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Distribution and Identification of the Parasitic Nematode *Contracaecum spp*. in Planiliza Abu (Heckel, 1843: Mugiliformes, Mugilidae) From Razzaza Lake, Karbala Province, Iraq

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Abstract: The Anisakidae family's most important genus, Contracaecum, contains various host species participating in their life cycles and considerably negatively influencing humans' health. *Planiliza abu* specimens from the local market in Karbala, Iraq, were examined for *Contracaecum spp*. in the abdominal cavity. A survey on parasites of Planiliza abu of Razzaza Lake was carried out over three years, from November 2019 to December 2021. A total of 148 fish were collected and tested for infection in 2019, 277 in 2020, and 577 in 2021. The prevalence in November and December 2019 was 48.73%, 65.08% in 2020, and 9.6% in 2021, respectively. Microscopically, it revealed larval type Contracaecum L3 (only in all fish). Baghdad University's Iraq Natural History Research Center and Museum confirmed the parasite as *Contracaecum spp.* infection rates were greatest in January and February and lowest in July and August 2021. Regarding third-stage larvae, female fish (n=25) had a higher infection rate than male fish (n=8), with 40 and 18, respectively. This study showed that industrial pollution, human activity, and seasonal patterns all influenced infection rates. The molecular diagnosis of *Contracaecum spp*. in different hosts is very important for studying their population ecology and biology, and this should be looked into in future studies

Keywords: *Contracaecum spp.*, Light microscopic examination, Planiliza abu, Risk factors

Irak, Kerbela Eyaleti Razzaza Gölü'ndeki Planiliza Abu'da (Heckel, 1843: Mugiliformes, Mugilidae) Parazitik Nematod *Contracaecum spp*'nin Dağılımı ve Tanımlanması

Özet: *Anisakidae* familyasının en önemli cinsi olan *Contracaecum*, yaşam döngülerine katılan ve insan sağlığını önemli ölçüde olumsuz etkileyen çeşitli konukçu türleri içerir. Irak Kerbala'daki yerel pazardan alınan Planiliza abu örnekleri *Contracaecum spp*. karın boşluğunda. Razzaza Gölü'ndeki *Planiliza abu* parazitleri üzerine Kasım 2019'dan Aralık 2021'e kadar üç yıl boyunca bir anket yapıldı. 2019'da 148, 2020'de 277 ve 2021'de 577 balık toplandı ve enfeksiyon için test edildi. Prevalans sırasıyla, Kasım ve Aralık 2019'da %48.73, 2020'de, %65.08 ve 2021'de %9.6 idi. Mikroskobik olarak, larva tipi Contracaecum L3'ü ortaya çıkardı (sadece tüm balıklarda). Bağdat Üniversitesi Irak Doğa Tarihi Araştırma Merkezi ve Müzesi, parazitin *Contracaecum spp* olduğunu doğruladı. Enfeksiyon oranları Ocak ve Şubat aylarında en yüksek, Temmuz ve Ağustos 2021'de en düşüktü. Üçüncü aşama larvalarla ilgili olarak, dişi balıklarda (n=25), erkek balıklardan (n=8) (sırasıyla 40 ve 18) daha yüksek bir enfeksiyon oranına sahipti. Bu çalışma endüstriyel kirlilik, insan faaliyetleri ve mevsimsel kalıpların hepsinin enfeksiyon oranlarını etkilediğini gösterdi. *Contracaecum spp*.'nin moleküler teşhisi farklı konaklarda popülasyon ekolojilerini ve biyolojilerini incelemek için çok önemlidir ve gelecekteki çalışmalarda incelenmelidir.

Anahtar Kelimeler: *Contracaecum spp.*, Işık mikroskobik inceleme, Planiliza abu, Risk faktörleri

1.Introduction

Contracaecum spp. is a parasitic nematode of the Anisakidae family. They have been shown to have extremely pathogenic effects on animals (fish, birds, and marine mammals) and humans(1). Anisakiasis is a parasitic infection caused by nematode larvae from the genera Anisakis, Pseudoterranova, and Contracaecum of the Anisakidae family (2). For the first time, Contracaecum spp. larvae were found in ten different fish species from various locations in Iraq(3). Adult worms of such larvae have been found in several aquatic birds in Iraq (4). Anisakis species have a complicated and heteroxenous life cycle (the ultimate definitive hosts of these nematodes are marine mammals with a complex life cycle), which includes a variety of marine creatures at various levels of the trophic web in the marine environment. They are parasites that live in the larval stages of many fish species across the globe, some of which are commercially important (5). Anisakidosis has largely been documented in areas where uncooked fish consumption is common. The third-stage larvae of anisakid worms are primarily devoured

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when fish feed on intermediate crustacean hosts or teleostean transit hosts (6). These nematode parasites exploit various crustacean and fish species as intermediate or paratenic hosts, with humans serving as accidental hosts (7). The parasitic Anisakidae family comprises eight unique parasite genera and 46 distinct parasite species. The Anisakidae parasites mostly infect marine animals, although they may also infect paratenic hosts such as molluscs, small crustaceans, and fish (8). There are around 100 species in this genus. *Contracaecum spp*. is comprised of 142 species (9,10). Anisakidosis is a unique disease that affects humans and is caused by members of the Anisakidae family. Since the discovery of these parasites in humans in the early 1960s, anisakid nematodes have spread globally and infected many invertebrates and vertebrates throughout their life cycles (11). The presence of anisakid larvae in fish endangers people and discourages consumers from purchasing infected products (12). Fish-borne parasitic zoonoses are a significant component of food-borne parasitic zoonoses. In several Asian nations, the number of fish-borne nematodiasis seems to be increasing (13). No studies have been conducted to determine the prevalence of Contracaecum larvae in Planiliza abu collected from different locations in Karbala and across Iraq. Given the above facts, this research aimed to determine the general prevalence and key risk factors related to the occurrence of Contracaecum spp. collected from the viscera of Planiliza abu in the local market of Karbala, Iraq.

2. Materials and Methods

2.1. Location

Karbala, also known as Kerbala, is the capital of the Karbala Governorate in central Iraq, about 100 kilometers southwest of Baghdad, and has an estimated population of 1 million pilgrims that come each year. Milh Lake, also known as Razzaza Lake, can be found in western Iraq, just west of Karbala (32°41′N, 43°40′E). It is Iraq's second-biggest freshwater lake and was previously a major source of fish. The lake, which covers an area of 1810 square kilometers and is 40 meters above sea level, can store 26 billion cubic meters of water. Some of the water from Habbaniyah Lake is sent into Razzaza Lake through a controlled exit route or canal from the Euphrates River (Fig. 6).

Figure 6: Map of Iraq, Karbala province and Razzaza Lake location.

2.2. Fish collection and inspection

The specimens were obtained fresh from Karbala's local market. Count, measure, and weigh all the fish. Following traditional dissection, each specimen was examined for anisakid larvae. Each specimen's viscera and flesh were investigated individually in two different Petri dishes. After a visual examination, the viscera and flesh were dissected under a stereoscopic microscope, and the worms were counted for each sample.

2.3. Morphological identification

Using a light microscope, examine the morphology of isolated larvae. The larvae of each fish species were rinsed in saline solution for 30 min, briefly fixed in alcohol, and then inspected under a light microscope. All the larvae were morphologically evaluated. Nematode larvae were collected and cleaned in physiological saline from fish body cavities, and lactophenol was used to clear the nematodes for morphological evaluation. In all fish, light microscopic inspection showed a larval type compared with the Contracaecum type (Figs. 1-5). The fish parasite was confirmed as *Contracaecum spp*. by the Iraq Natural History Research Center and Museum at Baghdad University.

2.4. Morphological characteristics of Contracaecum

As the genus name implies, these worms have two oppositely oriented ceca as part of their digestive system (Fig. 1). They also have an excretory orifice at the front of their bodies. Because they are the most consistent at all developmental stages, they should be regarded as the most relevant morphological traits for distinguishing Contracaecum species from the rest of the anisakid nematodes (1).

Table 1: Size range, number of infected and uninfected fish, and infection rate in the *Planiliza abu* sampled in 2021 in Karbala, Iraq.

		Fish length (range cm)	Total no. of fish	No. of infected fish	Infection rate	No. of nematodes in fish
2019	**November**	10-18	71	18	25.35	64
	December	5-18	77	18	23.38	95
2020	**January**	8-15	40	13	32.50	115
	February	9-15	68	15	22.06	92
	March	*	*	*	*	*
	April	*	*	*	*	*
	May	*	*	*	*	*
	June	*	*	*	*	*
	July	*	*	*	*	*
	August	*	*	*	*	*
	September	8-15	49	14	28.57	46
	October	10-13	40	4	10.00	5
	November	10-15	45	10	22.22	31
	December	12-19	35	15	42.86	37

Table 2: Size range, number of infected and uninfected fish, and infection rate in the *Planiliza abu* sampled in 2019 and 2020 in Karbala, Iraq.

						Fish sex			
		Fish length (range cm)	No of non-infected fish	No. of infected fish	Infection rate	Male	%	Female	%
	January	6.5-11	43	6	12.2	11	22.4	38	77.6
	February	8-12	41	5	10.9	9	19.6	37	80.4
	March	7.5-11	41	3	6.8	8	18.2	36	81.8
	April	6.5-10	59	4	6.3	16	25.4	47	74.6
	May	5.8-10	39	3	7.1	7	16.7	35	83.3
	June	7.5-10.5	45	2	4.3	4	8.5	43	91.5
2021	**July**	8-11	38	0	0	4	10.5	34	89.5
	August	7.5-11	42	0	0	2	4.8	40	95.2
	September	8-11	45	3	6.3	11	22.9	37	77.1
	October	8-11	50	2	3.8	10	19.2	42	80.8
	November	7.5-11	55	2	3.5	10	17.5	47	82.5
	December	7.5-11	46	3	6.1	5	10.2	44	89.8
	Total	6.5-12	577	33					

3. Results

In Planiliza abu, only Contracaecum spp. third-stage larvae (L3) were discovered, and there were no Contracaecum spp. fourth-stage larvae (L4) found. The bodies of the third-stage larvae were rather substantial. The cuticle becomes annular and forms a collar at the front end, and an excretory orifice opens at the anterior end. The esophagus ends in a short, sub-globular ventriculus. The intestinal cecum is much shorter than the ventricular appendix; the intestine is large and fills the rest of the body. The tail is highly pointed and has a spine. January and February had the highest infection rates, while July and August had the lowest. Female (n=25) fish had a greater infection rate than male (n=8) fish, with 40 and 18 third-stage larvae collected, respectively (Table 2).

A survey on nematode parasites of Planiliza abu of Razzaza Lake was completed for three years, from November 2019 to December 2021. Because of the COVID-19 pandemic, no data was obtained between March and August 2020. A total of 148 fish were collected and tested for nematode infection in 2019, 277 in 2020, and 577 in 2021. The infection rate in November and December 2019 was 48.73%, 65.08% in 2020, and 9.6% in 2021, respectively (Tables 1 and 2).

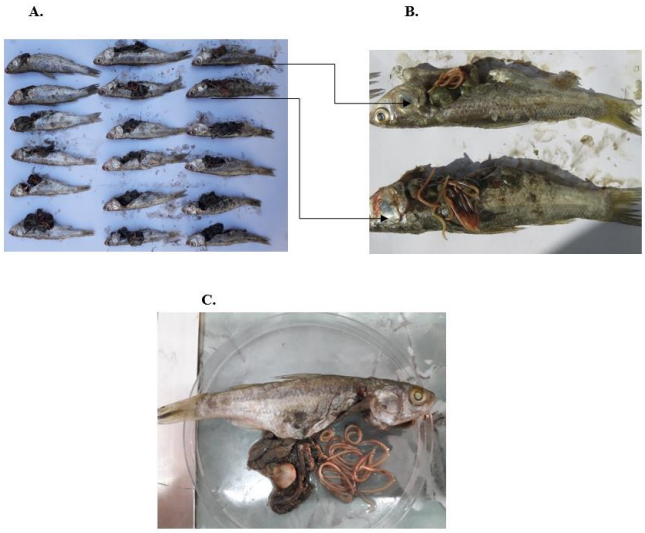

Figure 1: A, B, C. Counting the third-stage larvae of *Contracaecum spp*. in the viscera of dissected Planiliza abu.

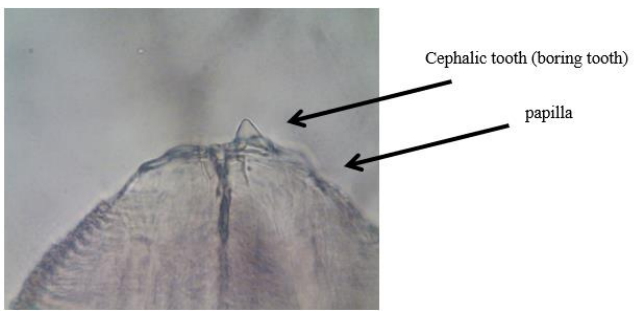

Figure 2: The anterior part of a third-stage larva of *Contracaecum spp*. provided with a ventral cephalic tooth (arrow (Scale bar= 0.10 mm).

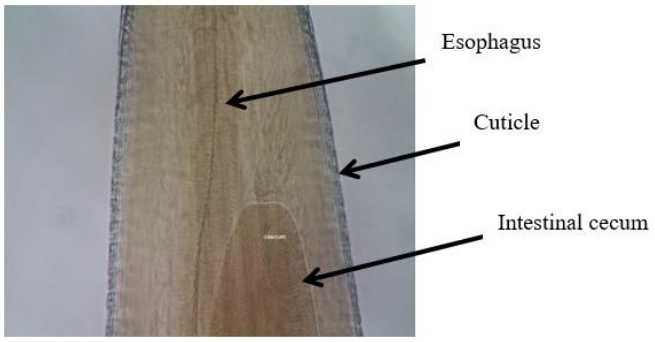

Figure 3: The anterior region showing the esophagus (OE), intestinal cecum (IC), and cuticle (C) (Scale bar = 0.10 mm).

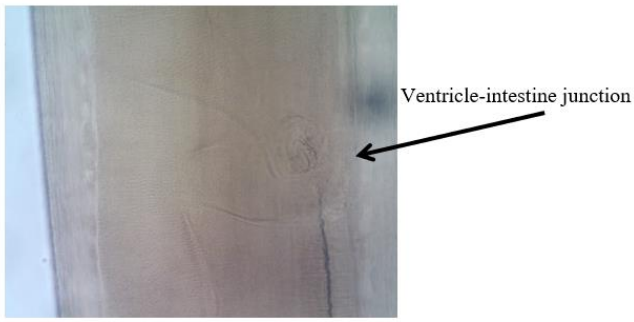

Figure 4: The middle region showing the ventricle-intestine junction (Scale bar = 0.10 mm).

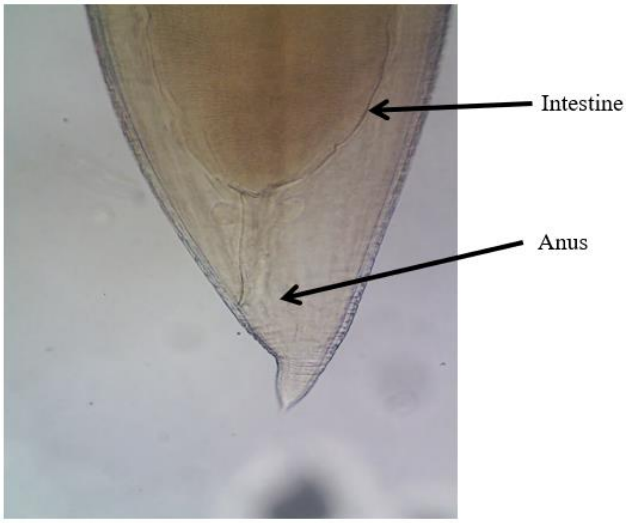

Figure 5: The posterior part of a third-stage larva of *Contracaecum spp.* showing conical tail, intestine, and anus (arrow (Scale-ar= 0.10 mm).

4. Discussion and Conclusion

Contracaecum, the most important nematode genus in the Anisakidae family, has a wide range of host species, a huge number of species, and a significant detrimental impact on human health. Contracaecum species in the abdominal cavity of Planiliza abu specimens from a local market in Karbala, Iraq, was studied. Over three years, from November 2019 to December 2021, researchers studied the nematodes that parasitize the Planiliza abu of Razzaza Lake.

A three-year survey on nematode parasites of Planiliza abu of Razzaza Lake was conducted from November 2019 to December 2021. In 2019, 148 fish were gathered and tested for nematode infection; in 2020, 277 fish were collected and tested; and in 2021, 577 fish were collected and tested for nematode infection. According to light microscopy, all the fish had the larval type of Contracaecum. The parasite was confirmed as Contracaecum spp. by the Iraq Natural History Research Center and Museum at Baghdad University. Planiliza abu contained only Contracaecum spp. L3 larvae. Infection rates were the highest in January and February 2021 and the lowest in July and August. Female fish (n= 25) showed a greater infection rate than male fish (n=8), with 40 and 18, respectively, in third-stage larvae. The prevalence of all species was higher in Atlantic fish than in Mediterranean fish. The parasite was more abundant in the viscera than in the flesh, although in the most parasitized species, the parasite was more prevalent in the flesh (14). In the present investigation, the parasitic nematodes were found in the viscera rather than in the flesh. The infections were found in the Tigris, Euphrates, and Shatt Al-Arab rivers, some of their tributaries, lakes, marshes, drainage networks, and many fish ponds and floating cages across Iraq (15). Razzaza Lake is a suitable home for many aquatic birds (both resident wading and migratory) since it provides ample food and warm water. Aquatic vegetation in the area offers an ideal habitat for such birds. Aquatic birds are susceptible to various parasite diseases, which may cause significant mechanical and chemical damage to host critical organs and a drop in host weight and egg production. Some aquatic birds are the final hosts for parasitic worms that utilize fish as intermediate hosts. During the development of their larval stages, nematodes of the Anisakidae family may infect a broad range of aquatic hosts, primarily marine mammals, aquatic birds, such as pelicans, and freshwater fish, such as crucian carp, which are the hosts where the life cycle is completed (8,16). The Anisakidae family's tolerance to diverse hosts and environmental circumstances enables a global distribution. Six adult Contracaecum species, as well as some unidentified species of this genus, were isolated from 17 different bird species in Iraq, with the Eurasian bittern, Botaurus stellaris, and the pygmy cormorant, Microcarbo pygmaeus, infected with a maximum of three Contracaecum species and unidentified species of this genus (15). An overview of the numerous parameters influencing parasite assemblages in fish hosts is provided. These variables are roughly classified as biotic or abiotic. Host age and size, host, and parasite size, host specificity, host nutrition, and host sex, as well as their effect on parasite abundance and dispersion, are all studied and emphasized. Seasonality and other environmental conditions may favor parasite establishment and growth in host populations (17). We found that industrial waste, human activities, and seasonal changes all affected infection rates. The largest infection rate occurred during the winter, coinciding with migrating birds arriving in early October and departing in early March. However, no infection was detected during July and August 2020. The average high temperature in Karbala in August is similar to that of July, at 44,4°C. Additionally, birds are drawn to fishing because fishermen throw out a lot of dead fishes. The utilization of invertebrates as paratenic and/or intermediate hosts is a core component of larval transmission in most anisakid species. Anthropogenic factors may increase Contracaecum parasite populations (1). This might be because of the fish's lower eating activity at cold temperatures, which reduces the possibility of infection by copepods (18). In natural environments, this guarantees that the parasite does not kill the intermediate host and makes it to the final host to complete the life cycle. These conditions may limit the probability of Contracaecum L3 larvae being transferred to their final host, reducing the overall infection (18).

In Chile, the most common endoparasite species observed were Contracaecum rudolphii s. l. (72/80, 90%) in the neotropic cormorant Nannopterum (Phalacrocorax (brasilianus (Suliformes: Phalacrocoracidae (19). Based on morphological descriptions in the proventriculus, recovered nematodes were identified as Contracaecum spp. 100(8.0% to 81.8%) piscivorous birds (Black Night Herons, Nycticorax) perished in their habitats at the Wild Duck Lake in Yanqing, northwest of Beijing, China (20). Birds are considered a necessary element of the life cycle. Many wading and migratory birds were affected by nematodes, particularly in Razzaza Lake in Karbala. These areas were found during an investigation of helminth infections in 663 aquatic birds in the Bahr Al-Najaf depression in mid-Iraq. These collected and examined birds from 11 different species were infected with nematodes. Contracaecum spp. were found in the proventriculus of six aquatic bird species: E. alba, E. garzetta, A. ralloides, B. stellaris, A. purpurea, and C. rudis, with incidences of 14.5%, 15.6%, 20%, 13.1%, 16.7%, and 3.4%, respectively (21). In the current study, the infection rate of Contracaecum spp. in Planiliza abu (Heckel, 1843) in November and December 2019 was 25.35% and 23.38%, respectively. In January and February 2020, the percentages were 32.50% and 22.06%, respectively, while in November, it was 22.22%, and in December, it was 42.86%. In addition, in 2021, January and February were 12.2% and 10.9%, respectively, while July and August were 0%, November 3.5%, and December 6.1%. The low parasite prevalence might be attributed to a disturbance in the infection cycle since piscivorous birds, the parasite's ultimate hosts, do not feed on C. gariepinus in Chivero Lake. In terms of ranges, the findings agree with prior studies. Only one study has described the endoparasites of fish in Al-Husainia Creek, Karbala Province, mid-Iraq. The study agrees with other studies in ranges, but only one epidemiological study represents the endoparasites of fishes, including L. abu, with an incidence of 0.8% in Al-Husainia Creek, Karbala province, mid-Iraq. L. abu has the most parasite species of the fish studied (six species). The third-stage larval forms of Contracaecum spp. were found in the mesenteries and liver of A. vorax with a 0.7% incidence, in the intestinal wall of B. grypus with a 0.6% incidence, in the body cavity of both B. sharpeyi and B. xanthopterus with a 0.4% and 0.3%, respectively, and in

the intestinal wall and gonads of L. abu with a 0.8% incidence (22). In the north of Iraq, in Sulaimani province, Kurdistan region, Iraq (23), 44 third larval stage Contracaecum were found in 13 infected freshwater fish species from 966 fish species, one species from each of the Bagridae, Heteropneustidae, Mastacembelidae, Mugilidae, Siluridae, and Sisorid. The infection rates ranged from 0.92% to 19.35%, and morphological and genetic analysis (ITS1, ITS2, and COX2) revealed that all the Contracaecum larvae belonged to the same species (Contracaecum rudolphii B). The percentage of contracaecum spp. was 8.74% in 183 samples of freshwater fish Planiliza abu collected from the Al-furat river in the-Qar Governorate in southern Iraq (24). Mhaisen et al. 2018 linked the most widely distributed nematode larval forms of the genus Contracaecum in Iraqi fishes and found infection in 44 freshwater and marine fish species. Contracaecum rudolphii Type-B and Contracaecum septentrionale Kreis, 1955 were among the infections. Globally, 42.6% of Clarias gariepinus were collected from Chivero Lake, Zimbabwe (18), 6% of European sea basses showed Anisakis infection (25), 50% of Contracaecum osculatum in wild Atlantic cod (Gadus morhua) in Denmark (26), and 30% of Contracaecum spp. Globally, 42.6% of Clarias gariepinus were collected from Chivero Lake, Zimbabwe (18), 6% in European seabasses showed a prevalence of Anisakis infection (25), 50% of Contracaecum osculatum in wild Atlantic cod (Gadus morhua) in Denmark (26), and 30% of larvae of Contracaecum spp. were detected in fish species from Nasser Lake, Egypt (27). The larval nematode intensity considered low in crustaceans, the first intermediate hosts (7). In these studies, only one nematode (Contracaecum spp.) was found in L. abu, which agrees with (28), who researched fish parasites in the Bahr Al-Najaf depression in mid-Iraq for two years, from January 1995 to December 1996. A total of 6992 fishes from 11 different species were infected with only one nematode, Contracaecum spp. The weight of the fish is unaffected by infection or gender. While host length was significantly correlated with infection, (29) found that infection with Contracaecum spp. larvae was positively correlated with fish length as it increased gradually in larger fishes in Planiliza abu collected between June 2001 and May 2002 from three locations (Hilla river, Al-Furat fish farm, and Al-Mahaweel drain), Babylon province in central Iraq. According to the present research, the fish length (range in cm) in 2019 was 10-18 cm in November, 5-18 cm in December, 8-15 cm in January and 9-15 cm in February, and 10-15 cm in November, 12–19 cm in December, and 7.5–11 cm in November and December. These ranges are comparable to other studies by (30), who collected 180 fish (Planiliza abu) from a fish market in Al-Diwanya city and found a correlation between the length of fish and the infection rate. The infection rate was higher in 180 fish (Planiliza abu) with a length of 16 cm than in fish of 6-10 cm and 11-15 cm, respectively. Al-Husainia Creek in Karbala province is a tributary of the Al-Furat river (Euphrates river) that feeds Razzaza lake. The present research differed from another study (29), in which monthly samples related to season changes of infection with Contracaecum spp. were 11.9% in winter and 47.6% in summer. Because in the present research, no samples were collected in 2020, and none were collected in 2021. However, it was higher in the winter. In November and December 2019, the infection rate was 48.73%, 65.08% in 2020, and 9.6% in 2021, respectively. Human activities, increases and decreases in paratenic or intermediate hosts, birds, and environmental pollution might all be responsible for this study's fluctuation.

In conclusion, the preventive education of stakeholders is necessary to reduce the danger to public health. Consumers should evaluate fish species, origins, and risk factors, such as crustaceans and birds in the same region. If contracaecum larvae are found in the abdominal cavity of a fish, they should be mechanically isolated. To maintain food safety, parasitological surveys for contracaecum larvae in consumed seafood, especially fish hosts, are critical. To limit the danger of human diseases, worms must be killed using heat or cold.

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Ethical Statement

Ethics required are approved by the Ethical Committee of University of Kerbala/ College of Veterinary Medicine.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authors' Contribution

Marwa Jawad: Conceptualization, Methodology, Writing-Original draft preparation, Funding acquisition, Investigation, Data curation, Formal analysis. Zahraa Mohammed Ridha: Conceptualization, Funding acquisition, Investigation, Writing-Reviewing and Edit. Firas Alali: Writing-Reviewing and Edit, Software, Funding acquisition, Project Administration, Supervision, Resources. Saeed El-Ashram: Writing-Reviewing and Edit, Visualization, Funding acquisition, Validation, Supervision. Asaad Sh. M. Alhesnawi: Writing-Reviewing and Edit, Data curation, Formal analysis.

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