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Detection of some Helminthes and Protozoa Parasites in Different Fish ponds in Sulaimani Province.

Muqdad K. Ali[1] **Bahzad H.S. Mustafa[2]**

[1]*Animal Science Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, IRAQ.*

[2]*Animal Science Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, IRAQ.*

*Corresponding Author: muqdad.ali@univsul.edu.iq.

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ABSTRACT

This study aimed to identify specific types of gastrointestinal helminthes and protozoa parasites that infect the Cyprinidae (Cyprinus carpio). During the growing period, 400 fish were randomly separated from five spawning fish ponds in Sulaimani province: Mara Rash (P1), Qalachwalan (P2), Kandashin (P3), Kalawanan (P4), and Piramagrun (P5). Fish infection with parasites was found to be 30.25% prevalent overall, with a high distribution rate of infestation in the P3 region of 48.75%. Diphyllobothrium latum, Ligula intestinalis, and Bothriocephalusacheilognathi were identified as the three helminthes species,with an overall prevalence rate of 22.75%, P3 had the highest examination rate of helminthes, with 9.25% of the Ligula intestinalis species, 7.5% of Diphyllobothrium latum, and 6% of Bothriecephalusopsariichthydis (acheilognathi) species. The t-test revealed the largest differences, at p-value (Two-tailed) < alpha 0.05, between the helminthes and protozoa parasites' prevalence and pond area. Three protozoa species have been identified, including Cryptosporidium spp., E. histolytica, and Eimeria spp.All spawning fish ponds had an overall prevalence rate of 7.75% of fish infested with protozoa; the greatest percentage was 2.75% for E. histolytica, followed by 2.5% for Eimeria spp., and 1.5% for Cryptosporidium spp. Based on the ponds, the most common protozoa-infested fish were 5% of E. histolytica in P1, 3.75% of Cryptosporidium spp. in P3, and 3.75% of Eimeria spp. in P5. The investigation demonstrates that 1% of the mixed in various ponds were afflicted with protozoan parasites.

***Keywords*:** Parasite, Helminthes, Protozoa, Cyprinidae (Cyprinus carpio).

INTRODUCTION

Fish is an essential food source that plays a significant part in the world economy. It is undoubtedly the most important source of protein of the highest quality humans have access to, making up roughly 16 % of animal protein [1]. Carps are classified as members of the Cyprinidae family, a widely distributed family of freshwater fishes globally [2]. Bothriocephalosis is the intestinal infection of certain fish by the cestode Bothriocephalusacheilognathi (Yamaguti 1934), a Pseudophyllidean tapeworm. The infecting organism is also known as the Asian fish tapeworm and as the Chinese tapeworm and has had several synonymous scientific names, including.

BothriocephalusopsariichthydisBothriocephalusopsalichthydis,Bothriocephalusfluviatilis,Schyzocotlefluviatilis,Bothriocephalusgowkongensis,andBothriocephalusphoxini.Bothriocephalusacheilognathi, also known as the Asian tapeworm, is a freshwater fish parasite that originated from China and Eastern Russia.It is a generalized parasite that affects a wide variety of fish hosts, particularly cyprinids, contributing to its overall success [3].Dibothriocephalus latus (Linnaeus, 1758), commonly referred to as Diphyllobothrium latum (Cestoda: Diphyllobothriidea), is a zoonotic parasite that causes diphyllobothriasis in human hosts. It is spread by fish ingestion. Diphyllobothriasis is a zoonotic ailment caused by a cestode parasite primarily affecting the intestines. Diphyllobothrium, also known as the "fish tapeworm" or the "broad tapeworm," is contracted by humans by consuming fish containing infectious larvae from the genus Diphyllobothrium. In the context of Iraq, this particular fish species is often regarded as a highly valued and sought-after protein source. The cultivation of carp as a source of livelihood and income has gained significant popularity nationwide due to its high nutritional content [4]. It has been observed that a significant proportion of freshwater fish have substantial parasitic illnesses, which have a detrimental impact on their nutritional quality, as highlighted by [5]. According to [6], parasites can have many effects on the health of fish, including mechanical impacts, physiological disturbances, reproductive impairments, and even mortality. Several studies have documented the presence of parasite infections in the common carp (*C. carpio*) [7 and 8]. Furthermore, several forms of parasite infections have been documented in this particular species within the geographical region of Iraq [9]. Furthermore, several regions in Iraq, namely Salah Al-Deen province, Babylon, Al-Diwaniyah, Kurdistan, and Najaf al-Ashraf, have experienced infestations of carp caused by parasitic agents. These findings have been documented in various studies conducted by [10 and 11]. Due to its remarkable ability to adapt to diverse climatic and geographical situations, numerous

parasites have been identified within it. One parasite species included in the most comprehensive list of carp parasites is Cryptosporidium sp. [12]. This study aims to detect some gastrointestinal parasites in different fish ponds in Sulaimani province.

Material and Methods:

1. Area sample collection

A total of four hundred common carp (*Cyprinus carpio*) were gathered in Sulaimani province from five distinct ponds: P1 Mara Rash; P2 Qalachwalan; P3 Piramagrun; P4 Kandashin; and P5 Kalawanan. This investigation was carried out from July until the end of December 2023. Fish have a look about inside. The ventral section of the body wall was sliced, and the intestinal components—particularly the nematode worms and cestodes—were dissected and analyzed to identify the gastrointestinal parasites. Fish were moved to the lab after being gathered using a bag net. The fish was recognized by [13].

2- Examination for parasites: -

A- Identification of gastrointestinal parasites

A fecal examination was conducted to check for the presence of protozoan oocysts and helminthes eggs using the straightforward fecal centrifugation flotation procedure, as described by [14]. To put it simply, two grams of feces and sixty milliliters of sugar solution were combined; the combination was then poured into test tubes using a tea filter, and single-step centrifugation was run for ten minutes at 3000 rpm [15]. To get the mount, a plastic pipette was used to extract a few drops from the top layer. The techniques outlined by [16] were used to identify parasite eggs, oocysts, and larvae based on size and shape.

B-Direct inspection of fecal smears

The process of the direct smear approach involves mixing a small amount of excrement with a salt or water solution. The process is as follows: once the mixture is put onto a slide, a cover glass is placed over it. The whole smear is then examined under a low-power microscope. Inspect the mixture to see whether it contains worm eggs, larvae, protozoa trophozoites, or cysts. [17] and [18] state that the use of a small amount of feces and the presence of fecal debris impair the validity of this testing method.

3- Analysis of statistics:

To analyze the data, the percentage of those who tested positive for gastrointestinal parasites was computed. Applying a t-test, which was further discussed in detail.

Results:

Four hundred common carp (*Cyprinus carpio*) were harvested from five ponds Sulaimani province. A substantial difference (p-value < alpha 0.05) was seen in the prevalence rate of *C. carpio* with certain helminthes and protozoa. Fish infected with internal parasites (helminthes and protozoa) had an overall prevalence rate of 30.75%. Table 1 show that the highest documented prevalence of contaminated fish was 48.75% in Piramagrun (P3) and the lowest prevalence of 15% in Kandashin (P4).

Table.1: The prevalence rate of some species of internal parasites(both Helminthes,Protozoa) in Sulaimani province Fishpond.

Location/ pond	No. of fish exam	No. of fish positive with parasite (Helminthes and Protozoa)	Prevalence rate %	t (Observed value)	t (Critical value)
P1	80	32	40.0		
P2	80	21	26.25		
P3	80	39	48.75	3.957*	3.182
P4	80	12	15		
P5	80	19	23.75		
Overall	400	123	30.75		

P1= Mara Rash, P2= Qalachwalan, P3= Piramagrun, P4= Kandashin, and P5= Kalawanan

Prevalence rate: p-value (Two- tailed) 0.029< alpha 0.05

As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The location of several pounds of fish afflicted with certain helminthes differed significantly (p value < alpha 0.05) as shown in Table 2. Fish with helminthes had a prevalence incidence of 22.75%. The majority of fish infected with helminthes (38.75%) were found in Piramagrun (P3), whereas Kandashin (P4) had the lowest incidence (10%).

Table 2: The prevalence rate of fish infestation with some helminthes species according tosomeFishponds in Sulaimani province.

Location/ pond	No. of fish exam	No. of fish(+) with Helminthes	Prevalence rate %	t (Observed value)	t (Critical value)
P1	80	23	28.75		
P2	80	16	20		
P3	80	31	38.75	3.435*	3.182
P4	80	8	10		
P5	80	13	16.25		
Overall	400	91	22.75		

P1= Mara Rash, P2= Qalachwalan, P3= Piramagrun, P4= Kandashin, and P5= Kalawanan
Prevalence rate: p-value (Two- tailed) 0.041 < alpha 0.05

As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

A significant difference (p-value < alpha 0.05) between the locations of various pounds of fish afflicted with protozoa is shown in Table 3. The overall prevalence rate of protozoa-infested *C. carpio*is 7.75%. Protozoa-infested fish were more common in Mara Rash (P1), where the frequency was 11.25%, whereas Kandashin (P4) had the lowest incidence 3.75%.

Table 3: The prevalence rate of fish infestation with protozoa species in some Fishponds in Sulaimani province.

Location/ pond	No. of fish exam	No. of fish (+) with protozoa	Prevalence rate %	t (Observed value)	t (Critical value)
P1	80	9	11.25		
P2	80	5	6.25		
P3	80	8	10	5.284*	3.182
P4	80	3	3.75		
P5	80	6	7.5		
Overall	400	31	7.75		

P1= Mara Rash, P2= Qalachwalan, P3= Piramagrun, P4= Kandashin, and P5= Kalawanan
Prevalence rate: p-value (Two- tailed) 0.013 < alpha 0.05

As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

Several parasites, including Diphyllobothrium latum, Ligula intestinalis, and Bothriecephalusopsariichthydis, were detected, as shown in Table 4. This was the initial identification of fish gills in the province of Sulaimani. The total helminthes prevalence rate across all ponds was 22.75; the greatest prevalence was 9.25% for Ligula intestinalis, followed by 7.5% for Diphyllobothrium latum, and 6% for Bothriecephalusopsariichthydis. The pound (P3) had a high frequency of contaminated fish with Diphyllobothrium latum, Ligula intestinalis, and Bothriecephalusopsariichthydis at 38.75%. The highest is Ligula intestinalis, followed by Diphyllobothrium latum (13.75%) and Bothriecephalusopsariichthydis (8.75%). With 10% for each of the three helminthes species, the pond (P4) had the lowest overall prevalence rate.

Table 4: Distribution ofsomehelminthes species infested fish accordingto the FishpondinSulaimani province

Helminthes species	Total No. of Fish exam 400	Different Pound				
		P1	P2	P3	P4	P5
		No. of fish exam-80	No. of fish exam-80	No. of fish exam-80	No. of fish exam-80	No. of fish exam-80

	Total No.-	Total No (+) %	No. -	No. (+) %	No. -	No. (+) %	No. -	No. (+) %	No. -	No. (+) %	No. -	No. (+) %
Diphyllobothrium latum	309	30 7.5	57	8 10	64	7 8.75	49	11 13.75	72	1 1.25	67	3 3.75
Ligula intestinalis		37 9.25		9 11.25		5 6.25		13 16.25		4 5		6 7.5
Bothriocephalusacheilognathi		24 6		6 7.5		4 5		7 8.75		3 3.75		4 5
Overall		91 22.75		23 28.75		16 20		31 38.75		8 10		13 16.25

P1= Mara Rash, P2= Qalachwalan, P3= Piramagrun, P4= Kandashin, and P5= Kalawanan

Table 5 lists the three protozoa species that were found infected fish, along with a number of parasites. Among these species were Eimeria spp., Cryptosporidium spp., and *E. histolytica*. The total prevalence rate was 7.75%; Eimeria spp. had the largest proportion (2.75%), followed by Eimeria Spp. (2.5%), and Cryptosporidium spp. (1.5%). For mixed infestations, 1% was the lowestproportion. The degree of infestation varies among the fish affected in the pond: Mara Rash (P1) has the greatest infection, while Kandashin (P4) has the lowest, at 3.75%. 2.5% of the mixed populations of protozoa were observed in Mara Rash (P1).

Table 5: Distribution of some protozoa species in some regions in Sulaimani Province

Protozoa species	Total No. of Fish exam		Different Pound									
			P1		P2		P3		P4		P5	
	400 fish		No. of fish exam-80		No. of fish exam- 80		No. of fish exam- 80		No. of fish exam- 80		No. of fish exam- 80	
	No.	No.+ %	No.	No. + %	No.	No. + %	No.	No. + %	No.	No. + %	No.	No. + %
E. histolytica	369	11 2.75	71	4 5	75	2 2.5	82	3 3.75	77	1 1.25	74	1 1.25
Cryptosporidium spp.		6 1.5		1 1.25		1 1.25		2 2.5		1 1.25		1 1.25
Eimeria Spp.		10 2.5		2 2.5		2 2.5		2 2.5		1 1.25		3 3.75
Mixed infection		4 1		2 2.5		0 0		1 1.25		0 0		1 1.25
Overall		31 7.75		9 11.25		5 6.25		8 10		3 3.75		6 7.5

P1= Mara Rash, P2= Qalachwalan, P3= Piramagrun, P4= Kandashin, and P5= Kalawanan.

Discussion:

Four hundred fish had been randomly taken out of five spawning fish ponds during the growing period: Mara Rash (P1), Qalachwalan (P2), Piramagrun (P3), Kandashin (P4), Kalawanan (P5) in Sulaimani province. Studying the prevalence of these parasites, which include Diphyllobothrium latum [20], is crucial. The total percentage of fish infected with parasites was 22.75%. Protozoa and helminthes had prevalence rates of 7.75% and 30.75%, respectively. Three helminthes species were detected; two of them, Diphyllobothrium latum (distribution: 7.5%), and Bothriecephalusopsariichthydis (distribution: 6%), were found in the gills. Ligula intestinalis showed a high infection rate of 9.25%. Listed five species of cestodes; the two most significant ones, Bothriocephalusopsariichthydis, and Diphyllobothrium latum, were taken from the Tigris River [21]. Eight parasitic species were discovered in Iraqi cyprinid fish, including two cestode species (Proteocephalusosculatus

and Thriocephalusacheilognathi) and one protozoon (Trichodinamutabilis)[22]. The intestinal tract of C. carpio from several fish farms close to Baghdad was the source of the first documented case of B. acheilognathi in Iraq [23]. Two further species of Bothriocephalus have been reported to exist in Iraq, B. gowkongensis was discovered in the intestines of four different fish species, and B. opsariichthydis was detected in the intestines of six different fish species [24]. Leuciscus lepidus from the Greater Zab River and C. carpio from the Lesser Zab River were the sources of this parasite in the Kurdistan area [25]. According to references [26] and [19], B. acheilognathi is synonymous with B. gowkongensis and B. opsariichthydis. From a minimum of one cestode species in 15 fish hosts to a maximum of five cestode species in Silurustriostegus alone, the total number of cestode species observed for each fish host species varied. The total number of cestode species observed varied for each fish host species, ranging from a minimum of one cestode species in 15 fish hosts to a maximum of five cestode species in Silurustriostegus alone. The number of fish hosts reported for these cestodes varied, with a high of nine hosts for Bothriocephalusacheilognathi in Basrah, Iraq [27]. The first evidence of B. gowkongensis was found in Iraq in C. carpio from fish farms that are not identifiable [23]. It is believed that B. acheilognathi is interchangeable with B. opsariichthydis and B. gowkongensis [28]. There have been reports of an undetermined species of Bothriocephalus being hosted by four fish hosts in Basrah. These include C. carpio from the Garmat Ali River [30] and C. luteus from the Al-Hammar wetland by [29]. Cyprinus carpio was found to have three parasite species: Dactylogyrusminutus, Caryophyllaeuslaticeps, and Bothriocephalusacheilognathi. Throughout the year, Dactylogyrusminutus infections in fish have been reported in Turkey [31]. This study found that the overall prevalence of fish infested with three protozoa;Cryptosporidium spp., E. histolytica, and Eimeria spp., was 7.75%. Of these, E. histolytica had the highest prevalence (2.75%), followed by Eimeria spp. (2.5%), and Cryptosporidium spp. (1.5%). The three species mixed infestation was found in Mara Rash at 2.5%. The high prevalence rate of sporozoan infection observed in this investigation contradicts [19]. Five kinds of protozoan parasites were found in fish from the Tigris River in Tikrit City, Salah Al-Deen region, Iraq; the two most important species were Eimeria sinensis Chen and Apiosomamegamicronucleatum (Timofeev)[32]. The fish of the Al-Diwaniyah province belong to the genera Cryptosporidium and Eimeria, each of which has one unknown species, as illustrated in the following systematic classification by [33]. In the intestines of C. zillii and Planilizaabu, oocysts of Cryptosporidium sp. have been found by [34] and [35]. Two Cryptosporidium species have been found in Iraq; furthermore, three fish species have been found to harbor some unidentified Cryptosporidium species as far[36]. Coccidians belonging to the genus Eimeria, which includes species like E. subepithelialisMoroff and Fiebiger, infect internal organs and cause significant lesions in addition to causing nodular coccidiosis in carp. Fish that are afflicted by blood parasites known as coccidians may develop anemia, which manifests as pale gills [37]. There have also been reports from Iraq regarding two more species of Bothriocephalus: B. opsariichthydisYamaguti, 1934, and B. gowkongensis Yeh, 1955 [38]. Both of these taxa are regarded as synonyms of B. acheilognathi, according to [26]. There are now 21 host species in Iraq for B. acheilognathi including the two synonyms mentioned above [3]. Intestinal Ligula (L., 1758) Bloch, 1782, was seen in the internal organs of C. carpio and C. idella [39]. For the first time, L. intestinalis was identified in Iraq as a plerocercoid that was found in the body cavity of Leuciscus vorax, also known as A. vorax, from the Shatt Al-Arab River [40]. There are now 13 fish host species for this species in Iraq [38]. The adult stage of Listeria intestinalis was found in the intestine of a moorhen named Gallinula chloropus that was found in the vicinity of Baghdad, Iraq [41]. In the intestine of both C. idella, BothriocephalusacheilognathiYamaguti, 1934, was discovered [42]. It is pertinent to note that [43] described this worm under the synonym B. opsariichthydis. In Iraq, the gut of L. vorax (also referred to as A. vorax) was discovered in Al-Tharthar Lake [44].

Conclusion:

The focus of this investigation has been *D. latum* plerocercoid larvae found in Kurdistan's lakes and ponds. As a result, the fish breeder has the responsibility for pond upkeep and the timely use of prophylactic treatments. Nonetheless, consumers need to be made aware of the risks involved with consuming infected seafood.

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

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الكشف عن بعض الديدان والاوالى الطفيلية المعوية في الأحواض السمكية المختلفة في محافظة السليماني

بهزاد حمه صالح مصطفى [1] مقداد كمال علي[1]

[1]قسم علم الحيوان، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، العراق.

الخلاصة

أجريت هذه الدراسة لفحص وتشخيص بعض أنواع الديدان الطفيلية المعوية والطفيليات الأولية التي تصيب فصيلة*Cyprinidae (Cyprinuscarpio)*. تم عزل 400 سمكة عشوائياً من 5 أحواض تربية سمكية؛مارا راش(*P1*)، قلاجوالان (*P2*)، كانداشين (*P3*)، كالاوانان (*P4*)، وبيرامگرون (*P5*) في محافظة السليمانية.بلغ معدل انتشار إصابة الأسماك بالطفيليات 30.25%، مع ارتفاع معدل توزيع الإصابة في منطقة *P3* بنسبة48.75%.تم ملاحظة ثلاثة أنواع من الديدان الطفيلية؛*Diphyllobothrium latum*، *Ligula* المعوية، و*Bothriocephalusacheilognathi*، مع معدل انتشار إجمالي بلغ 22.75%، وارتفاع الإصابة بأنواع *Ligula* المعوية بنسبة9.25% تليها*Diphyllobothrium latum %7.5*، وأنواع *Bothriecephalusopsariichthydis (acheilognathi)* كانت6%.بينما تم فحص الإصابة العالية بثلاثة فحوصات للديدان الطفيلية المكتشفة في *P3*.عند إجراء اختبار *t*، أظهرت أعلى الفروق بين أحواض الأسماك تبعاً لانتشار الديدان الطفيلية والطفيليات الأولية، عند القيمة*p-value (Two-tailed) < alpha 0.05*تم التعرف على ثلاثة أنواع من الأوليات في الأسماك المصابة، بما في ذلك *E. histolytica*.*Cryptosporidium spp*و*Eimeria spp*.بلغت نسبة الانتشار الإجمالية 7.75% من الأسماك المصابة بالطفيليات، على النحو التالي:وكانت أعلى نسبة هي *E. histolytica*بنسبة 2.75%، تليها *Eimeria spp*. بنسبة 2.5%، و *Cryptosporidium spp*.وسجلت النسبة الأقل بنسبة 1.5% في جميع أرطال أسماك التربية.كان معدل انتشار الأسماك المصابة بالطفيليات الأولية وفقًا للوزن هو *E. Histolytica*في *P1* بنسبة 5%، و*Cryptosporidium spp*في *P3* بنسبة 3.75%، و*Eimeria spp*.، في *P5* بنسبة3.75% كانت المجموعة المختلطة مصابة بثلاثة أنواع مسجلة في مارا راش. أظهرت الدراسة أن الخليط المصاب بالطفيليات الأولية في أحواض مختلفة بلغ1%.

الكلمات المفتاحية : طفيليات،الديدان، الأولي الطفيلية، *Cyprinidae (Cyprinus carpio)*.