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Identification of Selected Cestodes Affecting Some Marine Fish

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ABSTRACT

In the last winter, a total of wild marine fish species were collected from two different marine water sources. Samples were clustered into 70 bysuria (Engraulis encrasicolus) fish that were collected from Mediterranean coasts of Damietta and 50 grouper (Epinephelus gigas) from Hurghada Red Sea coast. Bysuria fish were examined for the presence of abdominal and intestinal cestodes larvae. In grouper, larvae were detected in intestine, abdominal cavities, liver, spleen and gonads in grouper fish that can be seen by naked eyes. The total infestation rate of larval cestodes in examined fish was 50%. As 54% of the detected larvae in Bysuria fish were belonging to diphyllobothrid plerocercoid larvae, while 44 % of detected larvae in grouper were belonging to Trypanorhyncha Plerocrcoid larvae. The morphological characters of the detected larvae were discussed. Molecular analysis was performed to confirm the final ID of the detected diphillobothrid larvae.

INTRODUCTION

Fish are the most valuable, palatable and preferred food worldwide because of their easily digestible protein which is of high nutritional value. Marine fish are preferred due to its rich nature in trace elements (phosphorous and iodine). Bysuria fish is small with longitudinal stripe that runs from the base of caudal fin, while grouper are Teleosts, typically having a stout body and a large mouth.

Fish parasitic diseases considered one of the most important roles in the biology of fish and can affect their health and distribution(**Rohde, 1993**), marine fish play serious roles as intermediate or definitive hosts for a number of parasites that decrease the aqua resource production, reduction in fish growth and increase susceptibility of fish to other pathogens and raising mortality rates (**Shih et al. 2010).** Plerocercoids cause economic loses and find in intestine, muscle and peritoneum of red sea fish **(Olfat et al. 1998),** also migrate through out visceral organs and manifested with spleen damage, hepatic necrosis and gonadal damage that might has the potential to reduce the reproductive capability and survival of affected fish **(Elsayed and Faisal 2008)** also can detect any pathological abnormalities by naked eyes

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(Noga, 2010). Diphyllobothrium larvae are the agents of human diphyllobothriasis and are found in teleostean fishes, and the transmission occurs after the ingestion of raw, poor cooked or improperly frozen fish meat (**Knoff et al. 2008).**

Human diphyllobothriasis is a fish borne parasitic illness caused by cestodes of order; Pseudophyllidea, Family; Diphyllobothridae. Several species of Diphyllobothrium are described as pathogenic to man with Diphyllobothrium latum being considered as the more prevalent. The parasite has a complex life cycle with piscivorous animals, including man, being the primary host. There are two intermediate hosts; a copepod as the first intermediate host and a predatory fresh water or marine fish. Humans acquire these fish borne parasitic zoonoses through the consumption of infected raw, undercooked, or inadequately preserved fish **(Lima dos Santos and Howgate 2011).**

Trypanorhycha larval forms infect a wide variety ofmarine invertebrates and mainly use fishes as intermediate or paratenic hosts**(Palm, 2007)**. Their presence among infected fish causes marketing problems **(Peterson et al. 1993)** and allergic reactions **(Pelayo et al. 2009).** All trypanorhynch species larvae were isolated from muscles and abdominal cavities **(Mahmoud et al. 2015).**

The taxonomic identification of broad tapeworms (genus Diphyllobothrium) is remarkably obscure due to lack of critical morphologic criteria in its larval and adult stages. Thus, molecular analysis by PCR and sequencing represents the only reliable tool to identify these parasites to the species level **(Wicht et al. 2010); (Jeon et al. 2009).**

In the current study, two species of marine fish from different locations were examined for the possible identification of the larval cestodes parasites, their prevalence and its public health hazards.

MATERIALS AND METHODS

1- Fish samples:

The present study was done utilizing a total of 120fresh marine fish samples with different body weights. Samples were randomly divided into 70 bysaria and 50 grouper fish. Clinical and Post-mortem examination of fish were carried out with according to **Eissa (2016).** Fish samples were stored in insulated ice box containing crushed ice and brought to the Parasitology Lab in its most fresh state. Samples were visually inspected for the presence of worms using both naked eye and hand lens. Ultimately, isolated parasites examined with both stereo and compound microscopes according to **Phan et al. (2010).**

2- Parasitological examination:

Tapeworms collected from freshly examined fish were washed in saline. The specimens were fixed in 10% buffered formalin solution. Fixed specimens were washed with water, stained with Semicon's carmine, dehydrated in ascending grades ethanol, cleared in xylene and mounted in Canada balsam **(Kuchta et. al. 2009).** Mounted slides were examined under binocular microscope for morphological identification.

3-Genetic analysis:

The collected larvae were stored in 70 % ethanol at - 20 ° C until processed for molecular confirmation. The final identity of the reterived worms was done using PCR and sequencing of the 18S gene on of ribosomal RNA.

PCR technique:

DNA was extracted according to the protocol of tissue Gene JetTM Genomic DNA purification Kit ® (Fermentas). Due to its length, amplifying the 18S region was done in amplifying one overlapping fragment. The fragment of 1000 base pairs (bp) was obtained using primer 81 (5'-TTC ACC TAC AAC CTT GGA GTT ACG-3 ') and primer 83 (5'-GAT CTA GTT GTC ACC ACC CTG A-3'). This primer was previously described by **Mariaux (1998)**. The amplification reaction consisted of the following steps: five minutes denaturation at 94 ° C followed by 38 cycles comprising 30 s at 94 ° C, 40 s at 55 ° C, 1 min. 30 s at 68 ° C, with an elongation final 10 min. at 68 ° C. The PCR-amplified products were run on 2-3% agarose gels or 10% polyacrylamide gel to visualize the products.

RESULTS

Clinical examination:

External examination of naturally infested fish showed no pathognomic clinical signs. *Diphyllobothrium latum* larvae may be presented from the mouth of by saria fish Figure (1), while some infested grouper fish showed abdominal distension. The clinical findings were exihibited in the form of thinning of the wall with swelling in intestine. Moreover, presence of some encapsulated or free plerocercoid larvae with different shapes, sizes especially in liver as well as other internal organs causing adhesions, mild peritonitis, hemorrhages (particularly the liver) and ascetic fluid. These lesions resulted from the presence of large number and size of trypanorycha species in the abdominal cavity. They may also affect the gonads leading to infertility of infested fish. Severe infestations in juvenile fish can cause mortality Figure (5).

The prevalence of plerocercoid larvae in some marine fish:

Total prevalence of larval cestodes was 50% in examined fish as Diphyllothrium latum 54% (38/70) in Bysarea fish, while prevalence of Trypanorycha was 44% (22/50) in Grouper fish.

Parasitological examination:

Toxonomical classification of the larval cestodes parasites was carried out according to its morphometric features described by **Hoffmanand Dunbar (1961)** and **Khalil et al. (1994)** as follows:

Larval cestodes:

Order: Pseudophyllidea., Family: Diphyllobothriidae., Genus: Diphylobothrium

Species: Diphylobothrium latum

The larvae were collected from the body cavity of bysarea, intestine and the mouth of fish Figure (1), measured 9.6-10.5mm in average length and 0.45- 1.2mm in average width. This larva has thick cuticle. The gonads are not developed. Bothria are shallow and connected by a deep dorsal-ventral groove or invagination over the anterior end, but the strobila closely resembles Diphyllobothrium. This worm belongs in the family Diphyllobothriidae (**Liihe, 1910),** but cannot be further identified until life history studies are made Figure (2, 3).

Trypanorhyncha plerocercoid:

It was isolated from the body cavity of grouper fish Figure (4).The small protruded larvae from the blast cysts had elongate and acraspedote scolex. Auriculate, spinose, bothridia. Tentacles emerge from apex of bothridia; broad band of hooks present on external surface of tentacle; intercalary. Tentacle sheaths coiled. Retractor muscle originates in anterior half of bulb. Figure (5, 6).

Molecular confirmation:

The morphological identification of Pseudophyllidean, Diphillobothrid plerocercoid is very difficult due to the lack of critical morphologic criteria. Consequently, molecular analysis by PCR represents one of tool to identify these plerocercoid larvae which considered the major public health hazards cestodiasis. The amplification followed by sequencing double-stranded allowed us to obtain almost complete sequence 18S region (2101 bp) of the gene for rRNA. The molecular identification of D. latum plerocercoid using PCR and sequencing of the 18SrDNA.

Figure(1) Macroscopic detection Plerocecoid of Diphyllobothrium latum isolate from moth and body cavity of bysarea Fish.

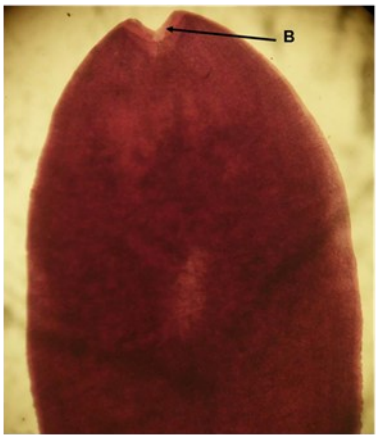
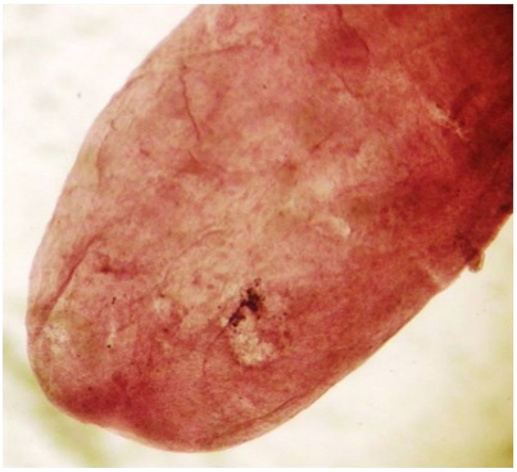


Figure (2) Microscopic identification of anterior part of Diphyllobothrium latum plerocercoid: B= Bothria or groove in plerocercoid scolex (x5).



Figure(3) Microscopic identification of Posterior end of Diphyllobothrium latum plerocercoid (x5).

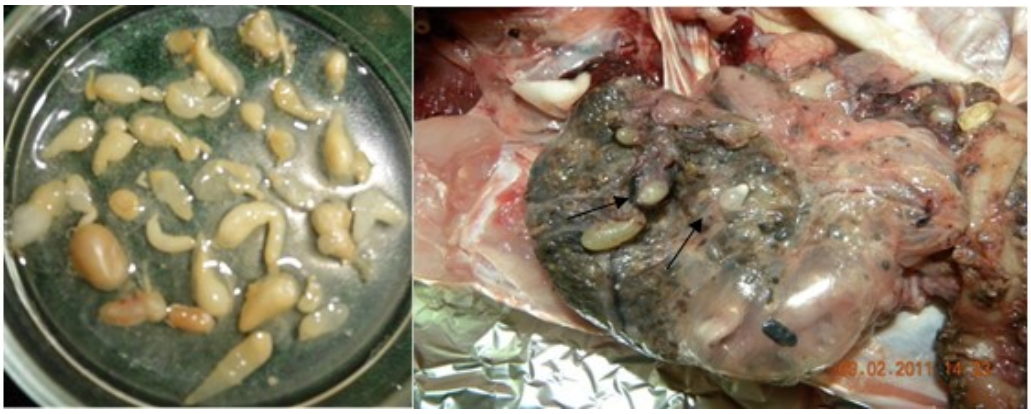


Figure (4)Macroscopic detection of Trypanorhyncha plerocercoid isolated from body cavity of grouper Fish.

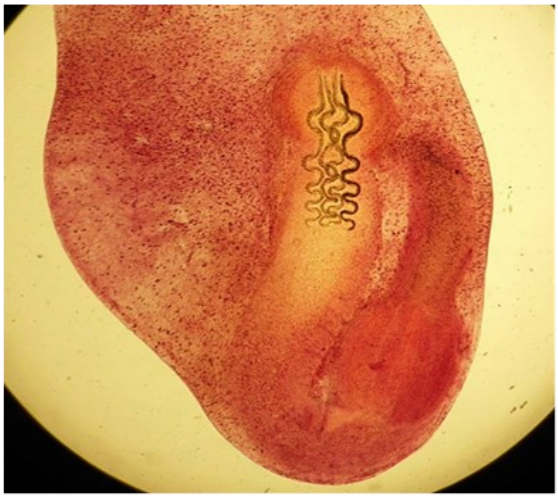


Figure (5) Microscopic identification of Encapsulated Trypanorhyncha plerocercoid (x100)

Figure (6) Microscopic identification of Protruded larvae from the blastocysts of Trypanorhyncha plerocercoid (x100). A-Parsbothridialis. B-Parsbulbosa.

DISCUSSION

Food from marine aquatic environments makes an important contribution to human nutrition and health and is also Background Health recommendations advocating increased fish consumption need **(Eric et al. 2019)**. Approximately 20% of protein is obtained from hydrobiological resources to fulfill human nutritional requirements in developing countries **(Freon et al. 2017; Mohanty et al. 2017; Tacon and Metian 2013)**. Direct human consumption of fish worldwide is 20.3 kg per capita (in 2016). In Peru, this value rose from 7 kg in 1990 to 14.5 in 2017 **(FAO 2018; Gestión 2017)**. Bysuria (Engraulis sp.) plays an important role not only in the marine trophic chain system but also supporting a successful fish oil and fishmeal industry. Protein values (approxi-mately 19%) and w-3 fatty acids (29.1–33.1%) are the main attractive nutritional characteristics of this species **(ITP/IMARPE 1996; Ayala et al. 2003)**. Epinephelus Spp. are known as coral reef species **(Ambak et al. 2012)** and rich in vitamin D and can serve as a good source of vitamin D **(Mohanty et al. 2017).**

Diphyllobothrium latum is one of the most common agent of diphyllobothriasis in human has complex life cycle : two intermediates (crustaceans and fish) and definitive hosts(fish –eating mammals) as human **(Guttowa and Moskwa. 2005)**Trypanorhyncha plerocercoids were widely spreeding within the world oceans using invertebrates as first intermediate as first with teleosts and some invertebrates as second intermediate or paratenic hostes **(Hassan et al. 2002).**

The clinical findings of infested fish showed no pathognomic leisons. Some infested showed thinning of the wall with swelling in intestine and presence of some encapsulated or free plerocercoid larvae especially in liver as well as other internal organs causing adhesions, mild peritonitis, hemorrhage(particularly the liver) and ascitic fluid These lesions are resulted from the presence of large number and size of trypanorhycha species in the abdominal cavity. They may also affect the gonads leading to infertility of infested fish. Severe infestations in juvenile fish can cause mortality. These clinical findings are similar to that recorded by **El-Ekiaby (2009)** and **Felizardo et al. (2010).**

In the present study, the overall prevalence of larval cestode larvae was50% among the two different species of fish. This result relatively agreed with **Eissa et al. (2001)** 51.25% in Euthynus affinis and lower than that obtained by **Mahmoud et al. (1995), Abo-Esa and Badawy (2006), Amer et al. (2007), Felizardo et al. (2010) and Eissa et al. (2020)** who recorded that a prevalence in infested marine fish was 59.06% among Mediterranean fish, 66.6% in Hamour fish, 100% (Atherina sp.), 93.3% (Paralichthys isosceles) from Brazil and 66 % (four species of marine fish) from Suez Canal in Ismailia province. In contrast, higher prevalence were recorded by **Mahmoud et al. (2015), Abo-Esa and Abdel-Mawla (2013) and Abdou and Palm, (2008)**from Mediterranean (42%), Red Sea (43.3%) and Red sea fish (20%) respectively

Regarding the detected plerocercoid larvae, Genus Diphylobothrium latum Larvae was isolated from Bysarea with prevalence of 54% lower that recorded by **Eissa et al. 2020** from Bysarea fish with prevalence75%, while this larvae recorded by **Andrea et al. (2016)** from 5 species of fish in four different Italian lakes with prevalence 6.6%, 25.4%, 7.6% of perch (Perca fluviatilis) from Lakes Maggiore, Como and Iseo respectively and 71.4 to 84.2 % and in 3.6–3.8% of burbot (Lota lota) from Lakes Iseo and Como. While Trypanorhynch. Larvae was detected from Grouper fish (44%) that was nearly agreed with **Mahmoud et al. (2015)** who recorded (46%) in grouper fish but higher than detected by **Hassan et al. (2002)** in sex species of fish was 7.73% in Saudi Arabia **(Al-Zubaidy and Mhaisen. 2011)** in seven fish species in Yemen from Red sea (12.5-43.6%), **(Carvalno and Luque. 2011)** in Trichiurus lepturus (12.5%) from Rio de Janeiro**(Haseli et al. 2011)** in seven teleosts from the Arabian Gulf (21.13%).

The difference between the prevalence of the two genuses of larvae may be due to predicted factors including locality difference, water salinity, degree of water pollution, feeding habits

of fish as well as the selected fish species.

Concerning the morphology of diphillobothrium latum was similar to that described by **Hoffman and Dunbar (1961)** and **Olfat et al., (1998)** in its general morphological feature. The occurrence of Diphyllobothrid plerocercoids in the internal organs and body cavity of fish was agreed to that recorded by **Meyer (1972) and Felizardo et al. (2010).** While detected Trypanorhynch sp. was supported by that mentioned by **Beveridge and Campbell (1988)**, **Khalil et al. (1994)** and **Abo-Esa and Abdel-Mawla (2013).**

The sequences obtained from the DNA of larvae studied are identical to partial sequences of larvae on the surface of filets referenced by **Nicoulaud et al. (2005)** in GenBank (accession number DQ181941) and proved identical to that of the adult worm expelled by a patient living on the banks of Lake Leman (number accession DQ181942). **Hanzelova et al. (1999)** showed that molecular identification of D. latum plerocercoid using PCR and sequencing of the 18S rDNA. Lane 1 and 2 were species-specific PCR products from DNA of D. latum plerocercoid (1000 bp). This finding is coincided with that recorded by **Nicoulaud et al. (2005).**

Conclusion

The current study extends the scope of morphometric and molecular assessment to one of the most deleterious zoonoticlarval cestodes "*Diphillobothrium latum* and *trypanorhyncha*". Further, some details about their public health significance were emphasized. The potential of zoonotic parasitic infection mainly depends on the abundance of intermediate hosts as well as suitable climatic / environmental conditions. It is worthy to mention that consumers should be efficiently educated about the health risks of raw fish consumption .

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