Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(5): 307 – 321 (2020)
www.ejabf.journals.ekb.eg

Advanced Studies on the Endo-Parasitic Diseases (Digeneasis and Larval Cestodiasis) Affecting some Marine Fishes in Ismailia Governorate

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ARTICLE INFO

Article History:
Received: June 24, 2020
Accepted: July 25, 2020
Online: July 29, 2020

Keywords:
Marine fish,
Digeneasis,
Larval cestodiasis,
PCR,
Prevalence

ABSTRACT

A seasonal study was performed on 500 marine fishes (100 Engraulis encrasicolus, 100 Sardina pilchardus, 100 Caranx rhonchus, 100 Tilapia zillii, 100 Dicentrarchus punctatus) that collected randomly from Suez Canal in Ismailia Province. Some examined fishes showed sluggish movement, emaciation, and hemorrhages all over the body, beside abdominal distension, congestion of internal organs, and enteritis. Encapsulated plerocercoids were detected in the abdominal cavity and liver. The total prevalence of parasitic infestation was 66.0% (highest in autumn (76.8%) and the lowest in summer 44.8%). The highest parasitic prevalence was recorded in Engraulis encrasicolus (75%), followed by Dicentrarchus punctatus (72%), Tilapia zillii (69%), Caranx rhoncus (67%) then Sardina pilchardus (47%). The isolated parasites were digenetic trematodes (encysted metacercariae, Erilepturus hamati, Erilepturus tiegsi and Clupenuroide sp.) and cestodal larvae (Callitetrarhyncus sp. and plerocercoid). Cestodal plerocercoids were the highest prevailing parasites (28.6%). A negative correlation between infestation and fish body weights were detected. Confirmation of Callitetrarhynchus plerocercoid was done using PCR. Histopathological changes were also recorded in the infested fishes. It could be concluded that infestation with digeneasis and larval cestodiasis might have negative impacts on fish health.

INTRODUCTION

Fish is a chief nutritional food source for about 60% of the world population; it contains low cholesterol and high protein levels **(Sichewo *et al.*, 2013)**. Developing countries fulfill 30% of their annual animal protein through fish **(Abisoye *et al.*, 2011)**. The aquaculture industry has been plagued with diseases caused by viral, bacterial, fungal, and parasitic pathogens **(Tokşen *et al.*, 2010)**. Parasitic infestations affect standard growth levels resulting in reduced size and changed behavior **(Thomas, 2014)**, weaken the host's immune system, and so increase susceptibility to secondary infections and cause severe economic losses. The annual global cost of parasites in finfish aquaculture ranged from $1.05 billion to $9.58 billion **(Shinn *et al.,* 2015)**.

Marine fishes take the upper hand as a good animal protein for man, animal, and birds. They are preferable to freshwater fishes due to richness in trace elements and minerals. Despite these benefits, they act as either final or intermediate hosts for some parasites causing injuries and high economic losses **(Eissa, 2002).**

This study aimed to diagnose the most prevailing digenetic and larval cestodal parasitic diseases affecting some marine fishes through recording the clinical picture, identifying the isolated parasites using traditional and advanced methods, together with recording the associated histopathological alterations.

MATERIALS AND METHODS

1. Fish samples:

A total number of 500 marine fishes (100 *Engraulis encrasicolus* (anchovy), 100 *Sardina pilchardus, 100 Caranx rhoncus*, 100 *Tilapia zillii* and 100 *Dicentrarchus punctatus* (spotted seabass) of different body weights were collected in different seasons (25/season) between October 2018 and September 2019 from Suez Canal area, Ismailia, Egypt. Fish samples transported alive in polyethylene bags containing 1/3 of its volume water and 2/3 filled with air to the lab of Fish Diseases and Management Department, Faculty of Vet. Medicine, Suez Canal University. Freshly dead fish samples were transported in a closely tight ice box filled with ice.

2. Clinical picture:

All fishes were grossly examined for the determination of any clinical abnormalities and/or internal lesions, according to **Noga (2010).**

3. Parasitological examination:

Fish specimens were examined macroscopically and microscopically for the presence of any internal parasites. Detected adult trematodes, larval cestodes, and encysted metacercariae (EMC) were fixed, stained with alumn carmin, and mounted according to **Pritchard and Kruse (1982).** Morphological details of the detected parasites were discussed.

4. Histopathological examination:

Small tissue specimens (0.5 cm thickness) were collected from the affected musculature, liver, stomach and intestins), fixed in 10% neutral buffered formalin, processed routinely, and stained with Hematoxylin and Eosin (H&E) according to **Takashima and Hibiya (1995).**

6. Extraction of DNA for phylogenetic analysis:

For molecular analysis, DNA from the preserved cestodal sample was extracted according to the protocol of tissue Gene Jet TM Genomic DNA purification Kit (Fermentas life sciences, Lithuania). The D2 variable region (~600 bp) of the nuclear, large subunit ribosomal DNA (lsrDNA) gene was sequenced to identify the plerocercoid. Polymerase chain reaction (PCR) was carried out to amplify the target D2 variable region of lsrDNA using the following primers: 300F (5-CAA GTA CCG TGA GGG AAA GTT-3) and ECD2 (5-CTT GGT CCG TGT TTC AAG ACG GG-3), as described by **Aznar *et al.* (2007).** PCR products were purified using standard techniques (Qiaquick PCR Purification Kit, Qiagen Company, CA) and run against a standard mass ladder (100 bp) on an agarose gel to estimate the concentration of DNA.

RESULTS

1. The clinical picture of naturally infested fishes:

Fishes with heavily parasitic infestations revealed abdominal distension with slight emaciation, loss of condition, and discolored or hemorrhagic skin with slimness**.** In some cases, fish showed congestion and inflammation of the eye, as shown in **Figure 1.**

Internally, the liver, gall bladder, kidneys, and spleen were enlarged and pale, but in some cases, they showed congestion. Liver infested with EMC showed greyish or whitish nodules of varying sizes embedded in its tissue. The stomach and intestine were congested in addition to enteritis**.** Some *Caranx rhoncus* revealed oval, whitish colored, bladder-like cysts encapsulating plerocercoids in the body cavity while *Engraulis encrasicolus* showed alive plerocercoids within the body cavity, as shown in **Figure 2.**

2. Parasitological examination:

As shown in **Figure 3**, microscopic examination revealed three adult digenean trematodes; they characterized by flattened elongated bodies with oral and ventral suckers:

Erilepturus hamati **Yamaguti, 1934,** was isolated from stomach and intestine of *Dicentrarchus punctatus* and sometimes from the intestine of *Caranx rhonchus* and characterized by a spindle-shaped body, short esophagus, conspicuous and broad caeca; extending into the ecsoma up to its length and muscular, coild uterus; opens into the hermaphroditic duct. The two testes were post-acetabular, round, symmetrical, and nearly equal in size. The genital pore is at the posterior ventral margin of the pharynx. The ovary was post-testicular، globular, and slightly median.

Erilepturus tiegsi **Woolcok, 1935,** was recovered from the intestine and stomach of *Dicentrarchus punctatus* and mainly characterized by sinus-sac possessing two muscular sphincters. They are around the genital atrium and not the sinus-sac. The seminal vesicle was undivided.

Clupenuroides sp. **Al-Yamani and Nahhas, 1981,** was isolated from the stomach and intestine of *Dicentrarchus punctatus* and *Caranx rhonchus*. It characterized by sub-terminal oral sucker, acetabulum at the beginning of the middle third, nearly twice the size of the oral sucker. The two testes were ovoid. The ovary was in the posterior end posterior to the left testis, vitellaria seven digitiform tubules chiefly post–ovarian. It lacks a seminal receptacle.

Microscopic examination revealed circular, varying sized, and double-walled cysts embedded in liver tissue and/or musculature. Morphologically, they are the EMC of different species.

Two species of plerocercoids were recovered from the body cavity: *Callitetrarhynchus species* **Linton, 1897,** was isolated from *Caranx rhoncus* and *Dicentrarchus punctatus*. It had lobulated scolex, showing two short lateral platform bothridia, supplied with four long cylindrical and sheathed tentacles. The tentacle sheaths were regularly coiled till the base of the bulb. The four bulbs were arranged symmetrically beside each other at the end of the scolex.

Cestodal plerocercoids **Diesing, 1863,** were isolated from *Engraulis encrasicolus*. They characterized by retracted scolex, anterior extremity with a dorso-ventral swelling, bothridial grooves present in the scolex reaching up 1/3 of the body length. Body surface wrinkled, not segmented, and without genital primordium. They had different morphological types.

3. Prevalences of parasitic infestation in the examined fishes:

3.1. Total and seasonal prevalence of parasitic infestations:

As shown in **Table 1**, the total prevalence of parasitic infestation was 66.0%. The highest prevalence was in autumn (76.8%), and the lowest was in summer, 44.8%. *Engraulis encrasicolu* was 100% in winter and spring and the lowest in summer (36%). *Sardina pilchardus*, was the highest prevalence (80%) in spring, while summer had the lowest prevalence (8%). *Caranx rhonchus* showed the highest prevalence (92%) in autumn, while the lowest was in summer (28%). In *Tilapia zillii* and *Dicentrarchus punctatus,* the highest prevalence was in autumn (100%), and the lowest were in spring (36% and 44%), respectively.

As recognized in **Figure 4,** the total prevalence of EMC, adult digenean infestations, and cestodal plerocercoid infestations were 24.2%, 13.2%, and 28.6%, respectively. The highest prevalence for plerocercoid larvae was seen in *Engralus encrasicolus* (73%), while there was no infestation with adult digeneans in *Engralus encrasicolus*. Also, adult digeneans and larval plerocercoids did not record in both *Sardina pilchardus* and *Tilapia zillii*.

3.2. prevalence of parasitic infestations in relation to body weights

The highest prevalence was 70% at body weights equal to or smaller than 50 gm, and the lowest was in weights ranged from 451-550 gm with a prevalence of 28.5%, as shown in **Figure 5.**

4. Results of Molecular identification (PCR):

The Callitetrarhynchus larvae samples were identified by PCR assay. It was based on primers for lsrDNA gene. It revealed amplification products of expected molecular size at ~165bp, which is specific for Genous, Callitetrarhynchus (**Fig. 6**).

5. Histopathological findings:

As showed in **Figure 7,** muscles affected by EMC showed parasitic cyst embedded in between muscle bundles and surrounded by connective tissue capsule. Stomach infested with adult digeneans had a cross-section of the parasite with destruction, desquamation, and erosion of epithelial lining, lamina propria, and submucosa. Affected liver showed a longitudinal section of digenetic trematode within its tissue, while the affected intestine showed a cross-section of multiple parasites in the intestinal lumen with massive necrosis and desquamation of the epithelial lining.

Table (1) Total and Seasonal prevalence of parasitic infestations among the examined fishes

Season	**Winter n=25**	**Spring n=25**	**Summer n=25**	**Autumn n=25**	**Total n=100**
Engraulis encrasicolus	25 (100%)	25 (100%)	9 (36%)	16 (64%)	75 (75%)
Sardina pilchardus	18 (72%)	20 (80%)	2 (8%)	7 (28%)	47 (47%)
Caranx rhonchus	17 (68%)	20 (80%)	7 (28%)	23 (92%)	67 (67%)
Tilapia zillii	19 (76%)	9 (36%)	16 (64%)	25 (100%)	69 (69%)
Dicentrarchus punctatus	14 (56%)	11 (44%)	22 (88%)	25 (100%)	72 (72%)
Total	93/125 (74.4%)	85/125 (68%)	56/125 (44.8%)	96/125 (76.8%)	330/500 (66%)

Fig. 1: A photograph of (A) *Dicentrarchus punctatus* showing abdominal distension, (B) *Tilapia zilli* showing skin discoloration and hemorrhages at the base of the operculum, (C) *Tilapia zillii* showing excessive skin slimness and (D) *Sardina pilchardus* showing congestion and inflammation of the eye.

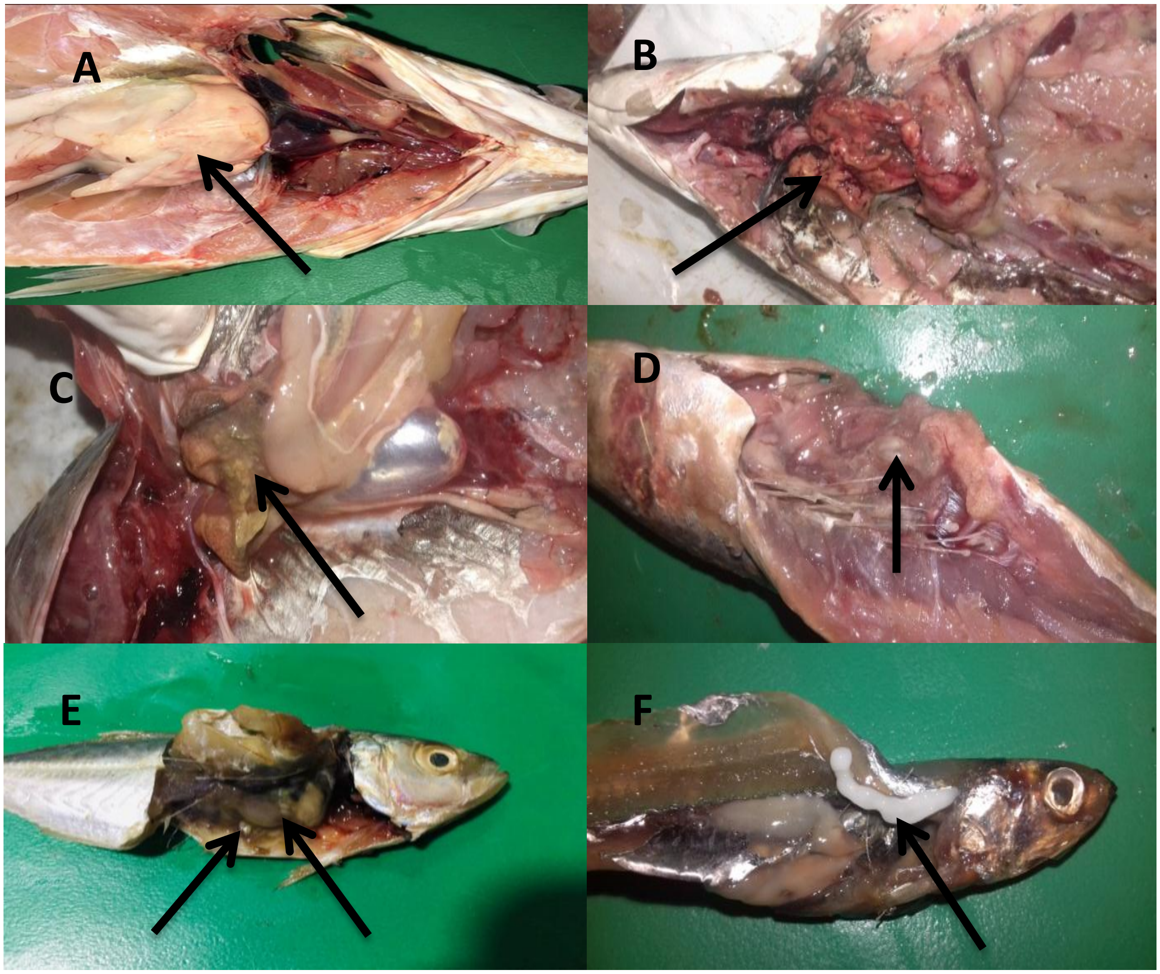

Fig. 2: A photograph of (A) *Caranx rhoncus* with enlarged, pale liver, (B) *Dicentrarchus punctatus* showing congested internal organs, (C) *Tilapia zillii* liver infested with EMC showing greyish embedded nodules, (D) *Dicentrarchus punctatus* infested with cestodal larvae, (E) *Caranx rhoncus* showing whitish colored cysts within the body cavity enclosing cestodal plerocercoid and (F) *Engraulis encrasicolus* infested with living cestode plerocercoid within the body cavity.

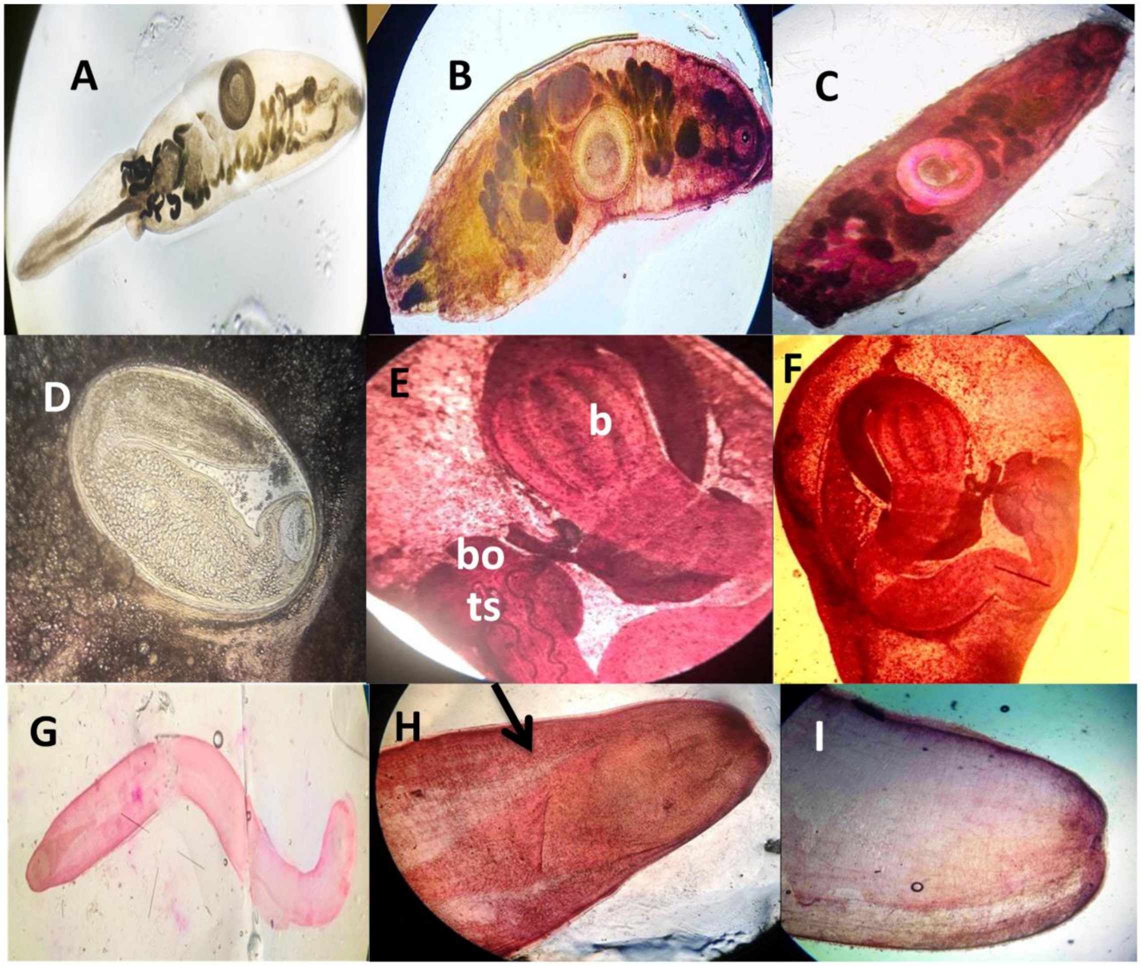

Fig. 3: A light photomicrograph of (A) Unstained *Erilepturus hamati* showing spindle-shaped body with broad conspicuous caeca and hermaphroditic duct, (B) Alumn carmine stained *Erilepturus tiegsi*, (C) Alumn carmin stained *Clupenuroides sp.*, (D) Unstained EMC in liver of *Sardina pilchardus* (E) Alumn carmin stained *Callitetrarynchus sp.*, .B: bulbus, TS: tentacle sheath, BO: bothria, (F) Alumn carmin *stained Callitetrarhynchus sp.* plerocercoid encapsulated in the blastocyst, (G) Stained whole cestodal plerocercoid, (H) Alumn carmin stained anterior end of plerocercoid showed distinct bothridial grooves and (I) Alumn carmin stained posterior end of the cestodal plerocercoid.

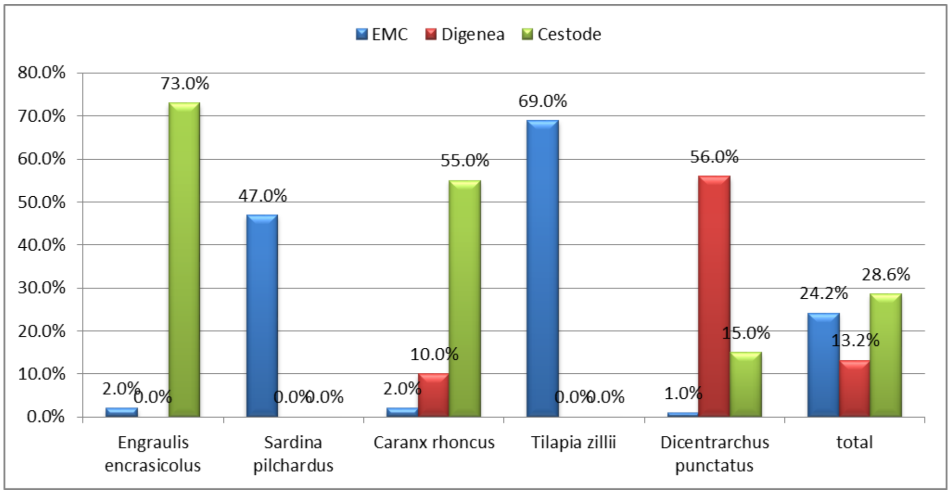

Fig. 4: Prevalence of different parasitic infestations among the examined fishes

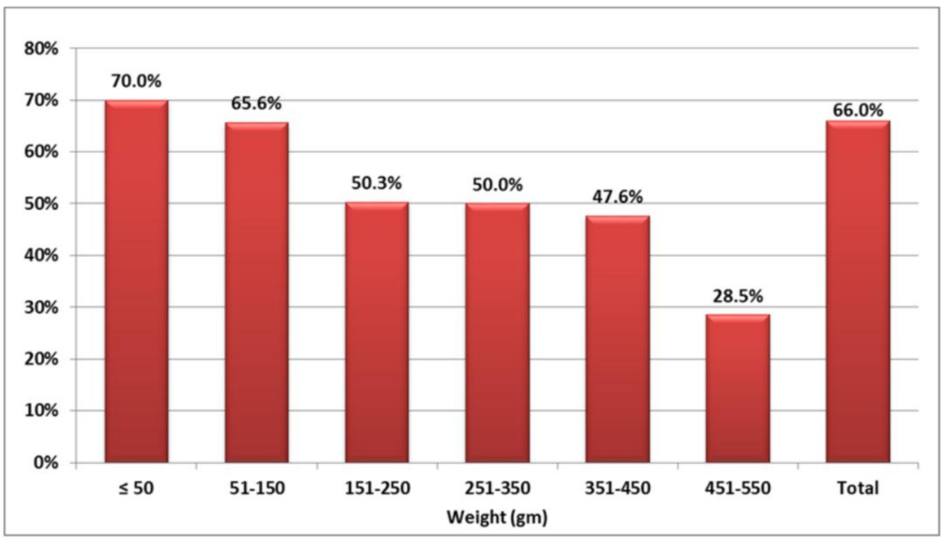

Fig. 5: Prevalence of parasitic infestations in relation to body weights.

Fig. 6: Molecular identification of *Callitetrarhynchus* species plerocercoid using PCR , L(bp): M ladder is from 100 to 600 bp , Neg: negative control, Pos : positive control , 1: An approximately 156 bp fragment of 1srDNA.

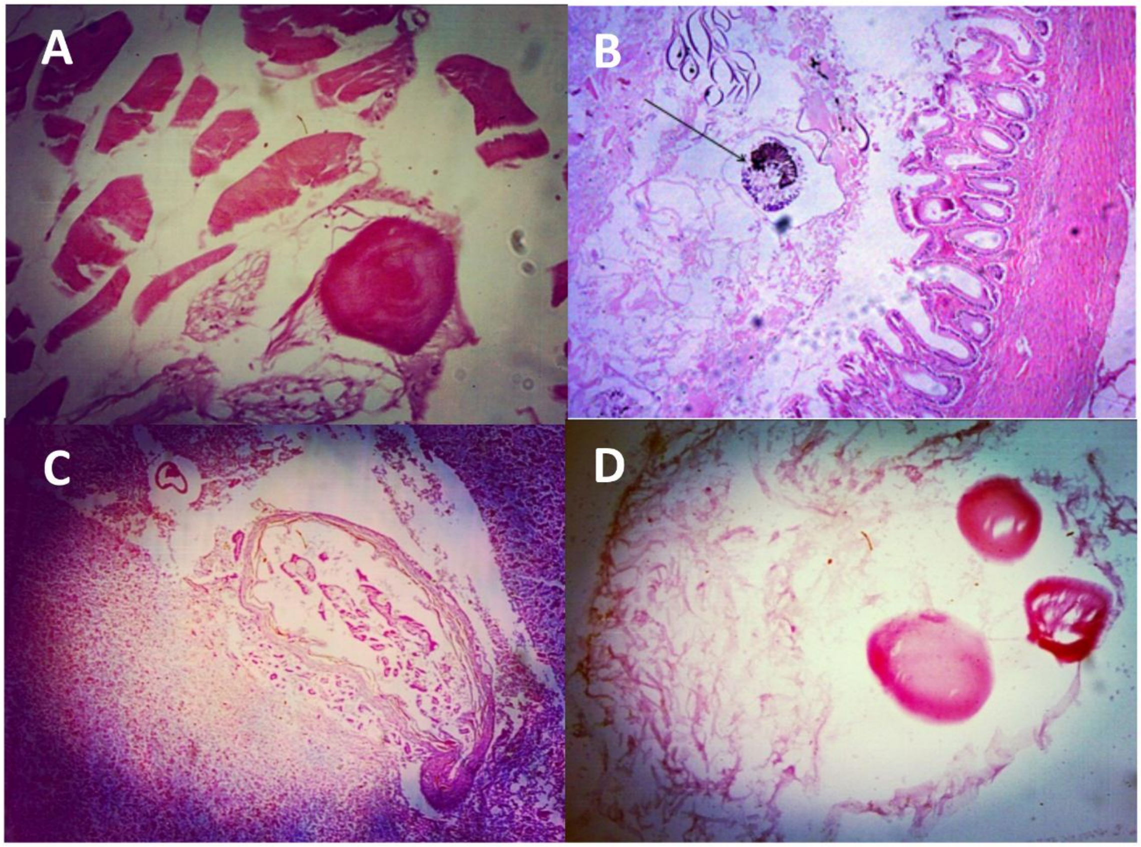

Fig. 7: Photomicrograph of (A) Musculature of *Engraulis encrasicolus* showing cross-section of a parasitic cyst embedded in between muscle bundles and surrounded by connective tissue capsule, (B) Stomach of *Dicentetrachus punctatus* infested with digenea showing cross-section of the parasite with destruction, desquamation, and erosion of epithelial lining, lamina propria, and submucosa. H&E. X 200, (C) Liver of *Sardina pilchardus* showing a longitudinal section of digenetic trematode. H&E X200 and (D) Intestine of *Caranx rhoncus* showing cross-section of multiple parasites in the intestinal lumen with massive necrosis and squamation of the epithelial lining. H&E X200.

DISCUSSION

The main clinical signs observed in this study were anemia, loss of condition, sluggish movement, abdominal distension, and emaciation. Anemia and emaciation may result from the inability to take food. As a result of the parasitic impact on the stomach and intestinal tract, complete destruction of tissues and the mechanical blockage will occur, which in turn decreases absorption from the gut. This agreed with **El-Lamie (2007)** and **Eissa *et al.* (2017)**. Sluggish movement and loss of condition may be attributed to that plerocercoid encystations disturbed muscle function and interfere with the fish motion (**Eissa, 2002).** The current study showed hemorrhagic skin; this sign agreed with that obtained by **Khalil *et al.* (2014).** Postmortem lesions revealed congested or anemic organs. The liver sometimes had visible, embedded yellowish-white nodules. These findings were in agreement with that mentioned by **Eissa (2002) and Abdel-Mawla (2005).**

The morphological description of *Erilepturus hamati* was in agreement with **Abdou (2001)** and **Al-Salim (2013)**. Also, *Clupenuroides sp.* and *Erilepturus tiegsi* described in this study were in agreement with **Hassanin (2000)** and **Hutson *et al.* (2011)**, respectively**.**

The recorded morphological characters of *Callitetrarhynchus sp.* were similar to those recorded from sciaenid fish by **Palm (2004)** and **Pereira and Boeger (2005).** Also, the cestodal plerocercoid recovered from *Engraulis encrasicolus* is similar to that described by **Pereira and Boeger (2005)**

The total prevalence of endoparasitic infestation in our study was 66%. This is nearly similar to that obtained **by Khurram Khan *et al.* (2019)** but higher than that obtained by **Youssef and Derwa (2005**) and **William (2004**) and lower than that obtained by **Amer *et al*. (2007)**. This may be attributed to the difference in location, feeding ways, fish species, and the number of examined fishes, environmental conditions, and availability of intermediate and final hosts.

Concerning the total prevalence of encysted metacercariae, it was 24.2%, which is lower than that reported by **Laffargue *et al.* (2004)** but higher than that mentioned by **Youssef and Derwa (2005)**, these variations may be due to time of collection, fish species and fish number.

Concerning digenean infestation, the total prevalence was 13.2%, which is lower than that reported by **Luque and Alves (2001**) but strongly agreed with **Al-Zubaidy (2010**), which was 14.3%. The total cestode larval infestation was 28.6%, which is lower than that obtained by **Genc *et al.* (2005)** and higher than that reported by **Guagliardo *et al*. (2010)**.

Regarding the seasonal prevalence of endoparasitic infestation, it was the highest in autumn (76.8%) followed by winter (74.4%), then spring (68%) and the lowest was in summer 44.8% which is strongly similar to that mentioned by **Gautam *et al.* (2018)** who reported that the prevalence of trematodes, nematodes, and cestodes was at the peak during autumn, and agreed with **El-Lamie (2007)** who mentioned that the lowest prevalence was in summer 53.3%. On the other hand, it disagreed with **William (2004),** who reported that the peak was in summer (60.27%), followed by autumn (50%), then spring (47.20%).

Concerning infestation in relation to body weight, there was a negative correlation between infestation and body weight. The highest prevalence at fish weights of (≤ 50 gm) and (51-150 gm); were 70% and 65.6%, respectively while the lowest infestation was at weighs of 451-550 (28.5%), this finding may be due to the incomplete immune system of small-sized fish or the small number of samples at the highest weight which give inaccurate results. This finding came along with **Ozturk and Aydogdu (2003)** and

Tasawar *et al.* (2007). Meanwhile, this finding did not agree with **Yakhchali *et al*. (2012)** and **Taha and Ramadan (2017),** who reported a positive correlation.

In this study, PCR analysis supports the identification of Callitetrarhynchus plerocercoids by using the lsrDNA gene, which gave a positive band at 156bp. This result is in agreement with **Abdelsalam *et al.* (2016)**.

Concerning the pathological changes, muscles infested with EMC showed a cross-section of the parasitic cyst, which embedded in between muscle bundles and surrounded by connective tissue capsule, this agreed with **Aly *et al.* (2005)**. The liver with gross whitish nodules showed a microscopically longitudinal section of a digenetic trematode; this agreed with **Shareef and Abidi (2012)**. Intestine and stomach affected with digeneans showed massive necrosis and desquamation of the epithelial lining. These results were in agreement with that obtained by **Woo (2006)**.

CONCLUSION

In conclusion, digenetic trematodes and cestodal plerocercoids were detected in investigated marine fishes where the total prevalence was 66.0%, highest in autumn (76.8%), and lowest in summer 44.8%. *Engraulis encrasicolus* recorded the highest prevalence (75%), while *Sardina pilchardus* was the lowest (47%). A negative correlation between infestation and body weights was detected. The isolated parasites induced marked clinical signs and histopathological changes that resulted in negative impacts on fish health.

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