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A CONTRIBUTION ON THE EPIDEMIOLOGY OF SOME COMMON PARASITES INFESTING CULTURED SEA BASS *(DICENTRARCHUS LABRAX)* IN EGYPT

By

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ABSTRACT

This study was carried out to spotlight on the most common parasitic infestation in cultured Sea bass, *Dicentrarchus labrax.* A total number of 120 *Dicentrarchus labrax* were collected from two private marine fish farms in Damietta governorate from September 2016 to August 2017. The prevalence, seasonal variation, as well as the histopathological changes in infested tissues were estimated. the parasitological examination revealed four genera of protozoa including(*Trichodina,Epistylis*, *Amyloodinium* and *Microsporidia),*twogenera of monogenean (*Diplectanum* and *Pseudohaliotrema sp.),* one genus of crustacean (*Caligus minimus)*, unidentified digenetic encysted metacercariae , two genera of adult digenetic trematodes (*Timoniella* and *Bucephalus)* and one genus of Nematode (*conteracecum)* with total prevalence 84.2%. The seasonal prevalence was different from parasite to another. Histopathological examination revealed different pathological changes in the affected tissues as a reaction to parasitic infestation.

Keywords:

Dicentrarchus labrax, cultured, parasites, seasonal variation, Histopathology.

INTRODUCTION

Fisheries and aquaculture are highly important sources of nutrition, income and livelihoods for millions of people all over the world **(FAO, 2016)**. Aquaculture in Egypt is a promising field with a great capability for expansion and development **(Shaalan *et al.*, 2018)**. Marine aquaculture represents about 14.5 % of the total aquaculture in Egypt. About 80 % of marine aquaculture production in Egypt is located in Damietta Governorate, while the remaining

percent located in the coastal provinces namely, Port Said, Suez, and Alexandria **(Wally, 2016).**The European Sea bass, *Dicentrarchus labrax* , is the most important commercial fish cultured in Southern Europe, especially in the North Atlantic and the Mediterranean area **(FAO, 2014)**. In Egypt, Sea bass is one of The major marine fish species that are **c**ultured in semi intensive brackish-water ponds, often in polyculture with mullet, mainly in the Diba Triangle Zone located between Damietta and Port Said **(Rothuis *et al.*, 2013)**. Semi-intensive and intensive fish farming systems increase the importance of parasites as disease-causing agents due to their ability to evolve rapidly the most serious limiting factors in aquaculture because of the increased density of cultured fish populations in restricted bodies of water and the ease by which pathogens can be transmitted from one fish to another **(Antonelli *et al.*, 2016)** . Parasites are the most commonly found pathogen with a prevalence rate up to 80% of the total infections in fish on farms **(Shaheen *et al.*, 2013).**The common parasites infested marine fishes include monogenetic and digenetic trematodes,protozoa namely, *Ichthyophthirius multifilis*,*Trichodina spp.* and *Chilodonella* as well as the encysted metacercariae **(Aly, 2013).** The present work was done on cultured Sea bass, (*Dicentrarchus labrax*) from tow private fish farms in Damietta governorate to identify the most common parasites infesting cultured Sea bass, (*Dicentrarchus labrax*), study their prevalence, seasonal dynamics as well as the histopathological changes induced by detected parasites.

MATERIAL AND METHODS

Fish:

A total number of 120 Sea bass (*Dicentrarchus labrax*) with average body weight of 150 ± 30 g were collected alive from tow private fish farms in Diba triangle in Port Said- Damietta way from September 2016 - August 2017. The collected fish were transported in large tanks filled with water of the same sources and supplied with battery air pumps to Fish Diseases Department Lab., at Animal Health Research Institute, Dokki, and Giza, Egypt. The clinical signs, postmortem changes were recorded and the parasitological examination was applied according to **Noga (2010)**.

Clinical and post-mortem examination:

This examination was applied on spot in the fish farms from which the naturally infested fish were collected. External examination of fish including, the clinical abnormalities and post-mortem changes were recorded according to **Noga (2010).**

Parasitological examination and identification:

The *Dicentrarchus labrax* were examined externally with naked eye and with aid of magnifying glass for detection of parasitism according to **Noga (2010).** Mucous smears were immediately prepared from the skin and fins with the aid of microscopic slides and covers. Then they were examined under stereomicroscope at X 10, 40 and 100 magnification for investigation of external parasites. Fish were euthanized and gills were carefully removed and placed in separate petri dish containing sterilized marine water to remove any excess gill mucus, Afterwards they were examined for parasitic infection under stereomicroscope. These internal organs were examined by naked eye for the presence of encysted metacercariae or worm and small pieces of each was taken and mixed with few drops of saline, compressed between two glass slides and examined under the microscope to detect the presence of encysted metacercariae. Detected parasites were collected, examined, fixed and stained and identified according to **Lucky (1977)** ; **Paperna and Laurencin (1979); Lom (1995) and Paperna (1996).**

Histopathological examination:

Tissue specimens from different organs were fixed in 10% neutral buffered formalin. The fixed samples were washed in tap water overnight then exposed to ascending concentrations of ethanol (70, 80, 90 and 100%), cleared in xylene and embedded in paraffin. Tissue slides of 5µm thick sections were prepared and stained by hematoxylin and eosin (H&E). The histopathological preparation was performed according to **Bancroft *et al.* (1996)**

RESULTS

Clinical and post-mortem examinations:

The clinical picture of infested fish in the fish farms was restlessness, gasping of air, rubbing body against hard objects, swam near the surface of the water with increased breathing frequency, stretched gills covers, loss of appetite and increase in mortality rate. Fish infested with Protozoa, monogenea and crustacean showed haemorrhagic skin and fins, areas of detachment of scales and excessive mucous secretion on the body and gills. In case of internal helminthes and digenetic-encysted metacercaria, infested fishes appear emaciated and off food. The post mortem examination of examined fishes showed paleness of gills in some infested fishes. The recorded clinical signs and postmortem changes demonstrated in plate (1).

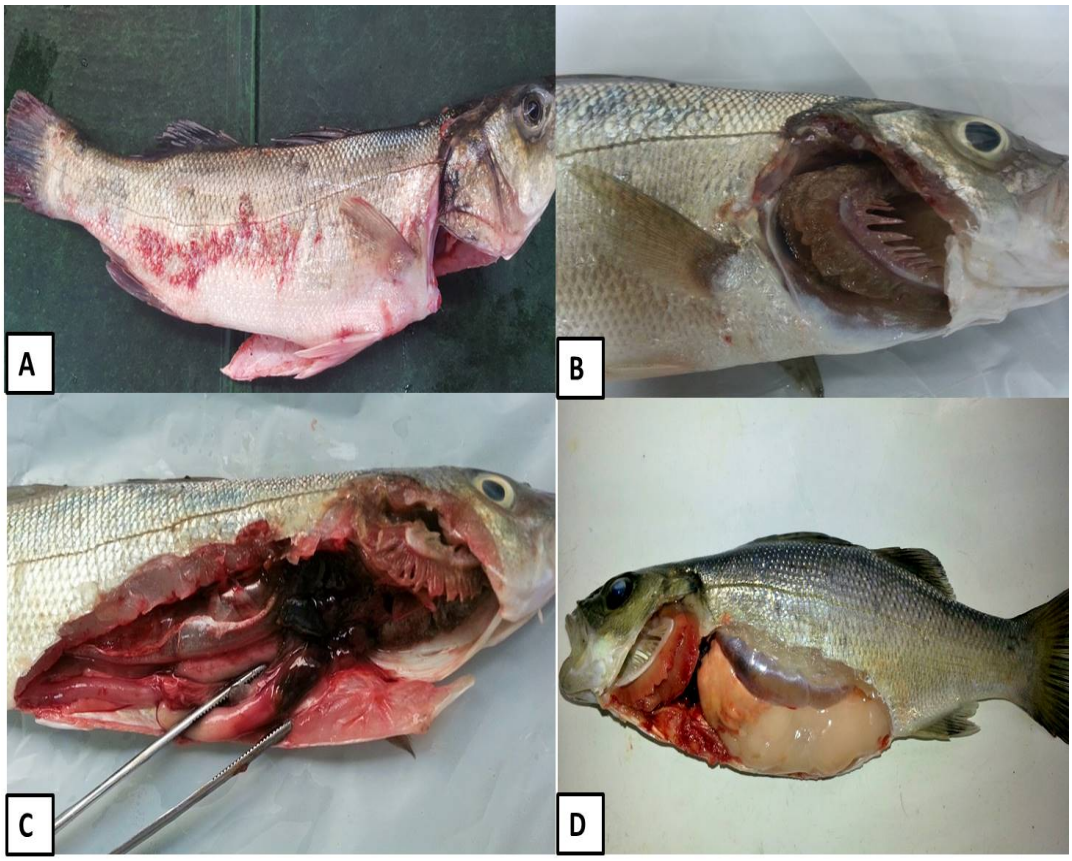

plate (1): A- *Dicentrarchus labrax* infested with mixed infection with *Trichodina heterodentata* and *Caligus minimus* showing haermorrhagic skin with detachment of scales.

B-*Dicentrarchus labrax* infested with a mixed infection monogenea and *Trichodina heterodentata* showing erosin of gill filamintes.

C -*Dicentrarchus labrax* infested with *Timoniella sp.* and *Bucephalus minimus* showing congetion in the gastro-intestinal tract and internal organs.

D-*Dicentrarchus labrax* infested with mixed infestation of *Amyloodinium ocellatum* and *unidentified encysted metacercaria* in internal organs showing pale and friable liver.

Parasitological examination:

The parasitological examination revalead four genera of protozoa including (*Trichodina, Epistylis*, *Amyloodinium* and *Microsporidia),* two genera of monogenean (*Diplectanum* and *Pseudohaliotrema sp.),* one genus crustacean (*Caligus minimus)* , unidentified digenetic encysted metacercariae,two genera of adult digenetic trematodes (*Timoniella* and *Bucephalus)* and one genus of Nematode (*conteracecum)* showed in plate (2,3).

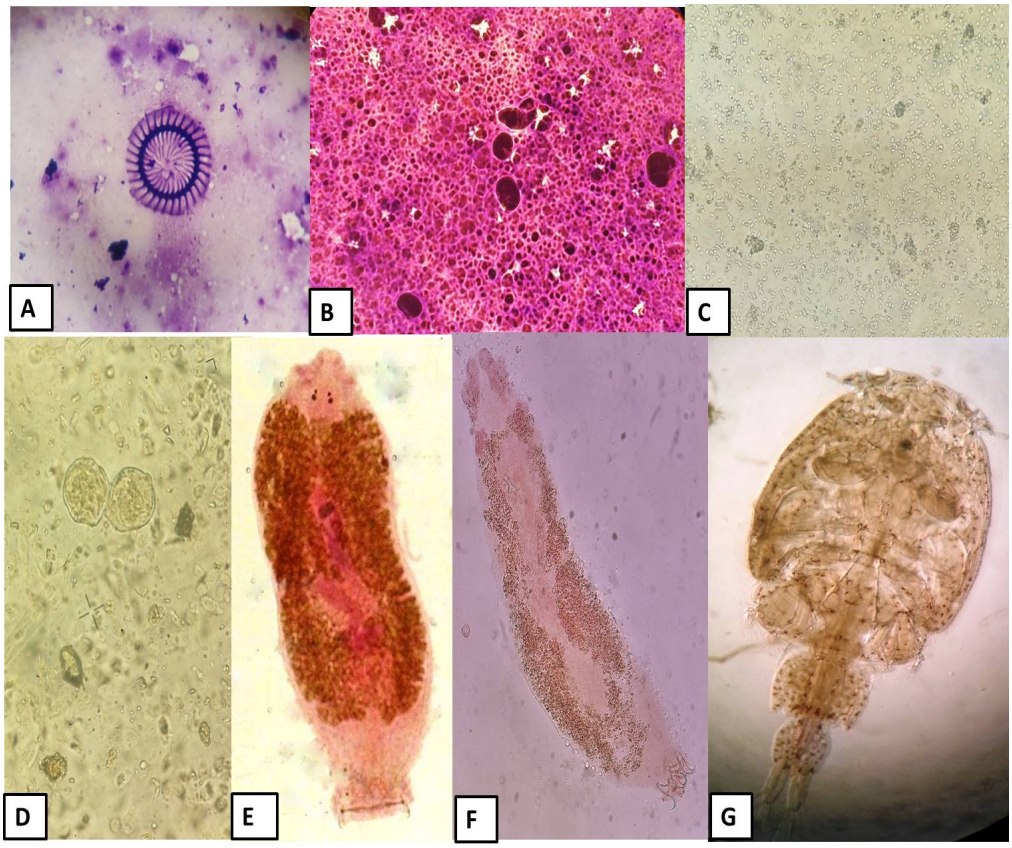

Plate (2): A- *Trichodina heterodentata* isolated from gills of *Dicentrarchus labrax* stained with Giemsa stain **(X1000).**

B- *Amyloodinium ocellatum* detected from *Dicentrarchus labrax* gills stained with Giemsa stain **(X400).**

C- *Microsporidia sp.* isolated from gills of *Dicentrarchus labrax* slide stained with Giemsa stain **(X400).**

D - *Epistylis sp.* wet preparation isolated from *Mugil cephalus* **(X400).**

E- Diplectanum sp. whole parasite isolated from *Dicentrarchus labrax* **(X100).**

F- *Pseudohaliotrema sp.* whole parasite isolated from gills o of *Dicentrarchus labrax* **(X100).**

G- Male *Caligus minimus* whole parasite isolated from skin of *Dicentrarchus labrax* **(X50).**

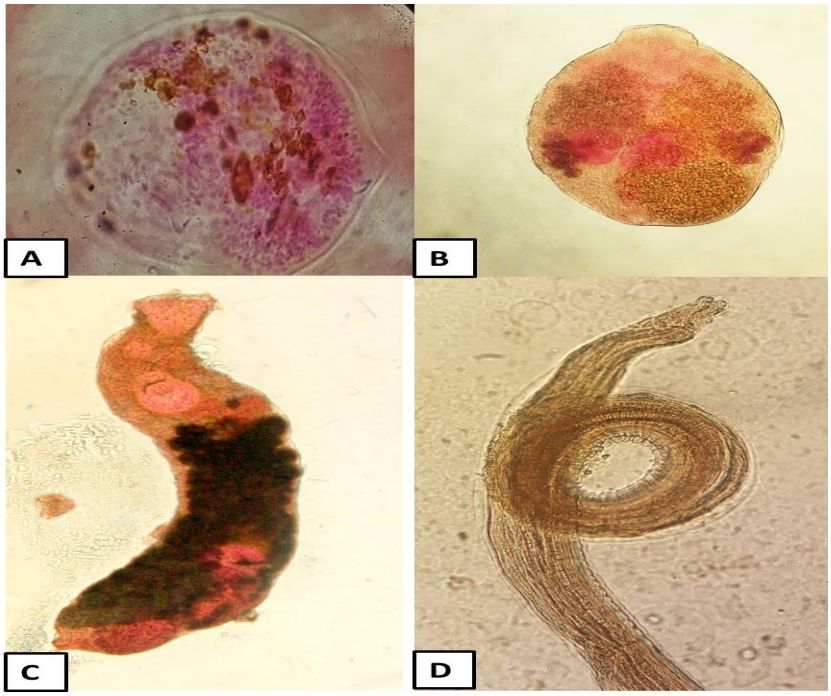

Plate (3): A- Unidentified encysted metacercaria detected from gills of *Dicentrarchus labrax* stained with acid carmine **(X400).**

B- Adult of *Bucephalus minimus* detected from intestine of *Dicentrarchus labrax* stained with acid carmine **(X400).**

C- Adult *Timoniella sp.* detected from intestine of *Dicentrarchus labrax* stained with acid carmine **(X100).**

D- *Contracaecum sp larvae* detected from intestine of *Dicentrarchus labrax* mounted in glycerin alcohol **(X100).**

Prevalence of parasitic infestation:

In this study, 101 out of examined 120 *D. labrax* (84.2%) were infested with parasites. The results of prevalence of parasitic infestation were illustrated in (Table 1)

Table (1): prevalence of parasites detected from *Dicentrarchus labrax from farm I and II.*

fish parasites	farm I (n=60)		farm II (n=60)		Total no of examined fish (n=120)	
	No. of infested fish	%	No. of infested fish	%	No. of infested fish	%
Trichodina heterodentata	17	28.3	17	28.3	34	28.3
Epistylis	8	13.3	9	15.0	17	14.2
Amyloodinium ocellatum	3	5.0	21	35.0	24	20.0
Microsporidia	3	5.0	7	11.7	10	8.3
Diplectanum	9	15.0	6	10.0	15	12.5
Pseudohaliotrema	11	18.3	0	0.0	11	9.2
Caligus minimus	10	16.7	18	30.0	18	15.0
unidentified encysted metacercariae	23	38.3	17	28.3	40	33.3
Timoniella sp.	13	21.7	8	13.3	21	17.5
Bucephalus minimus	12	20.0	9	15.0	21	17.5
Conteracecum larvae	3	5.0	2	3.3	5	4.2

*** n= Number of examined fish**

3.3 Seasonal prevalence of parasites:

Seasonal variation study of all detected parasite were shown in (Table 2).

Table (2): Seasonal prevalence of parasites recovered from examined *Dicentrarchus labrax*

parasites \ season		Autumn		winter		spring		summer	
		Farm I (n=15)	Farm II (n=15)	Farm I (n=15)	Farm II (n=15)	Farm I (n=15)	Farm II (n=15)	Farm I (n=15)	Farm II (n=15)
External protozoa	*T. heterodentata*	53.3	46.7	40	26.7	13.3	20	6.7	20
	Epistylis	33.3	26.7	13.3	20	6.7	6.7	0	6.7
	A. ocellatum	20	53.3	0	26.7	0	40	0	20
	Microsporidia	13.3	20	0	13.3	0	0	6.7	13.3
Monogenean	*Diplectanum*	26.7	20	13.33	6.7	20	13.3	0	0
	Pseudohaliotrema	33.3	0	13.3	0	26.7	0	0	0
Crustacea	*C. minimus*	6.7	6.7	0	0	13.3	13.3	46.7	33.3
Helminthes	EMC	40	26.7	33.3	20	26.7	20	53.3	46.7
	Timoniella sp.	26.7	20	40	20	13.3	13.3	6.7	0
	B. minimus	40	33.3	26.7	20	13.3	6.7	0	0
	Conteracecum larvae	20	13.3	0	0	0	0	0	0

The Histopathological findings:

The results of histopathological examination for different organs of naturally infested *D. labrax* revealed the following:

1- Gills of infested fish with *Trichodina* showing different sections of *Trichodina* between gills filaments while gills with Monogentic infestation showed monoginea attached into groove of fused gill lamellae. The most predominant changes of gills of examined fish were, lifting of respiratory epithelia, proliferation of basal undifferentiated cells, and partial and/or complete fusion of gill lamellae**.**

2- Muscle of infested fish with digenetic encysted metacercaria revealed cyst encountered by interstitial edema, some adjacent muscle bundles appeared atrophied with small caliber size.

3- Kidneys showing cystic formation of digenetic-encysted metacercaria within renal tissue, the surrounding tissues showed features of pressure atrophy.

4- Intestine demonstrated variable response to parasites including: massive sub-mucosal inflammatory cells infiltration, proliferation of intestinal villi and some intestinal villi appeared thick and short. Cross sections of parasite were detected in the Intestinal lumen. The results of histopathology showed in Micrograph plate (4).

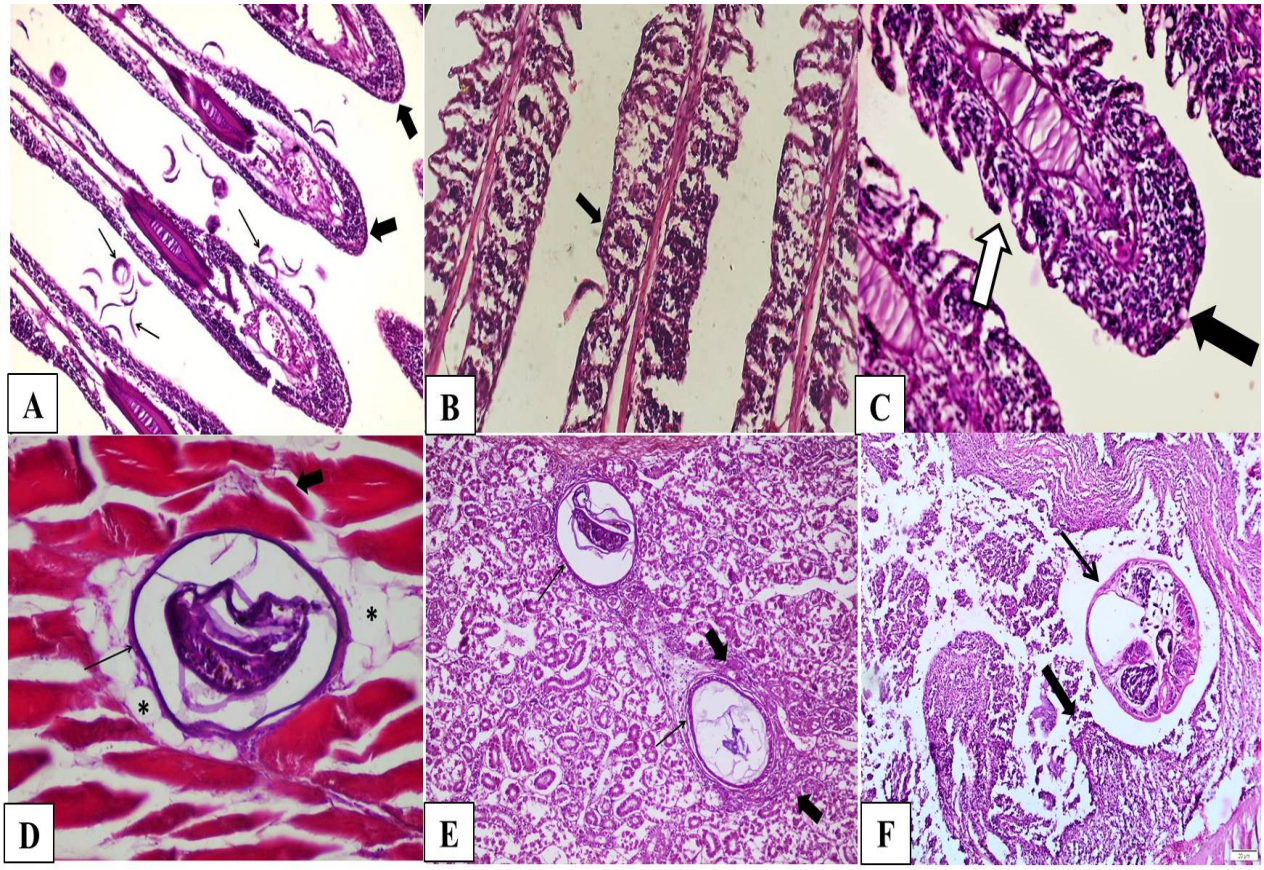

Plate (4): A- Sagittal section in the gill filaments of *Dicentrarchus labrax* demonstrating heavy parasitic infection with criteria of *Trichodina heterodentata.* Gill lamellae suffer from lifting of respiratory epithelium (thick white arrow) with partial fusion of secondary lamellae (thick black arrow). Gill lamellar tips were exhibiting complete fusion. **H&E X 100.**

B- Sagittal section in the gill filaments' of *Dicentrarchus labrax* revealing attachment of *Diplectanum sp.* to a groove within complete fusion lamellae (thin arrow). H&E X 100.

C- Gill lamellae of *Dicentrarchus labrax* exhibiting partial (thick white arrow) and complete fusion of lamellar epithelia (thick black arrow).**H&E.**

D- Longitudinal section of *Dicentrarchus labrax* muscle revealing section of digenetic encysted metacercaria cyst (thin arrow); the myogenic tissue showing interstitial edema (asterisks). Some muscle bundles were atrophied (thick arrow**). H&E X 400.**

E- Renal tissue of *D. labrax* showing cross sections of two digenetic encysted metacercaria cysts (thin arrows); surrounding tissues revealing evidence of pressure atrophy (thick arrows). **H&E X 200.**

F- Intestinal section of *Dicentrarchus labrax* infested with digenetic trematodes showing proliferated of intestinal villi (thick black arrow) and Cross section of digenetic trematodes (thin arrow). **H &E X200.**

DISCUSSION

Aquaculture is a globally important industry that provides essential food to a growing world population, with a major role in the supply of cheap animal protein. In Egypt there are very rapid developments have been occurred in aquaculture sector because aquaculture is considered as the mainly viable option for reducing the current gap between production and consumption of fish **(Soliman and Yacout, 2016)**. Parasites can seriously affect the marine resources being pathogenic to fish and zoonotic to consumers **(Kuhn *et al.*, 2011)**.

Concerning to the results of the clinical picture recorded in the fish farms, the naturally infected *D. labrax* showed restlessness, gasping of air, rubbing body against hard objects, swimming near the surface of the water with increased breathing frequency, stretched gills covers, loss of appetite, increase in mortality rate, haemorrhagic skin and fins, areas of detachment of scales and excessive mucous secretion in the body and gills.

These manifistation were in acordance with records of **(Noga, 2010; Noor El-Deen *et al.*, 2013; Hassanin, 2016 and Seoud *et al.*, 2017)**. Regarding of postmortem examination showed paleness of gills in some infected fishes friable and pale liver congestion and haemorrhagic patches in intestine this findings supported by finding of **(Ahmed, 2017)** who observed that *Dicentrachus labrax* and *D. punctatus* infested with *digenetic* trematodes and *encysted metacercariae* showed paleness in liver in some cases and congestion in others also Intestine showed congestion and inflammation.

The prevalence of protozoal infection among the examined fish revaled four genera namley, *Trichodina heterodentata ,Epistylis, Amyloodinium ocellatum* and *Microsporidia* were 28.3%, 14.2% , 20.0% and 8.3% respectivley , these results was supported by **(Pereira *et al.*, 2011)** reported that *Amyloodinium ocellatum* one of the most important ectoparasitic infectation in marine aquaculture and **Ihwan *et al.* (2016)** recorded that, that trichodinids are highly significant in aquaculture because their responsibility in decreasing of growth rate and immune response for vaccination and chronic mortalities.

Concerning to the results of the seasonal variation for protozoal infection in naturally infected examined fishes the infection rate showed the high peak during autumn in *Trichodina heterodentata ,Epistylis, Amyloodinium ocellatum* and *Microsporidia* infection they was (53.3%, 33.3 % , 20% and 13.3%) in farm I and (46.7%, 26.7 %,53.3% and 20% in farm II respectively. on the other hand there infection rate were decrease during summer (6.7% , 0%,

0% and 6.7%) in farm I and (20%, 6,7% ,20% and 13.3%) in farm II respectively , the difference between farm I and II in prevalence rate were very low these may be due to the two farms were located in the same area and use the same water source , also from these results can indicated that climatic changes have a strong effect in infection rate of protozoal diseases . These results in contrast with the record of **Noga (2010)** reported that all protozoan ectoparasites have a direct life cycle, which is faster at higher temperatures. This defrance may be attributet to difference in weather of countries and temprature in different seasons. The results of Monogenean infestation in this study reviled two genera of monogenean namely (*Diplectanum* and *Pseudohaliotrema)* with total prevalence rate 12.5% and 9.2% respectively. These results were supported the finding of **Ahmed (2017)** found that, the prevalence of *D. bocqueti* detected from *D. labrax* were 20% . On the other hand they were lower than the observation of **El-khatib *et al.* (2014)** found prevalence of *Diplectanum sp* . isolated from *D. labrax* collected from El - Fayoum governorate and were infected also with bacteria was 100 % the difference between the results may be due to difference in water quality between El Fayoum and Damietta . The result of prevalence of *Pseudohaliotrema* were lower than the finding of **(Abdel-Mawla and Abo-Esa, 2011)** found that, the prevalence rate of *Pseudohaliotrema sp*. detected from *Siganus revulatus* from Suez canal was 31.7 this may be due to difference in fish spices and locality of collected samples. The seasonal variation of *Diplectanum sp.* showed the highest peak of infestation during autumn (26.7% and 20 %) followed by spring (20.0% and 13.3%) respectively in farm I and farm II and not detected in both farms in summer. these results show some agreement with the observation of **Ahmed (2017)** concluded that, the prevalence of *D. bocqueti* affecting *D. labrax* and *D. punctatus* was high in winter followed by spring then in summer and not detected in the autumn the difference between the results may be due to difference in the locality of collected samples. In case of *Pseudohaliotrema sp.* infestation examined *D. labraxs* were detected only in farm I with high prevalence rate during autumn (33.3 %) followed by spring (36.7 %). these results was in contrast with **Abdel-Mawla and Abo-Esa (2011)** recorded that, the highest prevalence of *Pseudohaliotrema sp*. isolated from *Siganus revulatus* was in spring followed by summer while the lowest prevalence was in winter difference between result may be due to difference in fish species. Reading of infestation rate of crustacean ectoparasites infestation in naturally infested *D. labrax* revealed that only one genus, namely *Caligus minimus* with total prevalence rate of 15.0%. These finding was

higher than the results of **Wang *et al.* (2001) and El-Raziky (2009)** detected that, the infestation rate with crustacean ectoparasites from *Mugil cephalus* were 5.3% and 7% respectively, this difference may be due to difference in fish species. The Seasonal variation of *C. minimus* among examined *D. labrax* (farm I and II) showed that infestation rate increases in summer (46.7% and 33.3%), respectively and not detected in winter. These results in accordance with **(Eissa *et al.*, 2012) and Hassanin (2016)** reported that, the highest peak of *Caligus Spp.* infestation isolated from *D. labrax* and *M. cephalus* was in summer followed by autumn and decrease in winter. Increase infestation during summer may be due to increase in salinity of water in this locality during summer, this theory supported by **(Karlsbakk *et al.*, 2001)** reported that sea lice can cause disease outbreaks on non-salmonids fish when they are cultured in full salinity seawater. In case of infestation with unidentified encysted metacercaria (EMC) detected from naturally infested *D. labrax* the prevalence rate of infested with was 33.3 %. These results were higher than **Ahmed (2017)** recorded that, the infestation rate of *D. labrax* collected from private farm in Ismailia provinces was 19% , these fluctuation of prevalence may be due to the amount of intermediate host located in fish farm . infestation with EMC were detected all over the year with maximum prevalence during summer (53.3% and 46.7%) while the minimum prevalence were detected during spring (26.7% and 20.0%) respectively in farm I and farm II. These finding supported the finding of **Elsheikha and Elshazly (2008) and Ghobashy *et al.* (2010)** found that the highest rate was in summer , while they were in contrast with **Kotb *et al.* (2014) and Ahmed (2017)** proved that the seasonal prevalence of EMC was higher during winter followed by spring and decrease during summer. These differences could be explained by the season of migration of the migratory birds that act as definitive hosts for many of the metacercariae and presence of snails witch complete the life cycle of digenean. Concerned to the result of the adult digenetic trematodes detected from gastrointestinal tract of examined fish two genera (*Timoniella sp. and Bucephalus minimus)* were isolated with total prevalance rate 17.5% and 17.5% respectively. These results were nearly similar to observation of **Ahmed (2017)** noted that, the prevalence of *Timoniella sp.* affecting *D. labrax* was 25%, and also supported by **Pina *et al.* (2009)** recorded that, the adult stage of *B. minimus* develops in the Sea bass *Dicentrarchus labrax* as the final host the seasonal prevalence of *Timoniella* infestation showed the highest prevalence during winter (40.0% and 20.0%) in farm I and farm II

respectively while the lowest prevalence rate showed during summer (6.7% and 0.0%) in farm I and farm II respectively. These results partially disagree with **Ahmed (2017)** noted that seasonal prevalence of *Timoniella sp.* Affecting *D. labrax* and *D. punctatus* the highest peak was in spring followed by autumn and the lowest rate was in summer. On the other hand the infestation with *Bucephalus minimus* showed highest prevalence during autumn (40.0 % and 33.3%) followed by winter (26.7% and 20.0%) in farm I and farm II respectively and not detected during summer. these results was in contrast with the finding of **Pina *et al.* (2009)** recorded that prevalence of adult *B. minimus* isolated from Sea bass during spring and summer was 25%. This difference may be probably due to deference in geographical area of collected samples. In this study only one genus of nematode was detected namely *conteracecum* larvae from gastrointestinal tract of *D. labrax* with prevalence rate 4.2% , in the recent study of wild European sea bass, which reported prevalence ranging between 65 and 89.36 % in relation with body weight, the fish were caught from the northeastern Atlantic Ocean **(Bernardi, 2009)**. This may explain by the different in origin of collected fish samples. Infestation with nematode namely Conteracecum larvae detected only during autumn with prevalence rate (20.0% and 13.3%) in farm I and farm II respectively. On the other hand another study reported that, the high prevalence of *Conteracecum sp.* isolated from *Lethrinus lentijan and Lutjanus spp.* was in summer (36.7% and 30.0%) respectively **(Abo-Esa and Abdel-Mawla 2012).** This difference may be due to difference of locality of collected samples and fish species. Fish gills are highly sensitive organ that provide the largest surface area get in contact with external environment, so easily damaged **(Karlsson, 1983)**.Gills are incorporated in many vital functions; respiration, excretion and osmotic regulation, so histopathological changes in its structure will affect vitally on the fish health and behavior **(Cerqueira and Fernandes, 2002)** that approve the histopathological observations detected in these study, which represented in the form of lifting of respiratory epithelia, proliferation of basal undifferentiated cells, and partial and/or complete fusion of gill lamellae appearance of parasite or cyst of parasites in between gill filaments . Gill filaments epithelial hyperplasia and /or fusion and even aneurysm; that detected as a common feature in the present study, represent an indicator for infectious conditions **(Thiyagarajah *et al.*, 1996)**. This change give a false indication of increase surface area (in case of epithelial hyperplasia) **(Molnár *et al.*, 2016)**, as it lead to disturbance in blood fellow **(Stentiford *et al.*, 2003)** and less efficient respiration, but provide good condition for parasite transmission **(Molnár *et al.*,**

2016). In the present study, encysted metacercaria could demonstrate in different body organs including; muscle, kidneys, spleen and liver. as The fish defense mechanisms of fish against infestation nodular formation and melanin pigmentation were detected and end result were encapsulation of parasite, so the functions of the invested organ (s) not affected in most cases **(Molnár *et al.*, 2016)**. The present study demonstrated variable response to parasitic infestation including: massive sub-mucosal inflammatory cells infiltration, proliferation of intestinal villi and some intestinal villi appeared thick and short. Cross sections of parasite were detected in the Intestinal lumen. these results were supported by the findings of **(Ashour *et al.*, 2014; Dezfuli *et al.*, 2015)** recorded that a massive hyperplastic granulocyte response involving mast cells (MCs) and neutrophils and formed in close approximately to the helminths, meanwhile the associated desquamation of intestinal villi represent a common feature for intestinal parasitic infestation.

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