Studies on Prevailing Parasitic Trematodiasis Affecting Some Cultured Marine Fishes in Ismailia Governorate

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Abstract
This study was carried out to detect the trematodes affecting some marine fishes. Fish species collected were 100 *Dicentrarchus labrax* and 100 *Dicentrarchus punctatus* from private farms at Ismailia provinces. There were no pathognomonic signs in infested fishes except marbling of gills in fishes with gill monogenean infestations. Other signs and P. M. lesions were haemorrhages, abrasions on skin, sluggish movement, abdominal distension and emaciation, pale liver, congesion intestine with entritis in some cases. The higher infestation rate was in *D. punctatus* (52%) followed by *D. labrax* (35%). The isolated parasites in *D. labrax* and *D. punctatus* were monogenetic trematodes (*Diplectanum bocqueti*) and Digenetic trematodes (Acanthostomum sp. and *Allostomachicolina secundus*). Detection of Acanthostomum sp. using molecular biology (PCR) was recorded. Seasonal prevalence in relation to fish sex, body weight, body length and infestation, statistical analysis and histopathological picture was also recorded.

Introduction

The Parasitic diseases affecting marine fishes are numerous and they cause high economic losses in marine culture sector in Egypt. Parasites of fish are a concern since they often produce a weakening of the host`s immune system, thereby increasing their susceptibility to secondary infections, resulting in the nutritive devaluation of fish and subsequent economic losses. The aquaculture industry has been plagued with diseases caused by viral, bacterial, fungal and parasitic pathogens ***(Tokşen et al., 2010).*** Trematodes are very common parasites of all classes of vertebrates and may inhabit as adult, juvenile worms or encysted metacercaria, they are characterized by a sucker around the mouth and ventral sucker that is involved both in the attachment to host surfaces and in movement. Digenetic trematodes represent the largest group of all internal metazoan parasites as they include about 18,000 nominal species ***(Cribb et al., 2001).*** Monogeneans are the most important helminth group parasitizing the external surfaces of

the fish. Monogeneans are hermaphrodite and have a direct life cycle. Due to their life strategies and adaptations to parasitic life, they can be regarded as very successful parasites ***(Valigurová et al., 2011).*** Serious monogenean infestations through their attachment and feeding can induce a range of histopathological changes to the epithelium, which can facilitate the invasion and establishment of a range of secondary fungal, bacterial and viral infections ***(Yadimci and Pekmezci, 2012).*** The present study was directed towards further understanding of parasitic infestations of marine fish in Suez Canal region. The objectives were decided to throw the light on clinical picture of the naturally infested fish, isolation and identification of the causative agents by traditional and advanced techniques, the total and seasonal prevalence of the diseases in the examined fish and histopathological alterations induced by such diseases.

Materials and Methods

1.Fishes:

A total of 200 marine fishes of two species represented as "100 *Dicenrarchus labarx* and 100 *Dicenrarchus punctatus*" of different body weights (100-500g) collected in different seasons from marine private fish farms culture in Ismailia Governorate. They were collected between December 2015 to the end of November 2016.

2. Clinical examination:

First, body weight and total length of the examined fishes were recorded and then clinical examination was done on the live fish or freshly dead ones. Fish specimens under investigation were grossly examined for determination of any clinical abnormalities according to the methods described by ***Amlacker (1970).***

3. Postmortem examination:

For recording the internal abnormalities, the postmortem examination was performed on all fishes according to ***Amlacker (1970).***

4. Parasitological examination:

Fish specimens were examined macroscopically and microscopically for external and internal parasites as soon as possible after they were sacrificed.

4.1. Permanent slides smear preparations and staining:

Gills were placed in clean Petri dish containing saline till monognea detached then isolated in clean Petri dishes, Digenean endoparasites isolated from stomach and intestines placed into corked test tubes containing water and shaken vigorous. trematodes fixed in 5% formalin for 24 hours, washed in tap water, stained with borax carmine for 5 minutes, washed carefully in tap water. Differentiation occurred in acid alcohol and washed in distilled water, dehydrated in ascending grades of ethyl alcohol, cleared with clove oil, then xylene to remove the oil and mounted in

Canada balsam then left to dry in hot air oven according to ***Lucky (1977)*** and ***Schmidt and Roberts (1985).***

5- Detection of Acanthostomum sp. digenea using PCR.

Before the extraction of DNA, each parasite was kept in Eppendorf tube (0.5 ml).

Extraction of DNA according to QIAamp DNA mini kit instructions, Preparation of PCR Master Mix, Cycling conditions of the primers. Temperature and time conditions of the primers differentiate according to each primer (ITS2 and 28sDNA) during c PCR, DNA molecular weight marker by mixing the ladder gently by pipetting up and down. 6 µl of the required ladder were directly loaded. Then the final extraction performed for Agarose gel electrophoreses according to ***Sambrook et al., (1989)***.

6- Histopathological examination:

Small tissue specimens about 0.5 cm in thickness were collected from the gills, intestines, stomach and skin containing different parasites were rapidly fixed in 10% neutral buffered formalin . Five microns paraffin sections were prepared and stained with hematoxylin and eosin for microscopical examination according to ***Takashima and Hibiy (1995).***

Results

Clinical examination of naturally infested fish:

1-The main clinical signs revealed no pathognomonic clinical abnormalities. Some infested fishes showed swimming near the water surface, rubbing the body against hard objects, haemorrhages and abrasions all over the body, sluggish movement, abdominal distension and emaciation.

2. The postmortem findings revealed gill marbling with excessive mucus secretion**,** sticking of the gill tips and greyish coloration (Plate. 1). In some cases, liver was pale or with peticheal haemorrhage (Photo. 1), the intestines showed congestion and distension with enteritis in some cases (Plate 2).

Morphological description of the isolated parasites:

Gill monogeneans:

A- *Diplectanum bocqueti.* Oliver 1980 Family: Diplectanidae

It was isolated from gills of *Dicentrarchus labrax* and *Dicenrarchus punctatus,* is hermaphroditic, ranging 0.8 to 0.9 mm long and 0.33 to 0.38 mm wide. It is flat, with an anterior head bearing two pairs of ocular spots, a main elongate body and a posterior haptor. The digestive system includes an anterior muscular pharynx, and two lateral intestinal branches. The haptor in the posterior part of the body, is a specialized organ used to attach to the host which measures from 200 to 230 µm wide. The squamodisks are formed of 21 to 26 rows of imbricated sclerified pieces; they measure from 140 to 215 µm wide. The penis consists of a narrow sclerified tube, appearing

terminated by a tip Tapered; it measures from 82 to 95 µm long. (Plate 3)

2-Gastrointestinal digeneans:

Examination of mucosal scraping of the gastrointestinal tract revealed:

A- Acanthostomum sp. (Timoniella sp.): Loose 1899. (Family: Cryptogonimidae)

It was isolated from intestine of *Dicentrarchus labrax* and *Dicenrarchus punctatus.* It is medium-sized body, long, slender subcylinderical or spinulated. Has oral sucker terminal and fingerbowl-to funnel shaped, with a crown of spines. The prepharynx was long and in midway between tow suckers. Its esophagus is short, bifurcating a little in front of acetabulum ,ceca opening outside at posterior extremity. Acetabulum relatively small, located in anterior half of body. Testes tandem , or somewhat diagonal, intercecal , at posterior extremity. vesicula seminalis tubular, winding behind acetabulum and no copulatory organ . Gentile pore immediately in front of acetabulum .Ovary submedian or partically median , pretesticular. Uterus coiled from side to side in intercecal field between overy and acetaulum , eggs small , very numerous. Vitellaria extend from behind seminal vesicale to preoverian level. (Plate 4).

B-*Allostomachicolina Secundus* Yamaguti 1958. (Family: Hemiuridae)

Microscopic smears from the stomach showed small, smooth, elongated body. The oral sucker was well developed, subterminal and rounded measured 0.28- 0.65 mm in length and 0.26-0.62 mm in width. Prepharynx was very short. The pharynx was ovoid 0.095-0.17×0.083- 0.15 mm. The oesophagus was short and the intestinal ceca were termining to posterior extremity. Acetabulum located anteriorly 0.69-0.7×0.58-0.44 mm. The testes were smooth, ovoid and nearly tandem in position. The ovary was coarsely lobed, anteriolateral to anterior testes.

The uterus winding was largely and occupied most of the body. The vilellaria were well developed (Plate 3).

3- Encysted metacercariae (EMC):

They are spherical or sub-spherical, double walled, outer thick and inner hyaline and separated from the metacercariae by a potential space containing fluid in which the metacercariae was moving. Metacercarial cysts were found in gill, liver, heart and kidney (Plate 5).

Identification of family Cryptogonimidae (acanthostomum sp.) by using PCR.

The target gene trematode (Acansostomum sp.) was identifying by using universal ITS2 specific primers having the sequence (GGT ACC GGT GGA

TCA CTC GGC TCG TG) and (GGG ATC CTG GTT AGT TTC TTT TCC TCC GC). And 28S rDNA having the sequence (AGA GCG CAG CCA ACT GTG TGA) and (TGC CAC GTC CTA GCA TCA GCC). PCR amplification and agarose gel electrophoresis yielded a positive result of the used sample (Acansostomum sp.) of 539 bp for ITS2 and 450 bp for 28S rDNA (Plate 6).

Seasonal prevalence of trematodes infestation among different examined fishes:

From data in (**Table 1 and Chart 1**) it was revealed that the highest prevelance among the examined fish was in winter (60%) and the lowest was in summer (26%). The prevalence in *Dicentrarchus labrax* (35%) and (52%) in *Dicentrarchus punctatus* all over the year.

Seasonal prevalence of Monogenea, Digenea and encysted metacercariae infestation among different examined fishes:

The **seasonal prevalence of monogeneasis** was the highest in winter (34%) and the lowest in autumn (0%). In *D. labrax*, it was the highest in winter (36%) and the lowest in autumn (0%) and in *D. punctatus* the highest was in winter (32%) and the lowest in autumn (0%).(Table2, chart 2)

The **seasonal prevalence of digeneasis** was the highest in spring (24%) and the lowest in summer (8%). In *D. labrax*, it was the same in autumn and spring (12%) and the lowest was in summer (4%).

The **seasonal prevalence of encysted metacercariae** among *D. punctatus* and *D. labrax* were the highest in winter (40, 32%) respectively and the lowest in summer (12%). (Table2, Chart 2)

Seasonal prevalence of the recorded trematodes infestations in relation to sex among *D. labrax*

Table (3) and chart (3) showed the seasonal prevalence of the detected trematodes infestations in relation to sex among *D. labrax*, It was higher in female 40.5% while in male was 31.7%. The highest infestation in female was 70% in winter and the lowest prevalence was 16.6% in autumn.in male the highest infestation was 47% in spring and the lowest was 15.3% in autumn.

Table (4) and chart (4) showed the seasonal prevalence of the detected trematodes infestations in relation to sex among *D. punctatus*, It was higher in female (61.2%) while in male was (38.8%). The highest infestation in female was (83%) in autumn while the lowest prevalence was (25%) in summer. In male the highest infestation was 62.5% and the lowest was 15.3% in autumn.

Seasonal prevalence of the recorded trematodes infestations in relation to sex among *D. punctatus.*

Results of histopathological examination of the infested fishes:

Histopathological changes due to **monogenetic trematodes** were severe hyperplasia, destruction,

congestion, mononuclear cell infiltration and vacoulation of the epithelial lining of secondary lamellae (Photo. 2, 3). Intestine affected with **digenetic trematodes** showed mucinous degeneration and focal epithelial desquamation in epithelial lining with mononuclear cell infiltration infiltration in lamina proppria **(Photo. 4, 5**).

Plate (1): ***A*** *Gill of Dicentrarchus labrax showing marbling appearance.*
B *Gill of Dicentrarchus punctatus Showing sticking tips with greyish coloration.*

Photo (1): Dicentrarchus punctatus showing haemorrhagic liver.

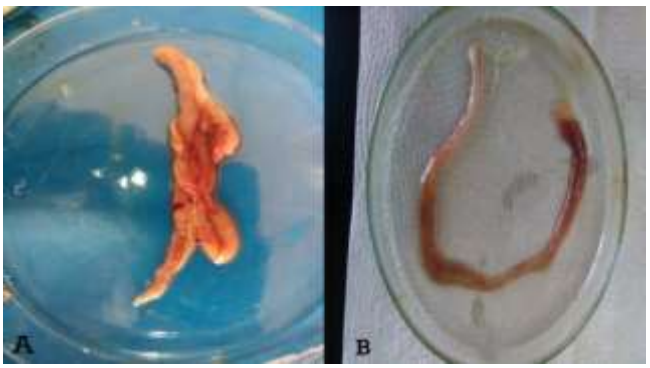

Plate (2): ***A*****-** *Dicentrarchus labrax intestine showing congestion.*
B**-** *intestine of Dicenrarchus punctatus showing congestion in the proximal part.*

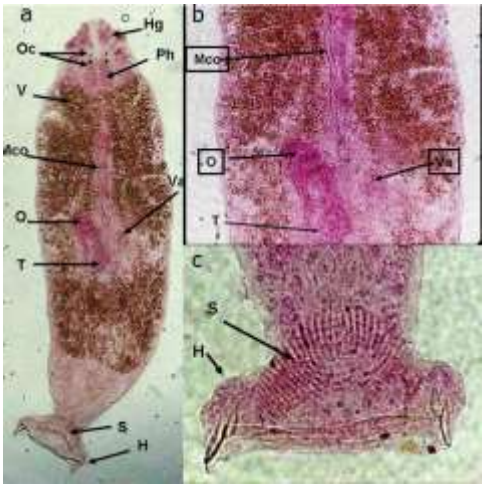

Plate (3): Light photomicrograph of *Diplectanum bocqueti*. **a.** Stained whole fluke, **b.** Copulatory organs of *Diplectanum bocqueti* : Mco: Male copulatory organ; Va: Vagina; O: Ovary; T: Testes. And **c**. Posterior part of *Diplectanum* sp. : H: Haptor; S: Squamodiscs.

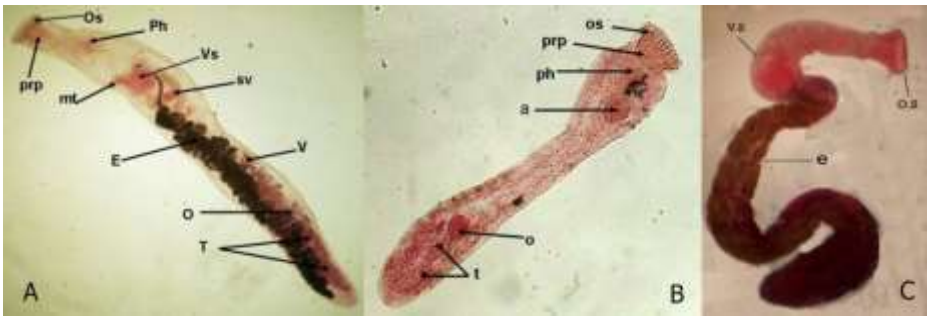

Plate (4): A: Light photomicrograph of Acanthostomum sp. os= oral spines; prp= Prepharynx; ph= Pharynx; mt= metraterm; ac= Acetabulum; e= eggs within the uterus; v= Vitellaria; o= Ovary; t= Two testes; sr= Seminal receptacle.
B: Light photomicrograph of ***Acanthostomum* larva.**
C: Light photomicrograph of *Allostomachicolina Secundus*. os= oral sucker ; vs= venteral sucker ; e = eggs.

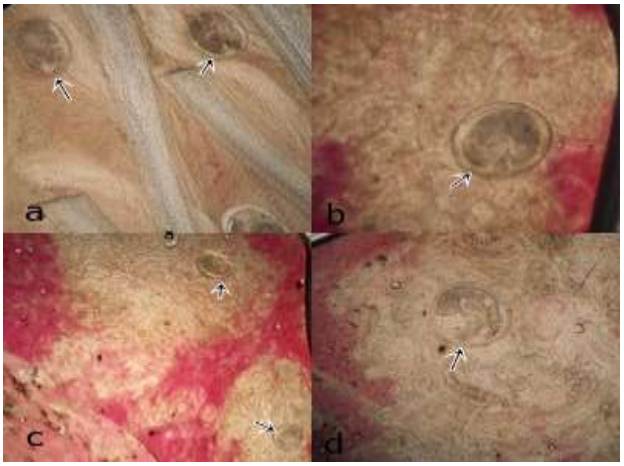

Plate (5): Light photomicrograph of metacercarial cysts embedded in **a**. gill filaments, **b**. in liver, **c** in heart and **d**. in kidney.

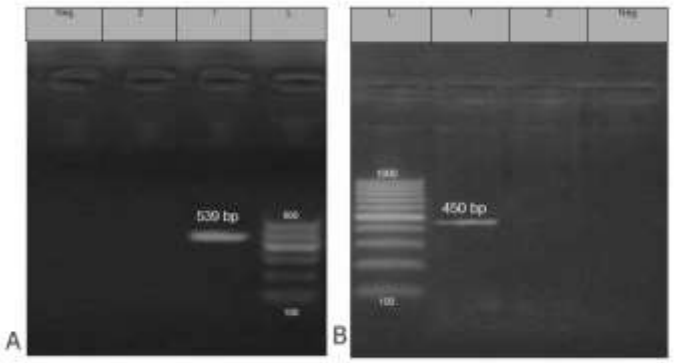

Plate (6): A- A representative gel displaying analysis of ITS2 region from adult specimens of Acanthostomum sp. lane (1) at 539 bp. Lane (L) represents the 100 bp DNA ladder as a marker (bp).

A representative gel displaying analysis of 28s DNA region from adult specimens of Acanthostomum sp. lane (1) at 450 bp. Lane (L) represents the 100 bp DNA ladder as a marker (bpz)

Table (1): *Seasonal prevalence of the total trematodes infestations affecting the examined fishes.*

	Autumn N= 25	Winter N = 25	Spring N = 25	Summer N = 25	Total N = 100
D. labrax					
Total parasitic infestation	(4) 16%	(13) 52%	(12) 48%	(6) 24%	(35) 35%
Monogenea	(0) 0%	(9) 36%	(8) 32%	(3) 12%	(20) 20%
Digenea	(3) 12%	(2) 8%	(3) 12%	(1) 4%	(9) 9%
Encysted metacercariea	(1) 4%	(8) 32%	(7) 28%	(3) 12%	(19) 19%
D. punctatus					
Total parasitic infestation	(0) 48%	(17) 68%	(16) 64%	(7) 28%	(52) 52%
Monogenea	(0) 0%	(8) 32%	(5) 20%	(2) 8%	(15) 15%
Digenea (worm)	(7) 28%	(6) 24%	(9) 36%	(3) 12%	(25) 25%
Encysted metacercariae	(5) 20%	(10) 40%	(8) 32%	(3) 12%	(26) 26%

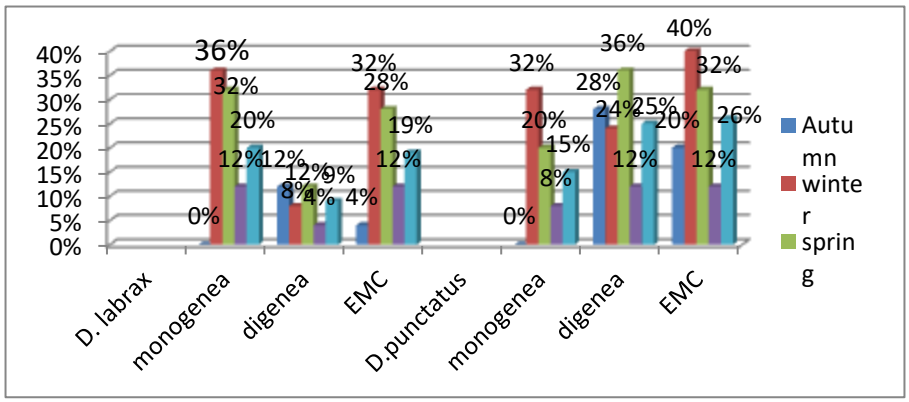

Chart (2): *Seasonal prevalence of Monogenea, Digenea and encysted metacercariea infestation among different examined fishes.*

Table (3): *seasonal prevalence of the recorded trematodes infestations in relation to sex among D. labrax*

season	Female			Male		
	No. of examined fish	No. of infested fish	%	No. of examined fish	No. of infested fish	%
Autumn	12	2	16.6	13	2	15.3
Winter	10	7	70	15	6	40
Spring	8	4	50	17	8	47
Summer	7	2	28.5	18	4	22.2
Total	37	15	40.5	63	20	31.7

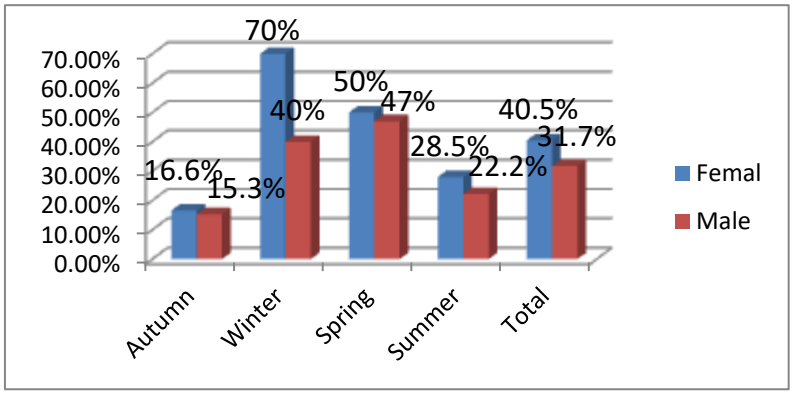

Chart (3): *Prevalence of the recorded trematodes infestations in relation to sex among D. labrax*

Table (4): *Prevalence of the recorded trematodes infestations in relation to sex among D. punctatus*

season	Female			Male		
	No. of examined fish	No. of infested fish	%	No. of examined fish	No. of infested fish	%
Autumn	12	10	83.3	13	2	15.3
Winter	17	10	58.8	8	5	62.5
Spring	17	12	70.5	8	4	50
Summer	16	4	25	9	3	33.3
Total	62	38	61.2	36	14	38.8

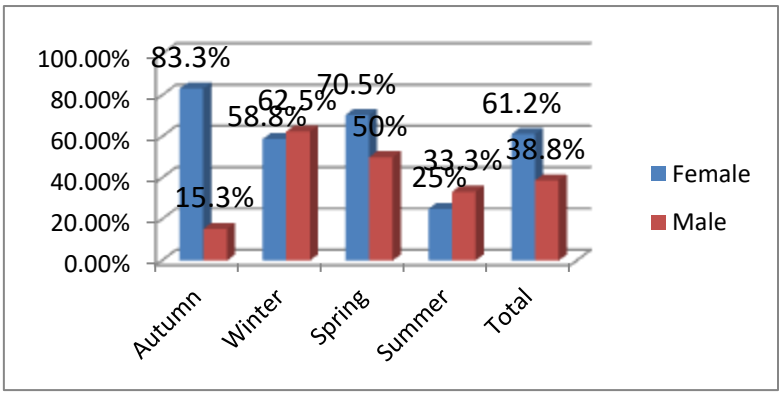

Chart (14): *Prevalence of the recorded trematodes infestations in relation to sex among D. punctatus.*

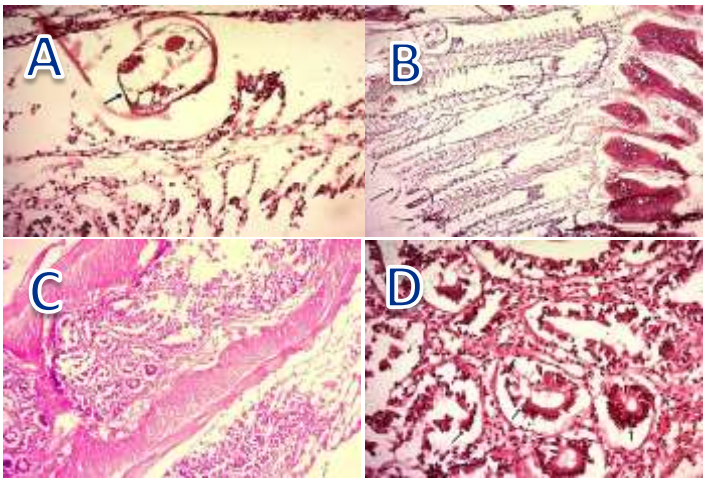

Photo. 2) Gill of *D. labrax* infested by monogenean parasite showing cross section of monogenean parasite with marked destruction in secondary gill lamellae. H&E x400.
(**Photo. 3**) Gill of *D. labrax* infested by monogenean parasite showing focal desqumation in the secondary lamellae with edema and mononuclear cells infiltration in both gill each and lamellae. H&E x 250.
(**photo. 4**) Intestine of *D. punctatus* infested by digenea showing muccinous degeneration and focal epithelial desquamation in epithelial lining with mononuclear cell infiltration in lamina proppria. H&E x400.
(**Photo. 5**) Intestine of *D. punctatus* infested by digenea showing massive epithelial sloughing with marked mononuclear cell infiltration. H&E x250.

Discussion

Fish diseases act as great effect either in culture or wild fishes and limit the full fish production especially in tropic countries like Egypt.

The main clinical signs observed in infested fish with monogeneasis infestations were marbling (mosaic) appearance, excessive mucus secretion and gill tips were sticking with grayish coloration, sluggishness and rubbing the body against hard objects and sides of aquaria to get rid the irritation induced by the parasites. Fishes gathered at water surface (surface breathing) with gulping the atmospheric air. These results agreed with those reported by ***Osman (2005)*** and ***Maather El-lamie (2007)***.

Any slight structural damage of gills can render fish very vulnerable to osmoregulation with respiratory difficulties as fish gills are responsible for regulating the exchange of salt and water and play a major role in excretion of nitrogenous waste products ***(Mahi Ghobashy, 2000).***These results may be attributed to the low respired oxygen of destructed gill epithelium which caused by feedin`g activity, attachment, fixation and locomotion of monogenea causing massive destruction of respiratory epithelial cells ***(Eissa, 2002).***

The main clinical signs observed in some infested fish with internal digenea were sluggish movement, abdominal distension and emaciation. As a result of parasitic impact on gastrointestinal tract resulting in destruction and alteration of tissue, mechanical blockage and in turn decrease absorption from gut. This result agreed with finding of ***Engy Abd Elglil (2009)*** and ***Mai Abd-El Azeem (2012)***.

Regarding the Postmortem examination of gills, it revealed areas of congestion and paler or marbling with excessive mucus secretions and sticking of the gill tips and grayish discoloration. This result agreed with ***Jithendran et al. (2005)*** and ***Osman (2005)***. Marbling appearance was common signs in examined fishes due to destruction of the efferent vessels by monogenea. Such phenomenon was caused by low blood pressure and extensive hemorrhages results in very hard blood clotting by which occlusion of the vessel occurs, thrombus is formed resulting in ischemia which in turn leads to necrosis in some areas that adjacent to areas of inflammation and congestion ***(Eissa, 2002).***

Postmortem examination in some infested fish with internal digeanean infestation revealed pale or anemic organs. Liver was haemorrhagic in some examined *Dicentrarchus labrax* while it was pale in some *Dicentrarchus punctatus*. Liver sometimes was congested. Stomach and intestine showed congestion and inflammation of their walls in some examined fishes. this agree

with ***Bassiony (2002)***, ***Eissa (2002)*** *and* ***Heba Abdel – Moula (2005)***.

Regarding the parasitological examinations the mononean trematode under discussion relate to family Diplectanid species *Diplectanum bocqueti* isolated from gills of *D. labrax* and *D. punctatus*. This result agreed with ***Banu and Zafer (2012)*** *and* ***Engi (2016)***

Regarding the parasitological examinations, the 1st digenean trematode under discussion relate to family Acanthostomatidae Acanthostomum sp. (Timoniella sp.) isolated from the intestine of *D. labrax* and *D. punctatus*. This result agree with ***Erik (2002)*** *and* ***Maather (2007)*** who isolated *Acanthostomum imbutiforme* from gastrointestinal tract of *D. labrax* and ***Kavch (2002)*** who isolated *Acanthostomum imbutiforme* from Round goby.

The 2nd digenean trematode under discussion related to family Hemiuridae species *Allostomachicolina secundus* isolated from the intestine of *D. labrax* and *D. punctatus*. These morphometric features were similar to that reported by ***Mahmoud (1990). El-Ekiaby (2009) and Abdel-Mawla and El-Ekiaby (2012)*** isolated this parasite from stomach of *Morone labrax* and ***Tadros (2004)*** *Scomberomorus commerson***.**

Unidentified species of encysted metacercariae in different body part these results were in agreement with that obtained by ***Mai (2016)*** who investigated a various encysted metacercariae

PCR is one of the most popular methods in biological and biochemical bench work today. The novelty of PCR is that it use the common chemical language among living things, the nucleic acid sequence, and increase it from small amount into million to billions. PCR is time saving and sensitive. ***Glennon and Cormican (2001).*** In this study molecular identification of (trematode) Acanthostomum sp. using PCR analysis of ITS2 size 539 bp and 28s DNA size 450 bp are a common molecular identification. The ITS2 amplicons size (539 bp) was agree with ***Bowles (1995)*** who identified trematode using PCR analysis of ITS2 size 539 bp.

The present study indicates that the total prevalence of parasitic infestation was 43.50%. This result was higher than that obtained by ***Eiman Youssef and Derwa (2005)*** which was 15.7%, ***Mai Abd-El Azeem (2012)*** which was 22%, and lower than that of ***Samar William (2004)*** which was 51% and ***Maather El-lamie (2007)*** which was 70% among marine fishes. This may be due to difference in fish species and the locality from which fish was obtained. The higher infestation rate was recorded in *D. punctatus* (52%) when compared with *D. labrax* (35%) It may be due to *D. punctatus* occurs on various kinds of bottoms, including sand,

muddy sand and rocks ***(Smith, 1990).*** Also the mud is high organic matter which increases susceptibility to disease. Or due to immune response of infested fish species.

The results of seasonal prevalence of the trematode infestation agreed with ***Maather El- lamie(2007)*** *and* ***Samah El Shafey (2016)*** and disagreed with ***Heba Abdel-Mawla and Walaa El-Ekiaby (2012).*** This may be attributed to the differences of the location from which the fishes were collected and the type of searched parasites.

The seasonal prevalence of monogenetic trematodes was the highest in winter in both fish species this result agreed with ***Rawson and Rogers (1972)*** who found monogeneans parasites showed peaks of abundance during cold seasons. This may be due to the lower levels of specific antibodies produced by the host during cold seasons ***Cloutman (1978)***. Also, life span of the free swimming monogeneans larvae is temperature independent ***Paperna (1980)*** and disagree with ***Maather El Lamai (2007)*** who recorded the highest prevalence of monogenea in sea bass was in spring.

Regarding to seasonal prevalence of digeneans among the examined fishes. It was the highest in Spring and Autumn in *D. labrax* and lowest in Summer. These results were disagreed with that of ***Maather El-Lamai (2007)*** who recorded the highest infestation in winter, This variation in prevalence may due to the differences in localities of samples, fish species and type of searched parasites. The change in prevalence in different seasons may relate to change in immune response of fish at different temperatures ***(Kennedy and Walker, 1969)***. This could be related to changes in abundances of plankton and food composition as well as food preference of *D. labrax* in different seasons. ***(Tekin et al., 2008).***

Concerning encycted metacercariae infestation in *D. labrax,* the prevalence was 19% while in *D. punctatus was* 26%.The higher prevalence recorded in winter this agreed with ***Kanda et al. (2005).*** The seasonal variation of metacercariae is due to a difference in time of exposure to parasites. Actually, release of trematode cercariae from snail host and successful transmission to the fish host is highly temperature dependent ***(Oshima and Nishi, 1963).***

The results explored that the prevalence of infection was invariably very high in the female host in compare to male; this result has similarities with ***Ozer and Ozturk (2004)*** because of the comparatively strong immunity carried by the male fish than female. The sex of the host has relevant impact on the regulation and periodicity of the parasites ***(Smith, 1969).***

The highly trematode infestation in female appeared at winter 70% and in male at spring 47% while the lowest infestation in female and male was at autumn 16.6 and 15.3% respectively. So, from these results there was no significant correlation between the host sex and the prevalence of parasites during the seasons. Their finding was similar with that obtained by ***Boungous et al. (2008)*** *and* ***Tombi et al. (2014)*** who observed no sex impact on the parasitic infestations.

The histoPathological changes due to monogenean infestation in gills of the examined fishes showed congestion of blood vessles, hyperplasia of lamellar epitheliul cells, degeneration, necrosis of lamellar epitheium and adhesion of lamellae and vacuolar degeneration. This result was in agreement with that obtained by ***Maather El-lamie (2007)*** *and* ***Engi El-Raziky (2016)***

Intestine of *D. labrax* with digeneans parasitic infestations showed severe congestion of the submucosal blood vessels a long with mucinous degeneration of the mucosal lining**.** These results were in agreement with that obtained by ***Heba Abdel- Moula (2005)*** *and* ***Maather (2007).***

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الملخص العربي

أجريت هذه الدراسة لاستبيان الامراض الناجمة عن الديدان المفلطحة التى تصيب بعض الأسماك البحرية و قد تم تجميع 100سمكة 100 قاروص و 100 نقط من مزارع خاصة من مدينة الإسماعيلية فى المواسم المختلفة.لم يتم الكشف عن علامات مرضية مميزة عدا الظاهرة الرخامية لخياشيم الأسماك المصابة بالديدان أحادية العائل على الخياشيم.العلامات الأخرى و الصفة التشريحية كانت أنزفة، خدوش بالجلد، بطء فى الحركة، انتفاخ البطن و الهزال . شحوب الكبد، احتقان الأمعاء مع الإلتهاب فى بعض الأحيان. أعلى نسبة إصابة سجلت فى أسماك النقط (52%) أتبعت بأسماك القاروص (35%). الطفيليات المعزولة كانت ديدان مفلطحة أحادية العائل(ديبلكتنيم بكوتي) و ديدان مفلطحة ثنائية العائل (اكانسوستومام و ألوستوماشيكولينا سيكوندوس) من أسماك القاروص والنقط.تم دراسة نسبة الاصابة الموسمية، العلاقة بين أوزان الاسماك وأطوالها و الجنس و نسبة الإصابة، كما تم تصنيف طفيل الاكنسوستومام باستخدام تفاعل البلمرة المتسلسل بجانب الصورة الهستوباثولوجية التي تم رصدها.