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Identification of *Cryptosporidium* sp. in common carp *(Cyprinus carpio)* in Tikrit city, Iraq

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Abstract :
The objective of the present study was to investigate the prevalence of *Cryptosporidium* sp. (Apicomplexa, Cryptosporidiidae) in the common carp *Cyprinus carpio* in Tikreet city and neighboring areas. 165 fish were examined for the presence of oocysts of *Cryptosporidium* sp. since 2010 until the end of May 2011 . At the time of arrival and intestinal washings with saline solution. Oocysts were concentrated by an ether-phosphate-buffered saline sedimentation technique .Smears were made with the sediment and submitted to modified acid-fast staining. The overall prevalence of *Cryptosporidium* sp. Oocysts was 16.9%. High prevalence of infection was in winter and spring 26.3% and 20.9% respectively.

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الكشف عن طفيلي البويغيات الخبيئة في الكارب العادي*Cyprinus carpio* في مدينة تكريت–العراق

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الخلاصة

تهدف الدراسة إلى الكشف عن انتشار طفيلي البويغيات الخبيئة *Cryptosporidium* sp. في سمك الكارب العادي *Cyprinus carpio* في مدينة تكريت والمناطق المجاورة لها . فحصت 165 عينة من الاسماك للكشف عن وجود اكياس بيض الطفيلي. حال وصول العينات الى المختبر غسلت الامعاء بالمحلول الفسلجي , ثم جمعت اكياس البيض باستخدام طريقة الترسيب بمحلول ether – phosphate – buffered solution .تم تحضير مسحات للراسب ثم صبغت بصبغة زيل – نلسن المحورة . كانت النسبة الكلية لانتشار اكياس بيض الطفيلي في الاسماك المفحوصة 16.9 % . اعلى نسبة للخمج كانت في فصلي الشتاء والربيع حيث كانت 26.3 % و 20.9 % على التوالي .

الكلمات الدالة :
طفيلي البويغات ، الكارب ، تكريت

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Introduction

The Common carp (*Cyprinus carpio*) is a widespread freshwater fish of eutrophic waters in lakes and large rivers in Europe and Asia (Taylor ,1977). Owing to its adaptation to a wide range of climatic and geographical conditions, many of parasites have been found in it. The most complete checklist of carp parasites records a total of 310 parasite species one of them *Cryptosporidium sp.* (Alvarez-Pellitero and Sitja-Bobadilla , 2002 ; Alvarez-Pellitero *et al*.,2004). The apicomplexan protozoal parasite *Cryptosporidium*, an intracellular extracytoplasmic protozoal parasite, was discovered by Clark in 1895 in the gastrointestinal tract of mice and has been identified within numerous vertebrate hosts, including mammals, birds, reptiles, and fish (Baruš *et al*.,2002; Fayer ,2009) . *Cryptosporidium* have a life cycle that involves both asexual and sexual reproductive cycles, which is completed within an individual host (O'Donoghue, 1995). Transmission from one host to another involves direct fecal-oral transmission often involving ingestion of oocyst-contaminated water(Franzen and Muller, 1999) .Cryptosporidiosis is recognized as a serious waterborne disease in humans and other animals which significant cause of diarrhea in humans and animals (Franzen and Muller,1999;Ryan *et al*., 2003 and Smith and Corcoran ,2004). Gastric and intestinal cryptosporidiosis have been previously identified in 14 species of marine and freshwater fish (Alvarez-Pellitero and Sitja-Bobadilla , 2002 ; Alvarez-Pellitero *et al*.,2004 and Xiao *et al*.,2004). Relatively little is known about the prevalence and geographic distribution of *Cryptosporidium* isolates that infect fish in Iraq (محيسن ,`1983 ; Al-Taee , 2008 and Ali and Al-Mahmood , 2009).

The aims of the present study were to document the prevalence of *Cryptosporidium* in common carp in Tikreet city and neighboring areas and to determine the seasonality of cryptosporidiosis.

Materials and Methods

In this study 165 fish of the common carp *Cyprinus carpio* were obtained from the local markets in Tikreet city randomly since 2010 until the end of May 2011 .Fish transferred to a laboratory of Microbiology at the Faculty of Veterinary Medicine, the samples examined on the day when it was obtained.

The intestine was dissected and placed in separate Petri dishes with physiological saline solution, and then washed with saline solution and the collected samples were centrifuged concentrated oocysts by ether-phosphate- buffered saline sedimentation technique and then separated by a density gradient centrifugation technique (Waldman *et al*.,1986). Smears were performed with the sediment and stained by a modified acid-fast(Henriksen and Pohlenz , 1981).

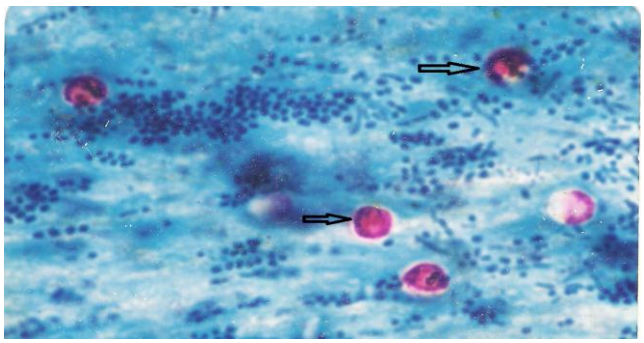
Result and discussion

Examination of stained intestinal contents smears from 165 common carp revealed that 28 fish (16.9%) were positive for *Cryptosporidium* sp. table (1) .(Hefnawy ,1989) reported similar percent (12 %) out of 80 fish were positive to *Cryptosporidium* species, while our finding was lower than (28.97 %) detected by (Al- Taee, 2008 and Ali and Al-Mahmood , 2009) in fish from Mosul and (20%) from Egypt (Hefnawy ,1989).

Table 1. Prevalence of *Cryptosporidium* sp. in common carp according to seasons

Seasons	No. cases	No. infected cases	%
Summer	44	4	9.1
Autumn	40	5	12.5
Winter	38	10	26.3
Spring	43	9	20.9
Total	165	28	16.9

The variation in the rate of infections may be due to the genus and numbers of fish, time of samples collection and examination, the rate of contaminated water. On the same approach, also proved that the coccidian incidence in fish was associated with age and sex of the fish examined , the methods used to make the diagnosis , climatic conditions and stress exposed. The appearance of detected *Cryptosporidium* oocysts after staining with modified Zeihl- Neelsen as bright red spherical oocysts with a diameter of 5.2 x 4.4 µm figure (1), and that comparable with the description by (Xio *et al*.,2004 ; O Donghue ,2005 and Fayer, 2009) .



Figure(1): The oocysts of *Cryptosporidium* sp. Staining with modified Zeihl-Neelsen 100X

Cryptosporidium lacks species specificity (Xio *et al*.,2004). Therefore, domestic and wild species of animals may be reservoirs of infection for susceptible human individuals, whether they are immunodeficient or immunologically competent, accordingly that the infected fish play a role as reservoirs and can shed massive amount of infective oocysts to the aquatic environments, so this pathogen has been isolated worldwide from rivers, lakes and other sources of water (Baruš *et al*.,2002 ; Smith and Corcoran ,2004 and O Donghue ,2005).

The results appeared the high prevalence of infection was in winter 26.3% and spring 20.9% table (1), while (Al- Taee, 2008) identified no significant difference between seasons. However the pathogen oocysts can survive for six months, suspended in natural surface waters (Smith and Corcoran ,2004),but the survival of this pathogen in the aquatic environment is significantly affected by temperature, with oocysts infectivity decreasing as temperature increases (Camphill *et al*., 1982 and Marahail *et al*.,1997). Many of the previously described *Cryptosporidium* infections in fish have not been associated with clinical signs of disease (O'Donoghue , 1995).However, evidence of acute cellular injury has been associated with infections with *Cryptosporidium* in marine fish (Ali and Al-Mahmood ,2009).

References

محيسن , فرحان صمد . (1983) . امراض وطفيليات الاسماك , البصرة . مطبعة جامعة البصرة : 121 – 143 .

Ali A. H. and Al-Mahmood S. S. (2009). Pathological effect of *Cryptosporidium mansoni* oocysts isolated from fish and treated with infra-red, microwave and ozone in common carp. Iraq J V Sc.23(1):187-192.

Al-Taee A. F.(2008) . Endoparasites of the fresh water fish *Liza abu* in Mosul, Iraq . Iraq J V Sc. 22(1):25-29.

Alvarez-Pellitero P. and Sitja-Bobadilla A. (2002). *Cryptosporidium molnarin*. sp. (Apicomplexa: Cryptosporidiidae) infecting two marine fish species, *Sparus aurata* L. and *Dicentrarchus labrax* L. Int J Parasitol 32:1007–1021.

Alvarez-Pellitero P., Quiroga M.I., Sitja-Bobadilla A, et al.(2004). Cryptosporidium scophthalmin. sp.(Apicomplexa: Cryptosporidiidae) from cultured turbot Scophthalmus maximus: Light and electron microscope description and histopathological study. Dis Aquat Organ 62:133–145.

Baruš, V., Peňáz, M. and Kohlmann, K. (2002). *Cyprinus carpio* (Linnaeus, 1758). In: P.M. Banaresku and H.J. Paepke (Eds.), The Freshwater Fishes of Europa. Cyprinidae, Carassius to *Cyprinus* Gasterosteidae AULA-Verlag: Wiebelsheim: 85-179.

Camphill L., Tzipori S., Hutchison G., Angus W.(1982). The effect of disinfectanls on survival of Cryptosporidium oocyts. Vet Rec . 111: 414–415.

Fayer R. (2009). Taxonomy and species delimitation in *Cryptosporidium.* Exp Parasitol. In press.

Franzen C. and Muller A. (1999). *Cryptosporidia and microsporidia: Waterborne diseases in the immunocompromised host. Diagn Microbiol Infect Dis 34:245–262.*

Hefnawy Y.(1989). *Cryptosporidium* affection of fresh water Nile fish in Assiut province. Assiut Vet Med J. 21: 130–133.

Henriksen A., Pohlenz J.F.L.(1981). Staining of *Cryptosporidium* by a modified Ziehl-Neelsen technique. Acta Vet Scand. 22: 594-596.

Marahail M.M., Naumorits D, Ortega Y, Sterling C.R.(1997). *Cryptosporidium parvum* in bornewater Protozoan pathogens. Chin Microbiol Rev . 10: 74–76.

O'Donoghue P.J. (1995). *Cryptosporidium* and cryptosporidiosis in man and animals. Int J Parasitol 25:139–195.

Ryan U., Xiao L., Read C., et al. (2003). Identification of novel *Cryptosporidium* genotypes from the Czech Republic. Appl Environ Microbiol 69:4302–4307.

Smith H.V., Corcoran G.D.(2004). New drugs and treatment for cryptosporidiosis. Curr Opin Infect Dis 17:557–564.

Taylor J.R. (1977). Hybridization of *Cyprinus carpio* and *Carassius auratus*, the first two exotic species in the lower Laurentian Great Lakes. Enviro Bio Of Fishes 1(2):205-208..

Waldman E., Tzipori S., Forsyth J.R.L. (1986). Separation of *Cryptosporidium* species oocysts from feces by using a percoll discontinuous density gradient. J Clin Microb .23:199-200.

Xiao L., Fayer R., Ryan U., Upton S.J. (2004). *Cryptosporidium* taxonomy: Recent advances and implications for public health. Clin Microbiol Rev 17:72–97.