكياس طفيلي الكربتوسبورديوم. ان هذه النسبة العالية تشير الى كفاءة طريقة ترشيح مياه الشرب بالاوزون لذلك نوصي بتطبيقها في محطات تصفية مياه الشرب. اما انتشار الطفيليات خلال اشهر الدراسة فقد سجل شهر نيسان و ايار اعلى نسب اصابة للكربتوسبورديوم وذلك لملائمة درجات الحرارة في هذه الاشهر والتي تتراوح بين (20-30)م°.