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Cestodes infestation in some marine fishes from Mediterranean coasts

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ABTRACT

Our practical side demonstrated the results of collecting marine fishes from Egyptian coasts of Mediterranean sea from Damietta to Marsa Matrouh for focusing on more prevalent cestode infestation in that area. Three cestodes were most prevalent existence. ***Ligula*** *intestinalis* larvae**,** ***Trypanorhyncha*** larvae *and* ***Tetraphyllidean*** Larvae. Out of 120 different collected and examined marine fishes were. ***Ligula intestinalis*** exhibited in body cavity and intestine of Silver-side fish (*Atherina boyeri)* by prevalence rate of **70%** and in *Pagrus pagrus* fish by prevalence rate of **15%,** while ***Trypanorhyncha*** found in the body cavity of *Thunnus thunnus*fish by prevalence rate of **62.5%** and finally ***Tetraphyllidean Larvae*** found in the intestine and stomach of *Pagrus pagrus* fish by prevalence rate **55%.** Histopathological examination of affected organs were applied given fully demonstrated lesions .Common lesions in some organs of infested fishes were associated with cestode infestation.

Introduction

Ligulosis is occurred by *Ligula intestinalis* cestode and named fish tapeworm. The parasite consider a permanent pathogen for native fish and wild fish as intermediate host If the final hosts (piscivorous birds) ingest the infested fishes , the parasites will localized their intestines and their body cavities. *L. intestinalis* is food-borne parasite, (**Ahmadiara, 2017).** Although *Ligula intestinalis* has been recorded in several types of fishes, available data on the parasitization of *Ligula intestinalis*

plerocercoids as mean intensity, prevalence and parasitic index in *Tinca tinca* was limited, **Ergonul & Altindag (2005).**

L.intestinalis affect fishes' genital organs, kidney and liver causing ovarian infiltration of inflammatory cells , fibrosis, atrophy of oocytes; testicular necrosis, fibrosis, hyperemia, and degeneration; kidneys necrosis, destruction of tubules, vacuolar degeneration; and liver vacuolar degeneration with biliary duct hyperplasia, necrosis, fibrosis and cholestasis with lymphatic vessel dilatation, **Bozorgnia *et al* ., (2016).**

Cestodes of order Trypanorhyncha cestodes are infest marine fishes and invertebrates parasites, especially in both tropical and subtropical regions. Adult's worm inhabit the gastrointestinal tract of elasmobranches while larvae localize the body cavity and visceral serosa of cephalopod, teleost and crustacean intermediate hosts. Trypanorhyncha larvae acquired significance because of their repugnant aspect, especially when present as severe infections in musculature and other organs, which can make commercialization infeasible due to sanitary inspection and/or consumer rejection, resulting in economic losses, **Diniz, *et al.* (2021).**

External examination showed no pathognomonic clinical signs. while the internal examination revealed heavy infestation of tetraphyllidean larvae especially in liver and musculature of the caudal peduncle, as other internal organs. The total prevalence was 51.52%: but in summer it was with heavy intensity The gills and internal organs showed pressure atrophy. degeneration and local necrosis beside some circulatory disturbances and inflammatory reactions with proliferation of melano-macrophages, **Eissa *et al.*, (2001)**

The damage caused by digenean was destruction of the mucosal epithelium of the villi. Degeneration and necrosis of epithelial cells were also declared. At the site of infection a high number of rodlet cells (RCs) and mucous cells were detected in the epithelium, with exhibiting cellulardischarge activity, **Dezfuli *et al.*, (2009)**

The histo-pathological examination of Trypanorhyncha revealed the presence of multiple parasitic larvae attached to intestinal serosa. Thehistopathological examination of the parasitic nodules revealed the characteristic shape of the anterior part of the cestodal larvae in tissue section. A thin layer of fibrous connective tissue surrounds the parasites and holds fast them to the intestinal tissue .Remnants of the larvae were also noticed in the hepatic tissue with

prominent melanophores aggregation. Some cases reported characteristic passage tracts formed of necrotic tissue with marked hepatocytes destruction. The parasitic larvae were wrapped with active thin layer of proliferative fibrous tissue, melanophores aggregation and atrophied hepatocytes. In such tracts, areas of hemorrhage were frequently noticed along with melanophores aggregates and mild to marked fibrosis .Mononuclear inflammatory cell infiltration and hepatocytes necrosis were common findings with multiple pyknotic nuclei in the affected hepatic tis-

sue. The examination of the spleen revealed marked activation of melano-macrophage centers while the homeopathic tissue showed depletion **Khalidah *et al*., (2014**)

Intestinal pathology associated with *tetraphyllidean* larvae infection was minimal. The damage caused was destruction of the villus mucosal epithelium. Necrosis and degeneration of epithelial cells were also observed, with both types of cells exhibiting discharge activity, **Dezfuli *et al.*, (2009).** *Tetraphyllideans*, adult stages are known to have ageographical distribution parasitizing various fishes, in the intestine and stomach of *chondrichthyan* fishes essentially all orders of rays and sharks. Nevertheless, similar larval stages of *Tetraphyllideans* were already found in Italy in *D.labrax* . During this survey, larval stages of *Tetraphyllidea* were located in the stomach and the intestine of wild D.labrax but detailed morphological examination for generic and specific identification wasn't performed on the specimens. No pathogenic effects were seen neither in the stomach or intestine of the examined fishes. However, there is no indication of pathological lesions by *Tetraphyllideans* in wild or reared D.labrax in the consulted literature, **Amel *et al.* (2016).**

The aim of the present study is to evaluate the occurrence of some cestodes in the north Egyptian marine coast of the Mediterranean Sea with full description of the accompanied pathological picture

Material and Methods

Parasitological Examination:

A total of 120 marine fishes collected from Egyptian Mediterranean coasts from Damietta to Marsa Matrouh were examined for helminth parasites (cestodes) from three species of fish, *Atherina boyeri*, *Thunnus thunnus* and *Pagrus pagrus.* Forty fish from each species.

Sample Examination and Parasites Identification:

All samples of fishes were clearly labeled with species of fish, and transported immediately into Animal Health Research Institute-Zagazig, Fish Diseases Research Unit.

Parasitological examinations involved the skin, fins, gills, alimentary tract , flesh and body cavity of fish. Samples were left for a few minutes into a petri dish containing saline solution, then opened and examined by light microscope. Cestodes were preserved in **10%** formalin for fixation**, (Marcogliese, 2001).**

The parasites were identified under light-microscope by using the standard keys in the literature, according to **(Bunkley-Williams and Williams, 1996;** and **Vernon, 2006)** for cestodes.

Postmortem examination

Collected fishes were sacrificed and external examination applied carefully then postmortem examination applied accurate. Registration of full data was applied.

Pathological examination

Tissue specimens:

Liver, kidneys, intestine, stomach, gill-sheart, spleen genital organs and muscles were collected from sacrificed *Atherina boyeri*, *Thunnus thunnus* and

Pagrus pagrus and fixed in 10% buffered neutral formalin. Paraffin sections 5 micron thick were prepared and stained with hematoxylin and eosin stain **(Survarna *et al.*, 2013)** and examined microscopically.

Results

In the present study, out of 120 fish were examined for helminth parasites (cestodes), 40 fish of each *Atherina boyeri, Thunnus thunnus* and *Pagrus pagrus* respectively. We found out of 28/40 *Atherina boyeri* fish and out of 6/40 *Pagrus pagrus* infested by ***Ligula intestinalis*** **Larvae, figure (A)**in thebody cavity and intestine with prevalence rate 85%, out of 25/40 fishes *Thunnus thunnus* fish infested by ***Trypanorhyncha*** **larvae, figure (B)** in the body cavity with prevalence rate 62.5% and out of 22/40 *Pagrus pagrus* fish infested by ***Tetraphyllidean Larvae***, **figure (C)** in the intestine and stomach with prevalence rate 55%, **Table (1) and Graph (1).**

Table (1) Prevalence rate of infection by different cestodes:

Fish / Cestode	*Ligula intestinalis* Larvae	*Trypanorhyncha* larvae	*Tetraphyllidean Larvae*
Atherina boyeri	**70%**	-	-
Thunnus thunnus	-	**62.5%**	-
Pagrus pagrus	**15%**	-	**55%**

Graph (1): Prevalence rate of infestation by different cestodes:

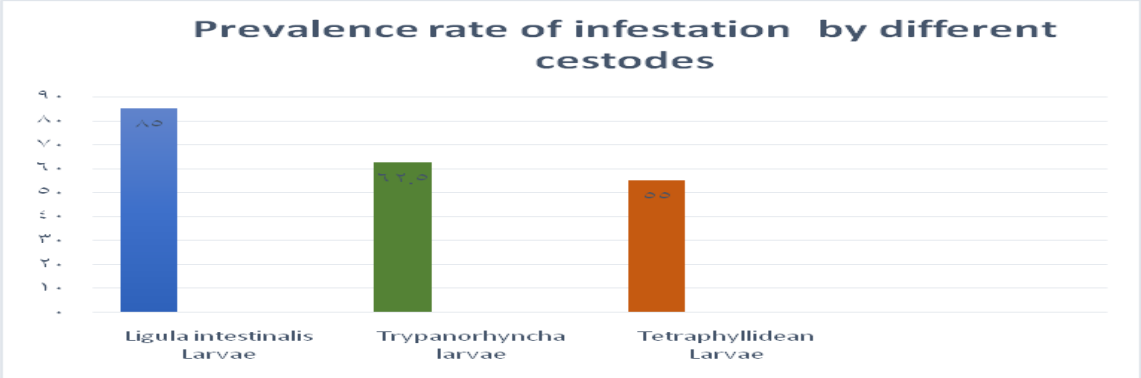

Figure (A) *Ligula intestinalis* Larvae in petri dish
Site of infection: Body cavity and intestine
Fish species: Atherina boyer-i*and pagrus pagrus*

Figure (B) *Trypanorhyncha* Site of infection: Body cavity
Fish species: *Thunnus thunnus*

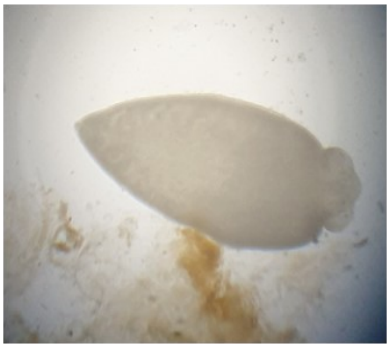

Figure (C) *Tetraphyllidean* Larvae Site of infection: Intestine and stomach
Fish species: *Pagrus pagrus*

II- Histopathological results

A-Postmortem examination

External examination didn't reveal any characteristic or pathognomonic macroscopical changes except mild to severe swelling of the abdominal lesions specially in case of *L.intestinalis* infestation, while the internal examination revealed existence of ***Ligula intestinalis*** larvae in the body cavity near gills **(Fig. 1a)** , heavy infestation of *trypanorenchya* covered most of internal organs **(Fig. 1b)** which exhibited moderate adhesion in most examined and infested fish and *tetraphyllidean* larvae especially in liver and musculature of the caudal peduncle in addition to other internal organs. The internal organs showed pressure atrophy with congestion in most infested fishes while focal or diffuse pale areas could be detected in other fishes.

B- Microscopical examination

1-*Ligula intestinalis* infestation affected most of internal organs of body cavity while liver ,kidneys and intestine were the most affected organs in most examined fishes and revealed some microscopical changes as hepatic lesions represented in congestion, hepatic necrosis in

addition to atrophied hepatocytes and cholestasis **(Fig. 1c)**. Diffuse vaccuolation of hepatocytesof mainly steatosis **(Fig.1d)** and biliary hyperplasia were also detected in heavy infested fishes. Melanophores aggregation in the hepatic tissue were also seen . Kidneys revealed degenerative changes to destruction of renal tubules as vacuolar degeneration of renal epithelium with melanophores aggregation in the renal medulla **(Fig.1e)** while intestine showed disturbance of mucosal epithelium to complete loss of intestinal villi. Inflammatory cells infiltration mainly eosinophil **(Fig.1f)** and fibrosis could be detected.

2-Trypanorenchya larvae infestation affected all the internal organs of body cavity even gills causing severe destruction of most of these organs as abnormalities in secondary lamellae of gills (curling, lifting and fusion) **(Fig.2a)**. Degeneration and local necrosis beside some circulatory disturbances and inflammatory reactions with proliferation of melano-macrophages were detected in affected liver. Embedded cyst of larvae ruminants (arrowhead) surrounded by a thin fibrous connective tissue capsule **(Fig.2b)**.Vacuolar degeneration of hepatocytes **(Fig.2c)** was also noticed while kidney suffering congestion of blood vessels with degenerative changes of renal tubules of mainly cloudy swelling **(Fig.2d)**. Intestinal lesions demonstrated nodular inflammatory reaction within intestinal mucosa **(Fig.2e)**. Other cases showed fusion of some intestinal villi **(Fig.2f)**.

3-tetraphyllidean larvae infestation affected mainly intestine and stomach causing mild to moderate lesion according to number of spreading larvae . Stomach showed submucosal edema **(Fig.3a)** was noticed ,diffuse congestion **(Fig.3b)** .Gastric mucosal infiltration with round cells in addition to submucosal vacillation **(Fig.3c)** were also noticed. Few cases showed destruction of the mucosal epithelium. Intestinal lesions were diffuse congestion of submucosal blood vessels **(Fig.3d)**, hyaline degeneration of intestinal submucosa **(Fig. 3e)** Denuded villar epithelium to denuded villi **(Fig. 3f)**,were noticed. Mucinous degeneration or metaplasia of enterocytes to goblet cells was seen in few cases.

Minimal alterations limited to mild congestion and minimal degenerative changes in other organs as heart ,spleen ,muscles and genital organs in all examined infested fishes fishes by the three cestodes.

Discussion

In the present study, out of 34/ 40 *Atherina boyeriandpagrus pagrus* fishes infested by *Ligula intestinalis* Larvae in the body cavity and intestine with total prevalence rate **85%.** *Ligula intestinalis* has been recorded in several fishes hosts, available data on *Ligula intestinalis* plerocercoids such as prevalence is limited. Low prevalent rates were detected in fresh water fishes when compared with our results were recorded as 40.01%, by **Ergonul & Altindag (2005)** and (21.4%) was determined by **Ozmen *et al.* (2021) and** 25.35 %, by **AlDulaimi *et al.* (2020).** Differences in type of water could be the cause of differences in prevalence **rate.** In the present study, ***Trypanorhyncha*** found in the body cavity of *Thunnus thunnus* by prevalence rate 62.5%. disagree with total of wild marine fish species was collected from marine water sources, Mediterranean coasts of Damietta, 44 % of detected larvae were belonging to *Trypanorhyncha* Plerocrcoid larvae, **Rehab *et al.* (2021).** The specimens of *Trypanorhyncha* larvae were obtained from subserosa of the anterior oesophagus, stomach, pyloric caeca, liver, ovaries and mesenteries of whiting caught by commercial fishing, in 268 fish were 18.66%, **Ahmet *et al* (2020).** Agree with constant presence of helminth larvae in the viscera and serosa of individuals of the species has been the subject of complaints among local

fish traders because of economic losses due to their repugnant aspect. Cestodes, identified as larvae of *Trypanorhyncha*, were found parasitizing the serosa of the stomach, mesentery and abdominal cavity with prevalence 61.25%, **Diniz, *et al.* (2021).**

In the present study, ***Tetraphyllidean Larvae*** found in the intestine and stomach of *Pagrus pagrus* by prevalence rate **55%.**Which nearly similar to that obtained by **Eissa *et al.* (2001)** who recorded **51.25% .** Other nearly similar prevalence rate was recorded by **Boukhari *et al.* (2020).**Who recorded **(63,46%)** which were caught in western Algeria (western Mediterranean) who explain the high prevalence rate according to the parasitic biodiversity of the Axillary seabream *Pagellus acarne* .Higher percent reach to (87.4%) was also recorded by **El-Ekiaby (2019)** from Red Sea at Suezprovince, Egypt **,** differences in prevalence rate may be due to differences in fish localities .

Considering the pathological effect of *L. intestinalis* on marine fish of north Egyptian coasts which could be explained on a base that fishes as the second host of larvae and affected with the most pathogenic lesions of *L.intestinalis,* because it invases the body cavity of the fish and can be stay there for several years **(Barber & Wright, 2005).** The detected lesions as swelling of abdominal cavity could be attributed to The rapid growth of the *L. intestinalis* in the fish's body cavity **(Geraudie *et al*., 2009).** Pathologic lesions including renal necrosis, destruction of tubules, vacuolar degeneration; and hepatic vacuolar degeneration, biliary duct hyperplasia, necrosis, lymphatic vessel dilatation, fibrosis and cholestasis were in partial agreement with those obtained by **(Rahmati *et al*., 2011)** reported cholangiohepat itis and metaplastic hyperplasiain liver, deformity of structure and function of hepatocytes, thrombosis in liver, decrements in intestine and with complete harmony with (**Bozorgnia *et al*., 2016**) who reported almost typical lesions.

Our macroscopical picture due to trypanorenchya infestation was far similar to those obtained by **Khalidah *et al*., (2014**) who congestion, edema and pressure with adhesion of internal organs .Similar gill, hepatic, renal and intestinal lesions were coordinated with those obtained by **Bamidele, A. (2007)**, C**amargoand Martinez, (2007)** and by **Khalidah *et al*., (2014**) while fusion of intestinal epithelium of villi was mentioned specially by **(Al-Niaeem, 1999).** The previously mentioned authors examined heart, spleen genital organs and muscles and registered some pathological alterations. These differences could be explained to many reasons as stage of and intensity of infestation beside species of the obtained worm and site of infestation.

Our external examination results of *tetraphyllidean* larvae were typical to that obtained by **Eissa *et al.* (2001)** who denied presence of path gnomonic clinical signs. Our microscopically lesions were limited to stomach and intestine as mentioned by (**Guagliardo *et al.,* 2009)** who declared that parasitic charges were more intense in the second portion of the digestive tract. .Intestinal lesions were far coordinated with those obtained by **Dezfuli *et al.,* (2009)**who mentioned destruction of the mucosal epithelium of the villi. with necrosis and degeneration of epithelial cells at the site of infestation with high number of rodlet cells (RCs) and mucous cells were observed in the epithelium. Gastric lesions were in partial agreement with those obtained

By **Santoro *et al*., (2013)** who mentioned inflammation associated with larval forms of helminths were located in gastric muscular is or liver and were associated with necrosis and mild to marked fibrosis.

We are in complete difference with that obtained by No pathogenic effects were observed neither in the oesophagus, stomach or intestine of the examined fishes. However, there is no indication of induced lesions by Tetraphyllideans in wild or reared D.labrax in the consulted literature, **Amel et al. (2016).**

Conclusion:

We registered a new prevalence rates of some cestodes larvae **(***Ligula intestinalis* larvae, *Trypanorenchya* larvae and *Tetraphyllidean* larvae) in Egyptian north coasts of Mediterranean sea from Damietta to Marsa Matrouh. Confirmation of the infestation determined after Identification of isolated larvae . Common pathological lesions in all infested organs were detected.

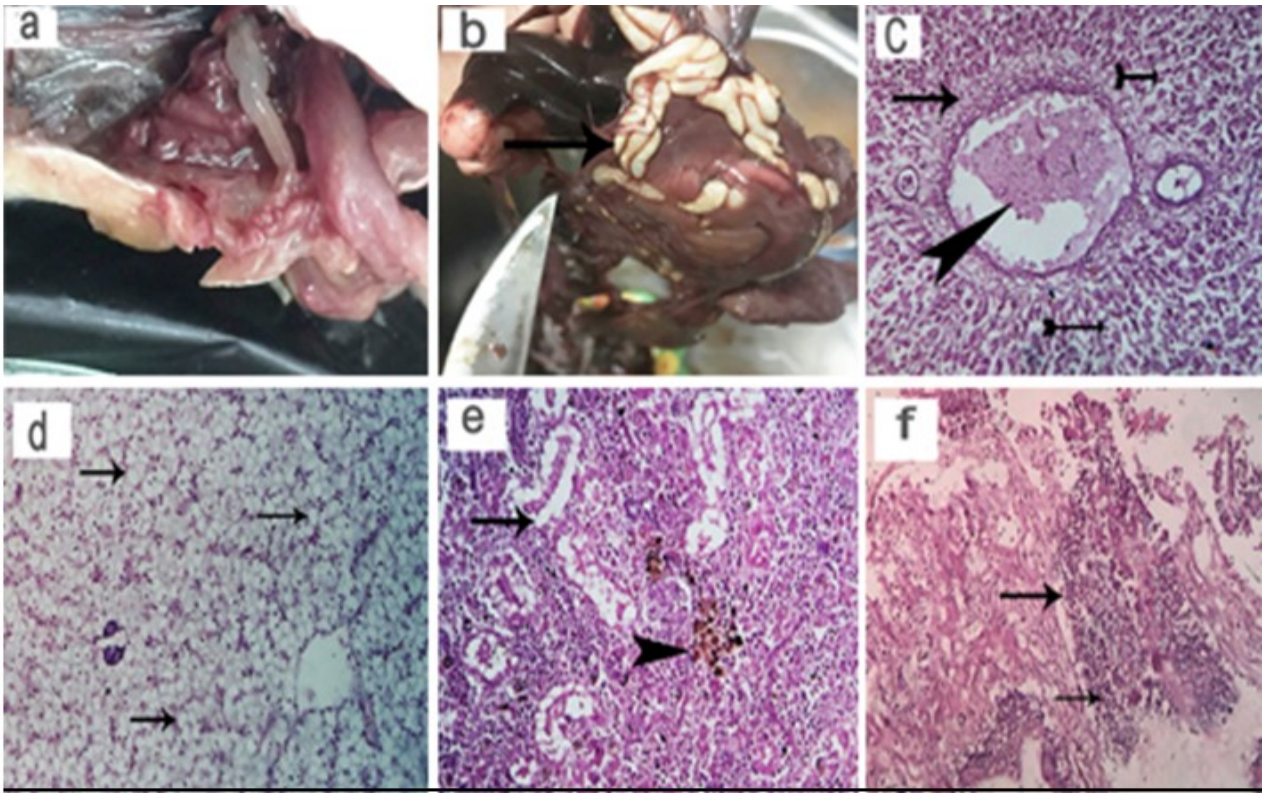

Plate 1.
(a): Opened *Pagrus pagrus* fish showing *Ligula intestinalis* larvae inside the body cavity near gills **.**
(b): Opened *Thunnus thunnus* fish showing heavy trypanorencya larvae covering the internal organs of the body cavity**.**
(c): Photomicrograph of liver of *Atherina boyeri* fish infested with *Ligula intestinalis* showing congestion of hepatic blood vessels (arrowhead) with per vascular hepatic necrosis (arrow) and atrophied hepatocytes (tailed arrows)(H&Ex 400)**.**
(d): Photomicrograph of liver of *pagrus pagrus* fish infested *with Ligula intestinalis* showing diffuse vacillation of hepatocytes of mainly steatosis (H&Ex 200).(e): Photomicrograph of kidney of *Atherina boyeri* fish infested *with Ligula intestinalis* showing vacuolar degeneration of renal epithelium with melanophores aggregation in the renal medulla (H&Ex 400).(f): Photomicrograph of kidney of *pagrus pagrus* fish infested *with Ligula intestinalis* showing inflammatory cells infiltration of almost eosinophil)(H&Ex 400)**.**

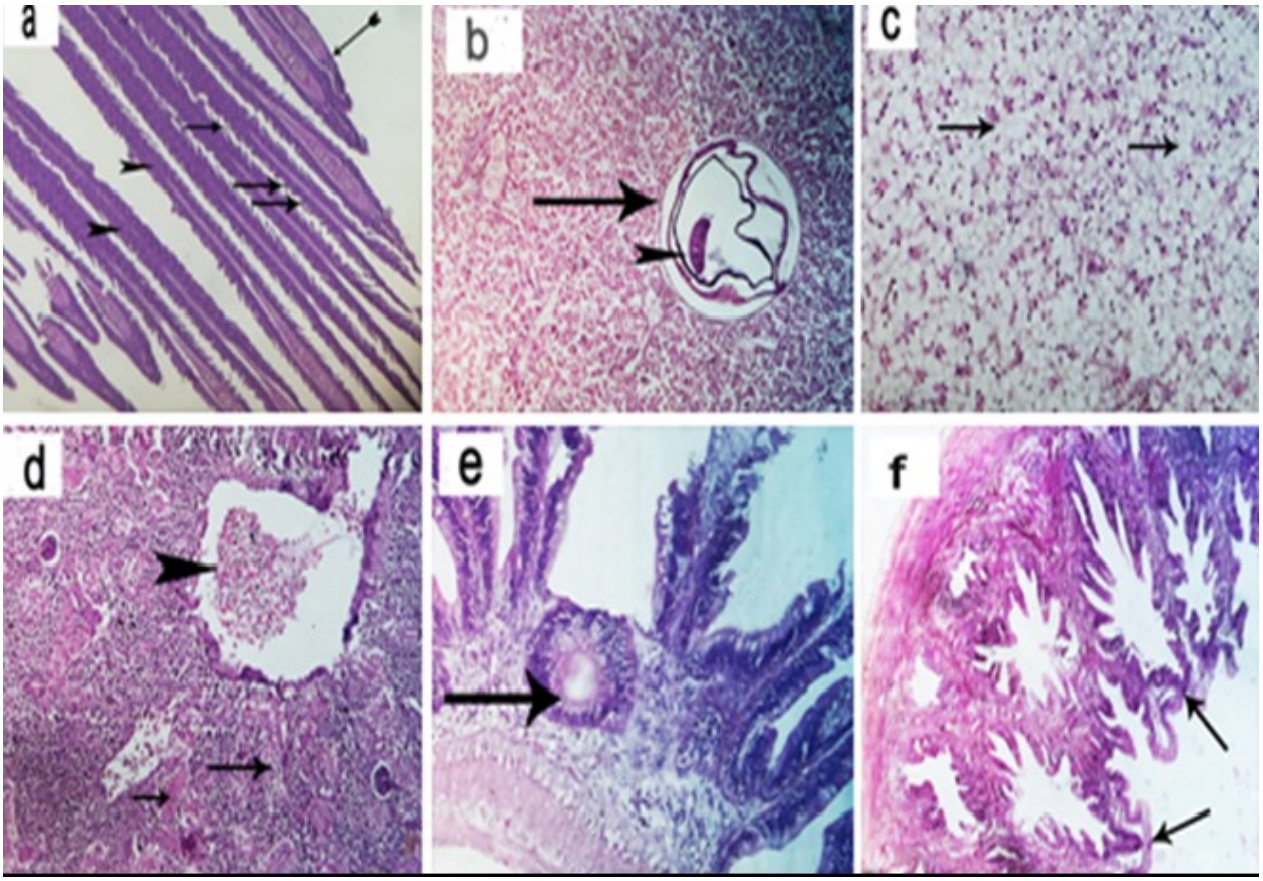

Plate 2.
(a): Photomicrograph of gills of *Thunnus thunnus* fish infested with *trypanorenchya* larvae showing hyperplasia of lamellar epithelium (arrowhead) , abnormalities of secondary lamellae (arrows) and complete absence of secondary lamellae (tailed arrow) **(H&Ex 400).**
(b): Photomicrograph of liver of *Thunnus thunnus* fish infested with *trypanorenchya* showing embedded cyst of larvae reminants (arrowhead) surrounded by a thin fibrous connective tissue capsule (arrow) **(H&Ex 200).**
(c): Photomicrograph of liver of*thunnus thunnus* fish infested with *trypanorenchya* showing vacuolar degeneration of hepatocytes (H&Ex 200)**.**
(d): Photomicrograph of kidney of *Thunnus thunnus* fish infested with *trypanorenchya* showing congestion of blood vessels (arrowhead) with degenerative changes of renal tubules of mainly cloudy swelling (H&Ex 200)**.**
(e): Photomicrograph of intestine of *Thunnus thunnus* fish infested with *trypanorenchya* showing nodular inflammatory reaction within intestinal mucosa (arrow) (H & Ex 200)**.**
(f): Photomicrograph of intestine of *Thunnus thunnus* fish infested with *trypanorenchya* showing fusion of some intestinal villi (arrows)(H& Ex 200)**.**

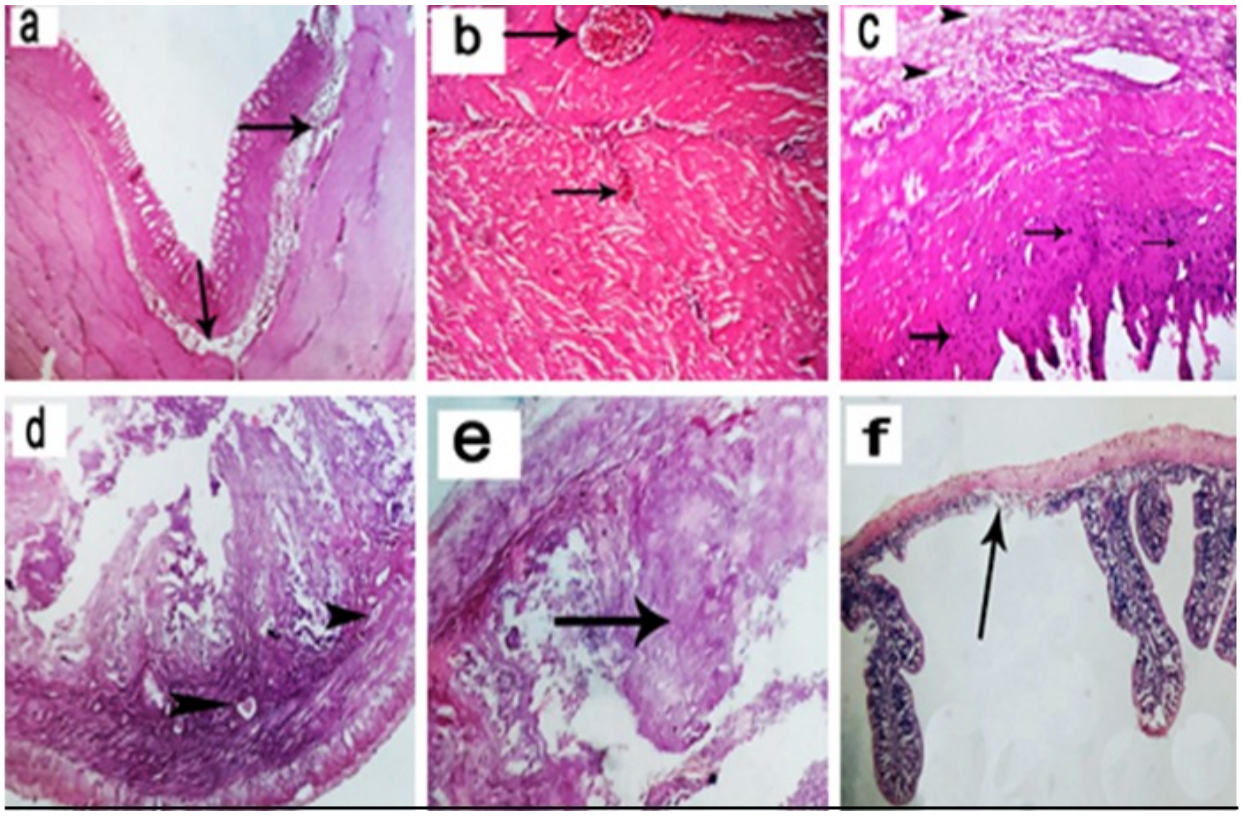

Plate:3.
(a): Photomicrograph of stomach of *Pagrus pagrus* fish infested with *Tetraphyllidean* larvae showing submucosal edema (arrow)**(**H&E x **200).**
(b): Photomicrograph of stomach of *Pagrus pagrus* fish infested with *Tetraphyllidean* larvae showing diffuse congestion (arrow) (H&E x 400)**.**
(c): Photomicrograph of stomach of *Pagrus pagrus* fish infested with *Tetraphyllidean* larvae showing mucosal infiltration with round cells (arrows) in addition to submucosal vaccuolation (arrowhead) (H&Ex **200**)**.(d):**Photomicrograph of intestine of *Pagrus pagrus* fish infested with *Tetraphyllidean* larvae showing diffuse congestion of submucosal blood vessels (H &E x **200).**
(e): Photomicrograph of intestine of *Pagrus pagrus* fish infested with *Tetraphyllidean* larvae showing hyaline degeneration of intestinal submucosa (H&E x 400)**.**
(f): Photomicrograph of intestine of *Pagrus pagrus* fish infested with *Tetraphyllidean* larvae showing focal area of denuded epithelium (H&Ex 200)**.**

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