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Morphology and Molecular Confirmation of Contracaecum spp. in Fish

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ABSTRACT

Contracaecum spp. is a parasite belonging to the Anisakidae family part of the nematode. It is characterized as a potential zoonotic parasite. In humans causing anisakiasis who consume undercooked seafood or raw infected with the larvae of this nematode. *Planilizaabu* specimens have been collected from Karbala city, Iraq (the local market). The survey on nematodes of *Planilizaabu* was done over eight months from May to December 2022. Prarsites were examined morphologically then confirmed the genus by molecular study for the presence of *Contracaecum* spp. A total of 493 fish were purchased and the incidence of larval type *Contracaecum* (L3) in the viscera was 21.50%. Morphological and molecular Confirmation of the parasite was done and the findings revealed that the prevalence rate of infection was greater in September and November than October. Molecularly, identification of *Contracaecum* spp. was conducted targeting *18S rRNA* gene. Length and number of nematodes, as well as number of infected fish was significantly recorded. This study indicated that the presence of different intermediate hosts, organic and nonorganic contamination. This is the first molecular report identifying *Contracaecum* spp. in Razzaza Lake, Karbala province, Iraq. In the future, molecular markers may be an effective tool for determining the Contracaecum L3 larval species, different the life-cycle, and population structure and transmission pattern.

1. Introduction

Anisakidae family members are responsible for the rare disease anisakiasis, which affects humans and discovered in the early 1960s [1]. *Contracaecum* and Anisakis species are zoonotic nematodes found in fish. Anisakidae species of *Contracaecum* are significant zoonotic fish-borne larval nematodes [2;3]. The fish may contain many intestinal zoonotic parasites. Given that parasitic zoonoses like anisakiasis can be dangerous to human health, affects people all over the world, and, are typically brought on by eating raw or undercooked fish [4;5;6]. Different species of fish can have a significant impact on how widely distributed anisakids are in the environment anthesis, and different species of fish can play an important as the source of the infestation in humans [7]. On the other hand, humans can become hosts accidentally and be infected with infective stage larvae (L3), this parasite causes different types of symptom nonspecific gastrointestinal symptoms or allergic reactions capable of causing anaphylactic shock[5], and also causes gastrointestinal tracts diarrhea, severe vomiting in vertebrate/invertebrate animals, humans [8]. To diagnose this parasite is now used molecular techniques due to Traditional diagnostic techniques typically having low sensitivity and /or specificity. The Anisakidae nematodes discovered were in stage 3 (L3), and the mitochondrial gene rRNA was molecularly characterized by PCR[9]. However, *Contracaecum rudolphii*. Samples collected from cormorant populations in Italy and Europe revealed two sibling species, *C. rudo rudolph* which was more prevalent in brackish water fish, and *C. rudolp rudolph*i was found infecting only freshwater fish identified by sequence analysis of the mtDNA cox2, and ITS region of rDNA gene loci [10]. To date, *C. rudolphii* complex has five currently recognized members A, B, C, D, E, and F [11]. All infected fishes by Contracaecum larvae in north Iraq's Sulaimani Province represented exactly one species (*C. rudolphii* B) through testing the sequences of ITS1, ITS2, and COX2 [12a]. In this study molecular studies of Contracaecum larvae in *Planilizaabu* from Razzaza Lake in Karbala, were used.

2. Methodology

Ethical approval

Ethics required are approved under decision (UOK.VET. MI.2022.058, Date: 10/01/2022). It is home to an estimated one million pilgrims who travel there annually. Razzaza or (Razaza Lake), is located in western Iraq, west of Karbala (3241N, 4340E), (Figure 1).

Figure 1 Map of Karbala province (Iraq)

Collection and examination of fish

Samples were obtained locally from Karbala markets, and the source of *P.abu* is Razzaza Lake of Karbala, Iraq. A total of samples 493 fish belong to one genus *Contracaecum* were captured between May 2022 and December 2022. All of the fish were counted, measured, and weighed. Each specimen was traditionally dissected, and then its anisakid larvae content was checked. Following inspection, stereoscopic microscope was used to dissect the viscera, and the number of worms was determined for each sample [7].

Morphological and parasitological identification

All of the isolated larvae were examined morphologically. Individual fish larvae were mechanically removed, and placed 70% ethanol. Lactophenol was used to clear the nematodes so that they could be morphologically evaluated. As suggested by the genus name, these worms' digestive system consists of two ceca that are situated in opposition to one another. The front of their bodies also features an excretory orifice (Figures 2-6).

Figure 2 Viscera of dissected Planiliza abu has three stages of larvae Contracaecum spp.

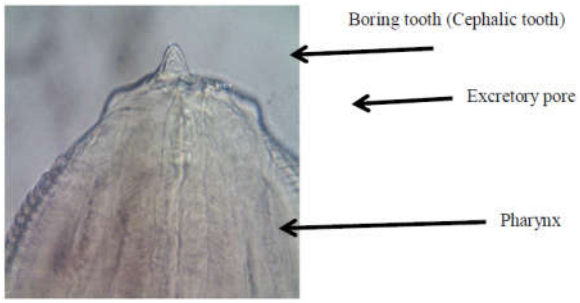

Figure 3 Anterior part of parasite

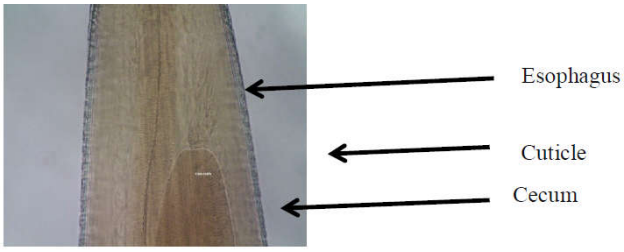

Figure 4 Anterior part of parasite

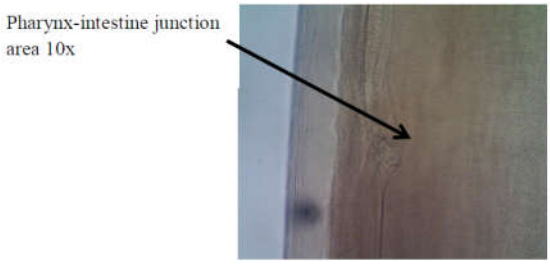

Figure 5 Middle part shows of parasite

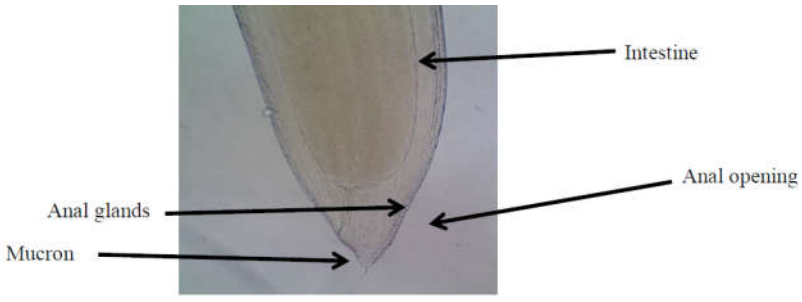

Figure 6 Posterior part of parasite

Molecular analysis of Contracaecum larvae

A total of were detected by the morphological examination used to detect eight third-stage larvae belonging to the *Contracaecum* genus of anisakid larvae. Then, eight *Contracaecum spp*. larvae were subjected to a molecular approach. DNA extraction from middle parts of *Contracaecum* larvae. And the length and weight were measured to give prevalence (P), and mean intensity, (mI) were calculated [9].

The molecular analysis was conducted on eight *Contracaecum* larvae. The larvae were selected randomly for each month. DNA extraction kit (Geneaid Biotech, korea) was used, and total DNA was recovered from the center region of the larvae. Amplifications were focused on the nuclear ribosomal regions of the small subunit of the mitochondrial ribosomal RNA (rrnS) gene. The rRNA gene was amplified using primers MH3 (5′-TTG TTC CAG AAT AATCGG CTA GAC TT-3′) and MH4.5 (5′-TCT ACT TTACTA CAA CTT ACT CC-3′) [14]. Briefly, the Amplification of DNA fragments of interest from genomic DNA was performed using the polymerase chain reaction (PCR). The reaction volume was 25 µl which included 55µl of a sample containing DNA, 7 µM forward primers, 7 µM of reverse primer, and double-distilled water to a final volume of 25 µl. The reaction was performed with an initial denaturation step at 94°C for 5 min, followed by 35 cycles at 92°C for 3 min. (denaturation), 52°C for 60 s (annealing), and 72°C for 1 min (extension), with a final polymerization step at 72°C for 7 min to ensure all amplification reactions had reached completion. The PCR products were analyzed by Safe-Red™ -agarose gel electrophoresis. PCR conditions were also used [14]. UV transillumination was used and visualized on .5% agarose of the amplified rRNA products (Figure 7).

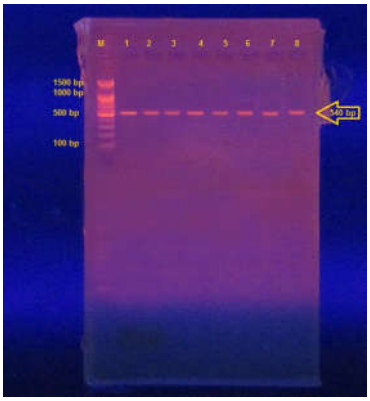

Figure 7 Agarose-gel electrophoresis of extracted DNAs

Statistical analysis

The GraphPad Prism Software was served to identify significant differences between values of study groups at P<0.05 (*),. Values were represented as Mean ± Standard Errors (M±SE) [15].

3. Results and Discussion

The nematodes were examined and identified as *Contracaecum* third larval stage in the current study using a morphology consistency molecular genetic approach. Generally, all the nematodes have been *Contracaecum* spp. using light microscopic inspection depending on the special characteristics feature of this parasite. This survey was completed during a period, from May 2022 to December 2022 to study nematode parasites of *P.abu* of Razzaza Lake. The total prevalence of 493 fish was 111(22.50%), (Table 1). Highly prevalence was in September / November while lowered at October to 2022 (Table 2). The relation among length, No. of nematode, and No. of infected fish have been signed at the level P≤0.05 according to (R) correlation (Table 3).

Table 1 Total prevalence with monthly Health status of fish

Months		Health status of fish		Total
		healthy	Infected	
may	Count	56	14	70
	% within Month	80.00%	20.00%	100.00%
Jun	Count	51	12	63

	% within Month	81.00%	19.00%	100.00%
July	Count	44	13	57
	% within Month	77.20%	22.80%	100.00%
August	Count	53	15	68
	% within Month	77.90%	22.10%	100.00%
September	Count	34	16	50
	% within Month	68.00%	32.00%	100.00%
October	Count	46	5	51
	% within Month	90.20%	9.80%	100.00%
November	Count	39	18	57
	% within Month	68.40%	31.60%	100.00%
December	Count	59	18	77
	% within Month	76.60%	23.40%	100.00%
Total	Count	382	111	493
	% within Month	77.50%	22.50%	100.00%
X^2	10.718	*P value*	0.151	Non-sig.

Table 2 Distribution number of infected and uninfected fish, weight, number of nematodes, Fish length and Prevalence in the Planiliza abu

Months	Total No.	Healthy	Infected	No. of parasite	Mean intensity	mean weight of fish	Fish length (mean cm)	Prevalence (%)
May	70	56	14	24	1.7	16.7	11.5	20.0
Jun	63	51	12	20	1.6	16.5	9.5	19.0
July	57	44	13	57	4.3	10.1	11	22.8
August	68	53	15	92	6.1	10.2	11	22.1
September	50	34	16	45	2.8	19.6	11.2	32.0
October	51	46	5	8	1.6	19.2	9.5	1.0
November	57	39	18	64	3.5	17.7	11.5	31.5
December	77	59	18	95	5.2	18.1	14	23.3
Mean	61.6	47.8	13.9	50.6	3.6	16.0	11.5	21.5

Tables 3 Correlations of number nematode, and number of infected fish with length

Correlations			
	Weight of fish	No. Nematode	No. of infected fish
Fish length cm	0.055	0.732*	0.750*
Weight of fish		-0.426	-0.043
No. Nematode			0.734*
No. of infected fish			
* Correlation is significant at the 0.05 level (2- tailed).			

Globally, *Contracaecum* spp. widely distributed with their larvae recorded in different fish species from different countries [15-25]. In this study was made, from May 2022 to December 2022, were conducted. Macroscopically was examined all the fish (were external and internal inspections). During this period, 493 fish were tested to reveal on 21.50% positives. microscopically, all larvas were *Contracaecum*. This result agrees with other study [6]. In September November, parasite showed great infection rates and low infection rates in October were 32.0%, 31.5%, and 0.1% respectively as recorded in Egypt [26]. In natural environments, the parasite hates to have the ability to kill the intermediate host and completes the life cycle on the final host [27]. These differences may be related to other factors [28]. The highest length of fish detected in December was (14 cm) while

the lowest in Jun and October was (9.5 cm). The fish length has been a significant effect on the number of infected fish and nematodes at the level P≤0.05. Another hand the weight of fish has been not significant with other factors (*P*>0.05). These agreements with Barson (2004) [27], who studied *Clarias gariepinus* from Lake Chivero, Zimbabwe, and these factors were non-significant with host size, prevalence, the intensity of the infection, and the body condition.

In general, all species were more common, even though, it found at lower abundance in flesh than viscera. *Contracaecum* found at greater abundance in viscera or abdominal cavity [29]. Currently, the total mean intensity was 3.6 and 1-15 worms per fish intensity of the infection this result agreement with Al-Zubaidy (2009) [30], and doesn't agree with Barson (2004) [27] who detected (1–7) worms per fish and mean intensity (2.2), this difference may be related with ecological environmental factors, location, size of sample and factors related with intermediate hosts.

These results of seasonal variations may affect the first, second, and final hosts' agreement with Iyaji, (2009)[28] who referred to seasonal variations and other environmental factors that may encourage the establishment and expansion of parasites in host populations. In fish, *P.abu* the seasonal variations have an impact on the prevalence of internal parasites [3]**.** People are put in danger and are deterred from buying contaminated goods when anisakid larvae are found in fish [31]. Because the zoonotic nematode Anisakidae family poses a risk to human health, it is crucial to identify fish [6].

Species from the three genera are also known to cause anisakiasis in humans can be caused by three genera after inadvertently ingesting live stage three larvae in unprocessed seafood, and may infect human who consumes raw or undercooked fish products. Infection or gender does not affect the weight of the fish. The previous studies in the same region (Razzaza Lake) about the same host (*P.abu*) were done by Al-Zubaidy (2009) [30]. They were included, 158 mugilid fish were sampled monthly and analyzed for *Contracaecum sp.* larvae, 41(25.9%). Another study by Al-Saadi*et al*. (2010)[32], describe *P.abu* fish endoparasites in Al-Husainia Creek, Karbala Province, central Iraq. Fish endoparasites, including only *Contracaecum spp.* third stage larval forms, which was 0.8%. While, Jawad *et al*. (2022)[6] who reported the prevalence only *Contracaecum spp.* third stage larval forms of 2019 was 48.73% out of 148 fish, 65.08% out of 277in 2020, and 9.6% out of 577 in 2021.

The current study used PCR to detect rRNA on eight *Contracaecum* larvae for eight months the selection was random and all the results have been confirmed as *Contracaecum* spp. PCR method was used to identify the ITS ribosomal gene, and the mitochondrial genes COX2 and rrnS were molecularly characterized in mullet fish (*Mugilcurema*) from the Chautengo Lagoon, Guerrero, Mexico, identified the *Contracaecum* sp. species in stage 3 (L3) with prevalence 283 (61.5%) out of 460 nematodes [4]. Shamsi *et al*. (2011)[7] conducted the molecular and genetic identification of various morphotypes of *Contracaecum* using (ITS-1 and ITS-2) in various species of fish (*mullet, Mugilcephalus, mackerel, Scomber australasicus, Arctocephalus* spp*, flathead, Platycephalus laevigatus, black cormorant and Phalacrocorax carbo*) found four different morphotypes of third-stage larvae including types I, II, III, and IV,

Eating raw fish in many traditional preparations was characterized by Chile has culinary preferences. The molecular method used to detect identification of collected anisakid L3 *Pseudoterranova cattani* and *Anisakis pegreffii* in food-borne anisakiasis in humans. Totally, of 180 fish samples in place of three different fish species, i.e. Chilean hake (*Merluccius gayi*), snoek (*Thyrsites atun*), and sea bream (*Brama australis*) [33]. Pekmezci and Yardimci [2] were confirmed the total number of marine fish was 475 and belong to 21 different species of larval *Contracaecum* species in marine fish from Turkish waters by molecular identification, thought to amplify RFLP targeting the ITS region and rRNA gene. Sequencing of *ITS* region, *rRNA*, and *COX2* gene in all *Contracaecum* L3 larvae. The *COX2* sequence analysis for the first time confirmed that all *Contracaecum* larvae were identified as *Contracaecum Overstreet* based also used to amplify the ITS region and *16S rRNA* gene from *C. Overstreet* by PCR-RFLP patterns [12].

Abdullah *et al*. (2021) [34] reported at the same region using the modern study as ultra-morphology and molecular studies ITS-1, *ITS-2*, and *COX-2* revealed from all infected fish species represented one species (*C. rudolphii* B) are collected in five Cyprinid fish species in this area. Verma *et al*. (2022) [35] have been more progressive detection of nematode parasites in the local markets for human consumption in the Western Ghats, India. SEM and genetic identification using mitochondrial COX subunit II assigned the *Contracaecum* genus. While, in Assiut Governorate, Egypt Thabit and Abdallah describe the larvae have used electron microscopy (Light and scanning) and (ITS1) and (*COI*) genes to identify species of larvae in Nile perch *Lates niloticus* collected from the Nile River [36].

4. Conclusion

As threats from fish-borne parasites like (*P.abu*)**,** zoonoses are extremely important for public health. Control measures and preventive education may be realized to reduce infections in fish stocks and thus the risk of human infections. Study of the diverse structure of the anisakid population is markedly important to more understanding of biology and ecology of the life cycle.

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