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A preliminary study on some larval trematodes parasites of marine snail *Cerithidea cingulata* (Gmelin, 1791) in Al-Faw Bay, South of Iraq

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Abstract

An examination of 500 snails (265 males and 235 females) of *Cerithidea cingulata* (Gmelin, 1791) collected from Al- Faw Port on the coast of the Arabian Gulf showed that they were infected with five species of larval trematodes of belong to the families : Schistosomatidae (5.3%) and Cyathocotylidae(47.7%) Haplosplanchnidae (21%), Echinostomatidae (8.3%) and Microphallidae (15.6%). The number of infected snails was 337 and the total infection rate was 67.4%, with 196 infected males (73%), 141 females (60%) and mixed infections were in 39 snails (11%) while the rest had a single infection.

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Introduction

C. cingulata is a marine and brackish water snail. It is found in tropical and subtropical regions. The estuaries of the rivers, salt swamps and open mud surfaces in marine beaches, where the coastal plant of Mangrove grows, is the natural environment for this type of snail (Printrakoon *et al.*, 2008). It is characterized by its rapid opportunistic growth and the lack of environmentally-competing animals and natural enemies. It exists in very large numbers that may exceed one thousand per square meter and it's a pest in most of the places where it lives (Zvonareva & Kantor, 2016). *Cerithidea* species is found in many parts of the world and serve as intermediate host of more than 50 species of digenetic trematodes. Its infections were recorded in California and Florida (Martin, 1972; Bush *et al.*, 1993), the Gulf of Mexico (Cable, 1956), Japan (Ito, 1956; 1957), India (Mani & Rao, 1993), the coast of Iran (Kalat-Meimari *et al.*, 2018), and Kuwait (Al-Kandari, 2000). Regardless of the presence and high density of *C. cingulata* in the Iraqi coasts, there are no studies on the cercariae that this snail harbors.

In general, the relationship of a snail with the larval stages in Iraq has not been studied by researchers in comparison to other regions of the world. This is evidenced by the lack of ongoing studies on the subject. AL-Mayah (1990) recorded the infection of *Melanoides tuberculata* with a species of avian schistosomes cercariae. Another study, AL-Mayah (1998), also found that *Lymnaea auricularia* was infected with six species of cercaria, *M. tuberculata* with one, *Melanopsis nodosa* with two and *Physa acuta* was not infected. Moreover, AL-Hussein (2000), in a study of the larval stages of the digenetic trematodes parasitizing snails of some Shatt al-Arab branches, has pointed out to the infection of *L. auricularia* with nine and *M. tuberculata* with seven and *M. nodosa* with four. Therefore, these three studies have dealt only with freshwater snails. In other words, there are no local studies on the infection of marine snails, so the current study is designed to shed light on this issue.

Materials and methods

The snails were brought to the laboratory by plastic cans with sufficient amount of water taken from the same area. The snails were laboratory-tested within two days of collection using Frandsen & Christensen's (1984) and AL-Mayah's (1998) method, known as Shading.

The Study Areas Description

The snails were collected at a distance of approximately 1km from the tidal area on the west coast of Al- Faw city at the beginning of Khor Abd Allah, specifically at the breakwater area of Al-Faw Grand Port south of Iraq (29°54'02.7"N 48°26'12.8"E). This area is affected by the tidal phenomenon. It is an open clay surface during low tide. The water level is about one meter during the high tide, which is a good biodiversity area, where there are many native and migratory shore birds, as well as different crustaceans, Annelids, and other aquatic invertebrates. 500 snail were manually collect during the months of March and April 2019.

The Cercariae Examination

The cercariae were examined using an stereomicroscope (WILD: MDG17) to determine their resting position in the water column and observe their behavior, such as how to swim and encystment on external substrates. After the emergence of cercariae they were examined using unstained and stained living cercariae in which morphological features are more easily observable than in unstained specimens. Stianing of living cercariae using very dilute solution of Neutral red and Methylene blue which are prepared by adding one or two drops of (0.1%) solution of dye to 50ml of water. The cercariae were then examined with a compound microscope (Leica DM500) under the oil immersion. A number of infected snails were crushed to discover the stage from which each kind of cercaria originated because some cercariae originate from the rediae while others from the sporocysts.

The susceptibility of cercariae to encystment after exiting the snail was examined by placing a number of plant leaves and pieces of cellophane in the dishes. They were observed under the stereomicroscope.

The walls of the dish were also examined to search for encysted cercariae.

The Cercariae Classification

The cercariae were classified based on the taxonomic keys proposed by Cable (1963) and Frandsen & Christensen (1984).

Results

The examination of 500 snails (265 males and 235 females) showed that they were infected with five species of cercariae belong to five families. The total number of infected snails was 337 and the percentage of infection was 67.4%, with 196 infected males (73%) and 141 females (60%). Mixed infection was also recorded in 39 snails (11%), whereas the rest had a single infection (Table 1).

Table 1. The percentage of Infection of *C. cingulata* with Species of Cercariae.

Species	The Number of Infected Snails
Schistosomatidae	18(5.3%)
Cyathocotylidae	161(47.7%)
Haplosplanchnidae	71(21%)
Echinostomatidae	28(8.3%)
Microphallidae	59(15.6%)

The following is a brief description and measurements of 25 specimens of each species of cercariae, noting that all measurements were taken in micrometers and in limited cases taken in millimeters.

Table 2. Comparison of the most Important Measurements of the Schistosomatidae *Cercariae* and their Intermediate Hosts.

Characteristic	Sample of Current Study	Sample of Abdul Salam & Sreelatha (2004)
Body length	302-340	310-350
Body width	100-120	105-130
Pharynx	Does not exist	Does not exist
Eyespots	Exist	Exist
Oral Sucker	Exist	Exist
Ventral sucker	Exist	Exist
Tail length	280-340	270-350
Length of Bifurcation	150-200	155-200
Spines	Cover body and tail	Cover body and tail
Intermediate Host	*C.Cingulata*	*P.Sulcatus,C. Cingulata*

Table 3. Comparison of the Most Important Characteristics of Cyathocotylidae recorded in other Regions with the Sample of the Current Study.

Characteristic	Cercaria of Current Study	Cercaria of Han *et al* (2012)	Cercaria of Cable(1963)
Body length	170-200	160-190	248-270
Body width	100-120	105-123	103-118
Pharynx	exist	Exist	Exist
Eyespots	Do not exist	Do not exist	Do not exist
Oral Sucker	exist	Exist	Exist
Ventral sucker	exist	Exist	Exist
Tail length	250-300	200-275	350-392
Length of Bifurcation	140-180	135-165	186-193
Spines	on body and tail	on body and tail	on body and tail
Finfold	Present	Present	Present
Intermediate Host	*C. cingulata*	*C. cingulata*	*C. cingulata*

Table 4. Comparison of the Most Important Characteristics of Haplosplanchnidae described by Abdul-Salam & Sreelatha (1995) with the Sample of the Current Study.

Characteristic	Sample of Current Study	Sample of Abdul Salam & Sreelatha (1995)
Body length	850-1000	910-1144
Body width	300-400	338-468
Pharynx	Exist	Exist
Eyespots	Exist	Exist
Oral Sucker	Exist	Exist
Ventral sucker	Exist and Prominent	Exist and Prominent
Tail length	900-1200	989.4-1326
Spines	Do not Exist	Do not Exist
Finfold	Does not Exist	Does not Exist
Intermediate Host	*C.cingulata*	*C. cingulata*

Table 5. Comparison of the Most Important Measurements of the Genus *Acanthoparyphium* and its Intermediate Host Recorded in Different Regions with the Cercaria of the Current Study.

Characteristic	Sample of Current Study	Sample of Abdul-Salam & Sreelatha (1999)	Sample of Mani & Rao (1993)
Body length	370-400	380-460	336-368
Body width	150-200	125-200	140-160
Pharynx	Exist	Exist	Exist
Oral Sucker	Exist	Exist	Exist
Ventral sucker	Exist	Exist	Exist
Number of Head Collar Spins	23	23	23
Tail length	200-250	250-400	288-304
Spines	Do not Exist	Do not Exist	Do not Exist
Finfold	Does not Exist	Does not Exist	Does not Exist
Intermediate Host	*C. cingulata*	*C. cingulata*	*C. cingulata*

Table 6. Comparison of the Most Important Characteristics of the cercariae of the Current Study with Studies from New Zealand and Australia.

Characteristic	Sample of Current Study	Sample of Australia Kudali & Cribb(2015)	Sample of New Zealand Martorelli *et al.* (2008)
Body length	95-135	87-122	75-85
Body width	50-70	40-66	20-35
Oral Sucker	Exist	Exist	Exist
Ventral sucker	Does not Exist	Does not Exist	Does not Exist
Stylet	Exist	Exist	Exist
Tail length	70-95	60-96	60-75
Spines	Cover the Body	Cover the Body	Cover the Body
Finfold	Does not Exist	Does not Exist	Does not Exist
Intermediate Host	*C. Cingulata*	*Posticobia brazier*	*Zeacumantus subcarinatus*

Schistosomatidae Cercaria

They are bifurcated-tail cercariae, (brevifurcate apharyngeate cercariae) swimming actively around the length of the body (head) 302 - 340 and width 100-120. They have two suckers, one is tilted to the ventral area in situ and is called oral sucker. It leads to a simple esophagus branching into two branches and the pharynx is absent. The ventral sucker is muscular and is close to the backside of the body. The body also contains a pair of eye spots clear in the front half. The tail is almost equal to the body at its length, which ranges from 280 to 340, where an excretory canal, The tail furcate, approximately 150 - 200 in length. The body and tail are covered with minute spines. These cercariae originate from sporocysts, with a length of about 0.9-1.3 mm.

Cyathocotylidae Cercaria

Is a bifurcated-tail cercaria, with a length of 170-200 and a width of 100-120. The oral sucker is small and sub-terminal equipped with structures similar to papillae. It has a short pharynx and esophagus but no eyespots. The ventral sucker is small and primitive and is approximately situated in the intermediate part of the body. The body and tail are covered with small spines. Tail length is 250-300. Bifurcation length is 140-180. There is a finfold on the tail bifurcation. Cercariae have several behaviors in motion such as contraction and expansion of the body or contraction and expansion of the tail, or both, or the oscillatory movement of the tail for rapid movement. A sporocyst is 2 - 2.5mm in length and was very elongated and contain cercariae with different maturation phases.

Haplosplanchnidae Cercaria

They have an elongated spindle-shaped body which is 850-1000 in length and 300 to 400 in width. The oral sucker has a sub-terminal position. They have a muscular and big pharynx and esophagus, as well as two clear eye spots located almost at the pharyngeal level. The ventral sucker is big and prominent to the outside. There are no spines. The tail is not bifurcated and is 900-1200 in length. The sporocyst is attached to the gastrointestinal tract in numbers ranging from 15 - 20 which are 1.5-2 in length. The cercariae were inactive and non-moving during the examination. They were attached to the dish by the ventral sucker.

Echinostomatidae Cercaria

The length of their body is 370 - 400 and the width is 150-200. The Oral sucker is prominent and is located in the front of the body and surrounded by collar of spines arranged in a row consisting of 23 spines. There is a long esophagus that breaks into two parts at the intermediate of the body and the two parts extend to the end and contain a short pharynx. The ventral sucker is slightly larger than the oral sucker and is located at the posterior half of the body. No spines are seen on the surface of the body or tail. The length of the tail is 200-250 and thick at the base with a tapered end and no finfold. Cercariae move by constriction and relaxation of the body. Ridia is with a length of 1.7 - 2mm, containing cercariae at various maturation stages.

Microphallidae Cercaria

is a cercaria with a slightly elongated oval-shaped body. Its length is 95 - 135 and its width is 50 - 70. The oral sucker is relatively large and clear. It is located on the front of the body. It contains the Stylet penetration organ. There is no pharynx or esophagus. Three or four pairs of penetration glands have been seen. There is neither a ventral sucker nor eyespots. The body is covered with minute spines. The tail is cylindrical that is not bifurcated, its length is 75-95.

There are no finfolds. Cercariae have a simple movement with the contraction and relaxation of the body and a simple movement of the tail. These cercariae produced oval- or bag-shaped sporocyst, whose length is about 0.2-0.25 mm. It has been observed that these sporocyst contain cercariae at different maturation stages.

It should be noted that all these cercariae are recorded for the first time in Iraq.

Discussion

The examination of *C. cingulata* showed that it is infected with five species of cercaria in a high percentage. This may be due to the availability of definitive hosts. The Arabian Gulf region is a winter destination of migratory and native waterfowl, so it is found in large numbers and species that gather at the tidal area, which is the same area that contains snails. The area is gradually covered with water to a height of about one meter. In this region, there are many species of crustaceans, snails, oysters and aquatic insects, which may be the second intermediate hosts of the cercariae. The salt concentration of water is relatively stable, which is during the collecting period is between 38-40 part per thousand, Therefore, the environmental conditions in the region are suitable for the completion of the life cycle of the trematodes, especially marine species. It is not surprising to find the snail *C. cingulata* infected with five species of trematodes, four of which were from the birds trematodes and one is attributed to Haplosplanchnidae, which intrude on fish. By reviewing the literature of the subject, it was found that this snail is characterized by an abundant number of cercariae, and it works as an intermediate host for several trematodes, especially those that intrude on birds that inhabit marine coasts. Therefore, the results of the current study if do not exactly match, it does not significantly differ from the results of studies at the nearby of the current study area. So, Abdul-Salam & Sreelatha (1991) found that in Kuwait that the snail itself was infected with six species of *cercaria*, most of which were bird trematodes. Al-Kandari *et al.* (2000) have also observed in Kuwait that the same snail was infected with 12 species of trematodes, 11 of them are trematodes that infect birds and only one that infects fish. They have pointed to the importance of the presence of definitive hosts of birds in the high rates of infection in snails. Moreover, Kalat-Meimari *et al.* (2018) reported that severe infections in the same snail in one of the coastal areas of the Strait of Hormuz was due to the abundance of seagulls that transmit the infection to the snail, while the reason for the high infection of *Heterophytes heterophytes* in another area was mainly attributed to sewage.

In the working methods, artificial light (electric) was used to stimulate cercariae to naturally exit the snail in order to obtain fully developed cercariae for the purpose of identification, because immature cercariae cannot be identified for their different sizes and lack of important characteristics and structures in the classification process. Besides, the shell of the snail contains protozoa and crustaceans that are difficult to distinguish from cercariae (AL-Mayah, 1998) when the crushing method is used. In order to determine the phases from which the cercariae originated, the shells of the snails were broken and their tissues were isolated. Moreover, Sporocysts or Ridiae were isolated. ridiae were distinguished from the sporocysts because the first had mouth, pharynx, and cystic intestines, while the second did not have these structures (Fried & Graczyk, 1997).

Both live non-stained and stained with vital stains cercariae, such as Neutral red and Methylene blue, have been used in the classification for two reasons. The first is the appearance of important structures, such as spines and finfolds, which can only be seen in live samples (AL-Mayah, 1998). The second reason is to observe the behavioral characteristics of cercariae, which are important in the classification, such as swimming and resting in the water column (Blair, 1977). To determine the rate of infection with cercariae and their species and whether the infection is Single or Mixed, the number of snails that naturally produced cercariae was calculated. These were examined under the microscope. Sporocysts or Ridiae have been observed. The rate of infection was 67.4%. This ratio is high compared to studies in other areas

of the world. For example in the coast of Kuwait, Al-Kandari *et al.* (2000) observed the infection of *C. cingulata* with 12 species and percentage of 49.9%, while Kalat-Meimari *et al.* (2018) found that in the coast of the Strait of Hormuz the snail itself is infected with ten species and a rate of 27.9%. These differences in infection rates and the number of species may be attributed to the nature of contact between the definitive hosts of birds and other vertebrates with *C. cingulata*, which is an intermediate host of helminths that parasitize these animals, or maybe due to the season of snail sample collection examined in the current study in comparison to the season of other studies.

In the present study, it has been found that mixed infections in the snails are low compared with the percentage of single cases, which is (11%), but this percentage is somewhat high compared to many studies of the same or other species belonging to the *Cerithidea* because Al-Kandari *et al.* (2000) discovered that mixed infection rate is 1.2%. Moreover, Kuris (1990) found that mixed infection rate is low, which reaches 3% in *Cerithidea californica*. Sousa (1990; 1993) also recorded a low mixed infection rate of 2% and 2. 5%, respectively. So that, the mixed infection does not happen randomly but is determined by the ability of infectious phases (Miracidia and Eggs) of the different species of trematodes to infect. The reason for the decrease in mixed infection could be due to antagonism (competition) (Sousa, 1993; Lafferty *et al.,* 1994). The difference in the rate of mixed infection of the current study from the above-mentioned studies may be because the species of current study cercariae did not have antagonism or because of the timing of sample collection and its size as the sample was collected between February and April, i.e. when the rate of infection is high. That is why Al-Kandari *et al.* (2000) pointed out that the highest infection rate in this period and attributed it to the abundance of migratory aquatic birds in this region in the winter months.

Cercariae highly differ in their phenotypic and behavioral characteristics. Therefore, their classification to Family or Genus could sometimes be possible, but the classification to lower levels requires completion of their life cycles in their appropriate definitive hosts in order to obtain mature worms or eggs (Frandsen & Christensen, 1984; AL - Mayah, 1998). Thus, the cercariae of the current study were classified to five families, all species are recorded for the first time in Iraq.

Schistosomatidae cercaria is distinguished from bifurcated cercariae because of its lack of pharynx; lack of pharynx is one distinguishing characteristic of schistosomatidae cercariae, and on the basis of the presence of eyespots, they have been identified as avian schistosomes cercariae (Scott & Burt, 1976; AL-Mayah, 1990). It is clear from the comparison of the present study cercaria with the avian schistosomes that have been found by Abdul-Salman & Sreeelatha (2004) in the *C. cingulata* and the *Planaxis sulcatus* in the coast of Kuwait (Table 2) there is a similarity in the characteristics and measurements, so they may be related to the same species, because the study areas are close, thus facilitating the process of the movement of aquatic birds, which are definitive hosts, between the two stations and contribute to the spread of infection among the snails. The human contact with these cercariae leads to Schistosoma dermatitis or what is called swimmer's itch (AL-Mayah, 1990).

Cyathocotylidae cercaria, the body of these cercariae is Lingui form, concave from the ventral side, with bifurcated long tail with finfolds, as well as the other characteristics of the cytocotylid group mentioned by Cable (1956). More specifically, they are characterized by active swimming through the tail in short periods. They spend long rest periods on the surface of dishwater after they are produced from the snail. They have produced from the sporocysts. The snail is a first intermediate host, while fish, amphibians and other invertebrates are secondary intermediate hosts (Yamaguti, 1958). Reptiles, birds, and mammals are the definitive hosts. The characteristics isolated from the snail in the present study are similar to those of the species described by Cable (1963) in Jamaica and of the species described by Han *et al.* (2012) in Korea (Table 3).

Haplosplanchnidae Cercaria it is clear from the comparison of the cercaria observed in the current study with the cercaria described by Abdul-Salam & Sreelatha (1995) from the same snail on the coast of Kuwait (Table 4) that there is a significant correlation between the two species. The numerous differences in the measurement of both samples are within the limits of heterogeneity. The cercariae of this species are spindle-shaped, had a long tail and a single caecum, a single testicle with no cirrus sac. The female reproductive system is made up of a single ovary and the vesicular vesicles had a V shape (Madhavi, 2005). The trematodes related to this family parasitized fish. Their life cycle includes only one intermediate host of snails. Whenever they are released from the snail, they encyst on the plants and algae available in their environment. Fish will be infected when eating these plants, that is why this cercaria infects herbivores fish (Huston *et al.,* 2017).

Echinostomatidae cercaria the characteristics of the family Echinostomatidae recorded by Ito(1957) and Frandsen & Christensen (1984) exactly match those of the current sample, the most important of which is the presence of a head collar with spines surrounding the oral sucker. The presence of a well-developed head collar bearing 23 spines arranged in a row is a defining characteristic of the genus *Acanthoparyphium* (Abdul-Salam & Sreelatha, 1999). Mani & Rao (1993) and Abdul-Salam & Sreelatha (1999) have previously recorded a species of cercaria in the same snail in India and Kuwait respectively.

From Table (5), it appears that there is a clear correlation in the measurements and number of collar spines as well as the intermediate host itself. The adult stages of this family are intrusive in the digestive tract of the aquatic birds and other vertebrates, while larval stages develop in hepatopancreatic gland of Prosobranche snails. Their cercariae encyst as metacercariae in Lamellibranchs or in the same snail (Abdul-Salam & Sreelatha, 1999).

Microphallidae cercariae are Monostom cercaria, i.e. they have only an oral sucker equipped with a stylet. These cercariae are produced in the oval-shaped sporocyst (Galaktionov and Skirnisson, 2007). These family spread throughout the world. They include approximately 47 genus (Deblock, 2008). The mature worms usually intrude into the digestive tract of vertebrates, especially birds and mammals. Their life cycle includes two intermediate hosts: the first intermediate host is Gastropods mostly brackish water and marine gastropods and the second intermediate host is Custaceans (Seo *et al.*, 2007; 2008; Guk *et al.*, 2008). Two species belonging to Microphallidae were recorded in Kuwait: Abdul-Salam and Sreelatha (2000) recorded *Probolocoryphe* and Al-Kandari *et al.* (2007) recorded *Maritrema eroliae*. Al-Kandari *et al.* (2010) have also molecular identified cercariae of the type *P. uca* isolated from the snail *C. cingulata*. and these studies did not provide a morphological description of cercariae. The description of the current sample is consistent both in characteristics and measurements with what was described by Martorelli *et al.* (2008) and Kudlai & Cribb (2015) (Table 6).

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