Comparative Studies on Some Prevailing Parasitic Diseases Cultured Freshwater Fingerlings and Adult *Oreochromis niloticus* on Some Fish Farms.

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Abstract: The present study was carried out on 400 specimens of fingerlings and adult *Oreochromis niloticus (O. niloticus)* in. They were randomly collected from some cultured fish farms in Egypt. The external protozoa (*Trichodina californica* and *Chilodenella hexatia,),* monogenitic trematodes (*Cichlidogyrus tilapiae and Enterogyrus cichlidarum*) *and* Adult flukes including *(Orientocreadium batracoides* and *Acanthostomum absconditum*) were isolated and identified. Also, some physico-chemical parameters of pond waters represented in pH, salinity, ammonia and sulphates were estimated in relation to the infestation rate with external and internal parasites. In addition, the histopathological alterations in the gills of infested fish were recorded. The more infestations were in adult fish than fingerlings and the highest prevalence was in Kaf El Sheihk followed by Alsharka governorate.

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Key words: *Oreochromis niloticus*, protozoa**,** monogenia, adult fluk, histopathology.

1. Introduction

Aquaculture is the hope for solving a part of protein shortage problem in Egypt. Global *Oreochromis niloticus* production has increased to over 1200000 tones / year by 2011 **(GAFRD, 2011).** Parasitic diseases have the upper hand in causing fish diseases, especially tilapia, where they shared fish diet and thus lead to retard in growth, marketing and high mortality in fry hatcheries and fingerling **(Eissa, *et al* 2013).** In addition, the external parasites cause mass mortilites and huge economic losses due to abrasions in skin and gills to allow fungal and bacterial invasion and that lead to a high mortality rate. Due to the length of periods of hot weather in Egypt, which is the ideal medium for reproduction of ectoparasites that cause adverse effects on fish **(Noor El Deen and Mona Zaki, 2010).** The more parasites prevalent in cultured tilapia in Egypt are single cells protozoa as well as monogenia as the speed of reproduction and they are need not intermediate host. Therefore, they can be easily spread among most fish causing mass deaths and huge economic losses **(Eissa, 2006).** Tilapias have been found to be most susceptible to parasitic diseases **(Philip *et al.*, 2008).** They can easily spread among most of tilapia fish **(Noor El-Deen, 2007)**. Uncontrollable or recurrent infestations with ciliated and flagelated protozoans are indicative of unhygienic husbandry problems **(Eissa *et al.*, 2011).** Also, monogenitic trematodes usually cause many problems in gills of cultured *Oreochromis niloticus* **(Osman *et al*, 2010).** Commercial aquaculture facilities require abundant clean water with oxygen, pH and nutrient levels at a suitable level to support the farmed species and temperature both at the initial source and in the production system **(Conte, 2004).**

This study was undertaken to investigate the prevalence of external parasitic infestations and histopathological alterations induced by the isolated parasites on *Oreochromis niloticus* (*O. niloticus*) fish collected from Kafr El Sheikh and Sharkia fish farms.

2. Materials and Methods

Fish:

A total number of 400 fish of cultured *Oreochromis* were collected from 2 different localities, Sharkaia Governorate (Abbassa region) and Kafr El- Sheikh Governorate earthen pond fish farms as 200 fish from each location. The collected were of 20±5g body weight average for fingerlings and 150 ±15 g and for adult fish ones. They were subjected to the clinical examination for detection of the prevalent ectoprotozoal monogenial and adult flukes infestations.

Clinical examination:

All fish were clinically examined according to the methods described by **Noga (2010)**.

Parasitological examination:

Smear scrapings from the external body surface and internal organs of the collected fish, were obtained especially the parts showing ulcers, wounds, petechial hemorrhages, slimness or sloughing of scales. Macroscopic parasites were collected by a brush; or dropper, then washed for several times in warm saline solution and left in the refrigerator until the specimens have been completely relaxed. The parasites were spread with a drop of normal saline, covered with a

clean cover slip, stained with Carmine stain and examined at low power magnification **(Lucky, 197).**

The gastrointestinal tract and internal organs were examined with naked eye. The alimentary canal of each fish was separated, dissected and divided into three main parts: foregut, mid gut and hindgut respectively. Each part was washed with physiological saline for several times to get rid of mucus and coarse particles that may be adherent to the parasites, then each part was opened and examined in a Petri dish under binocular dissecting microscope, then the helminthes were collected by Pasteur pipette or dissecting needle and transferred into Petri dishes containing warm saline solution for obtaining a fully relaxed and extended parasites **(Noga,2010).**

Preparation of permanent samples for Identification of parasites:

The obtained protozoa smears by scrapings were freshly examined, then fixed in methyl alcohol and stained by Giemsa which was diluted before staining with freshly prepared distilled water (one ml of the stain was added to 9 ml of distilled water). The fixed films were immersed into the diluted stain for 20 minutes. After staining, the film was rinsed with water and left to dry. The stained preparation were immersed for a few seconds into acetone. Cedar oil was then dropped on the dried preparation and examined with an immersion objective (Lucky, 1977).

The collected trematodes were left in refrigerator overnight to allow the worms to die; then compressed gently between to glass slides then fixed in 10% formalin. The samples were washed in running water then socked in alum carmine for few hours. After staining; the worms were washed in distilled water and passed through ascending grades of ethyl alcohol 50, 70, 90 and 100%, then transferred into xylol and clove oil respectively and finally mounted with Canda balsam and covered with cover slide, then slides were incubated at 60ºC for 24 hours for driving of the air bubbles (Kabata, 1985).

Identification of parasites:

The identification of the parasites was undertaken according to **Yamaguti (1985) and Paperna (1996).**

Physico- chemical analysis of water:

A total of 36 water samples equally distributed through the different seasons, were collected from the different fish ponds; simultaneously with fish specimens. For Determination of pH value, salinity, ammonia, alkalinity, oxygen and sulphate were recorded according to **Adams (1990).**

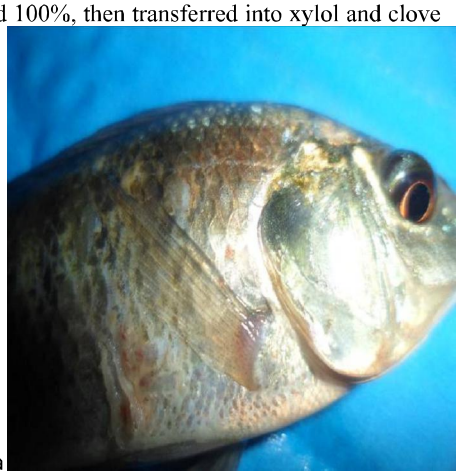
Histopathological examination.

It was carried out for the naturally infested fish. Specimens from skin were taken in different grades of alcohol, cleared in xylol then embedded in paraffin wax. Sections of 4-5 microns were obtained and mounted on glass slide and stained with Haematoxyline and Eosin (H & E), according to **Bancroft and Stevens (1996)**.

3. Results

Clinical examination:

The clinical examination of most examined fishes showed no or slight pathognomonic abnormalities except heavily naturally infested fishes (fingerlings and adult of *Oreochromis niloticus "O. niloticus"*), represented as respiratory manifestations, some aggregated on the surface, and accumulated at the water inlet of the pond. Others appeared dull with loss of escape reflex. Also, fishes showed emaciation, excessive amounts of mucus (slimness) covering the external body surface as well as scale sloughing (Plate 1).

a 

b 

Plate, 1: a, Showing *Oreochromis niloticus* with sloughed scales, ulcerations and haemorrhages on the body surface**, b,** showing *O. niloticus* with sunking eye.

Postmortem examination:

The postmortem findings of investigated fish revealed the presence of marbling appearance with numerous white dots in some gill filaments of infested fish. While, internal organs of examined fish appeared anemic with enlargement and congestion of spleen, liver with distended gallbladder, enteritis, haemorrhage and ulceration of stomach as well as intestinal mucous membrane **(Plate 2).**

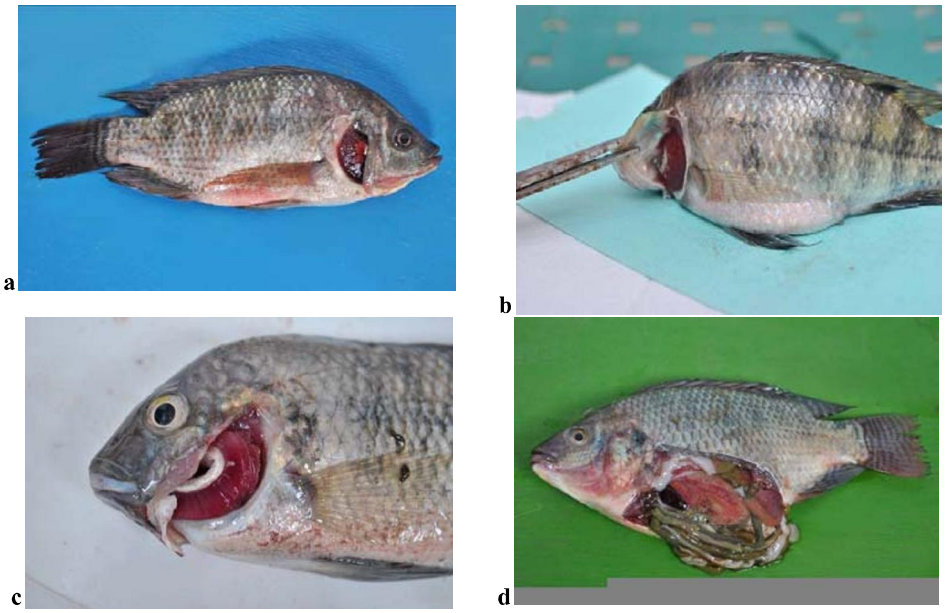


Plate 2: a, Showing *Oreochromis niloticus* with congestion of gill filaments and covered with slime and marbling appearance and **b**, white dots on gill filaments (arrow) (c), *d, O. niloticus* with slightly turgid stomach and inflammation of the intestines**.**

Parasitological examination:

Microscopic smears were taken from skin and gills of examined fish, showed a peritrichus ciliated protozoan. A denticulate ring of hollow conical structures was found with flat lateral projections. The centrifugal projections of denticles were semicircular. The macronucleus was large horseshoe shaped with a round micronucleus. Such ciliated protozoans were identified as *Trichodina californica* **Plate, 3 (a)** and another ciliate protozoa appeared as heart shaped. The ventral side was concave and ciliated were reduced to two longitudinal belts of cilia to body margins that arranged in parallel rows. The cytostome was distinct on the front part of venteral side with a funnel–shaped cytopharynex and two contractile vacuoles.A single oval to round macronucleus as well as round micronucleus were easily seen.Such ciliated protozoan were identified as *Chilodonella* **Plate,3(b).**

Adult worms were isolated from the gills of infested fish. They were flat and elliptical worms. Their anterior end (prohaptor) were divided into four cephalic lobed heads, with sticky, adhesive organs (cephalic glands and four black eye spots. The posterior end, appeared a dome shape and composed of one pairs of connecting bars (V-shaped) and seven pairs of small marginal hooklets. The intestinal limbs were connected, the ovary located in front to testes. Such adult worms are related to the phylum *Platyhelminthes*, class *Trematoda*, order *Mongenea* family *Dactylogyridae* and genus *Dactylogyrus* and identified as *Cichlidogyrus tilapiae* **Plate, 3(c).**

Regarding the internal parasites, adult worms were isolated from the stomach of infested fish. They were small dorsoventral flattened pear-shaped worms. The dorsal side was convex, while, the ventral one was concave. The cuticle was thick and striated transversally around all the body. There were two pairs of dense pigmented eye spots; the anterior pair is smaller than the posterior one. The opisthaptor appeared as a posterior fold, which armed with two pairs of central anchors, a transverse bar and marginal hooklets. The bar was visible as V-shaped. The marginal hooklets are 14. Such adult worms are trematodes belonged to phylum *Platyhelminthes*, class *Trematoda*, order *Monogenea,* and genus *Enterogyrus* and identified as *Enterogyrus cichlidarum* Plate,3(d).

Adult worms were isolated from midgut of infested fish, the body was elongated and cylindrical in shape and covered with fine spines. The gonads were located at the posterior third of the body. The two testes were round to ovoid in shape, smooth and followed the ovary in position. Vetilline follicles were extended laterally from the middle of the body till the middle of the anterior testis. Such adult worms are related to the phylum *Platyhelminthes*, class *Trematoda*, order *Digenea* and genus *Acanthostomum* and identified as *Acanthostomum abdconditum* Plate,3(e).

Adult worms were isolated from midgut of infested fish. They were elongated and the cuticle covered with fine spines. The oral sucker was subterminal and round. The ventral sucker was slightly larger than the oral sucker. The pharynxes well developed while oesophages illdevolped. The intestinal caeca were extended from the midway between the two suckers to the posterior body margin and surrounded laterally by vitelleria. Testes located behind the middle of the body. The external seminal vesicle was ovoid and extend behind the posterior margin of the ventral sucker. The ovary was located in front of the testes. The vetelline follicles were irregular in shape. The uterus was formed of irregular transverse coils and filed with eggs, which were yellowish brown, operculated. Such adult worms are related to the phylum *Platyhelminthes*, class *Trematoda*, order *Digenea* and genus *Orientocreadium* and identified as *Orientocreadium batrochoides* Plate,3(f).

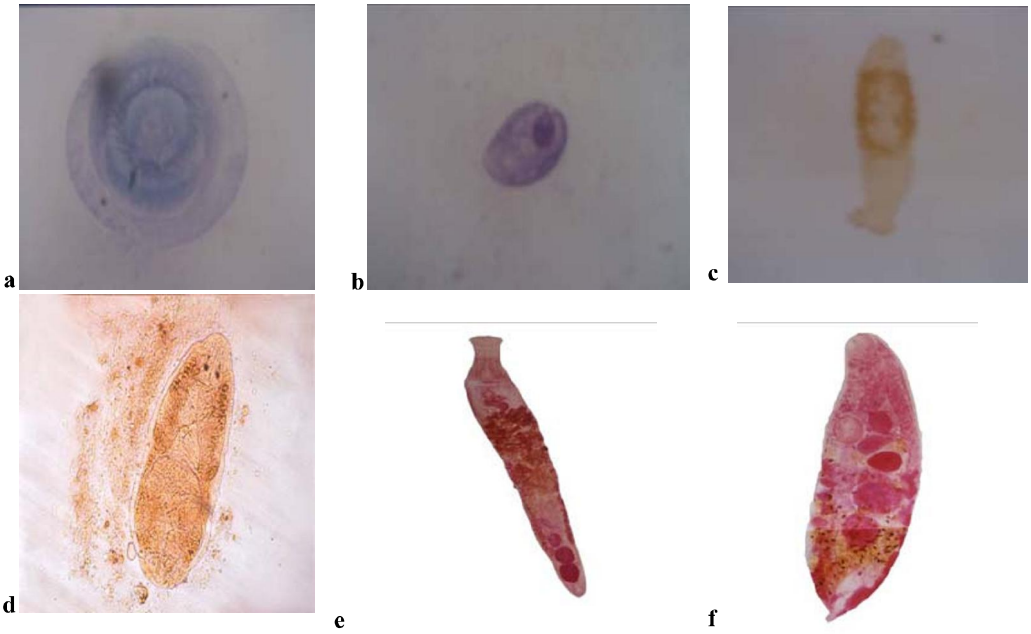


Plate 3: Magnified *Trichodina californica* (a), *Chilodonella hexastica* (b) Stain: Gimsa stain X 400, *Cichlidogyrus tilapiae* (c), *Enterogyrus tilapiae* (d) (wet mount), *Orientocreadium batracoides*(e), *Acanthostomum absconditum*(f). Stain: Acetic acid alum Carmine X 10.

Prevalence of infested fish.

In the present study, 400 various stages of cultured fish showed a great variation in the infestation percentage in (fingerlings and adult *O. niloticus*) from 2 distracts (Kafr El Sheikh and Sharkaia Governorate as in table,2&3.

Table (2): Incidence of parasitic infestations in different distracts fish farms in relation to different age.

Locations	Total No. of Examined Tilapia	Total No. of infested Tilapia		Age			
				fingerlings		Adults	
		No.	%	No.	%	No	%
Alshakaya fish farms	200	90	22.5	30	7.5	60	15
Kafr El Sheikh fish farms.	200	120	30	30	7.5	90	15
Total	400	210	52.5	60	15	150	30

Table (3): Prevalence of external and internal parasitic infestations among of adult and Fingerlings *Oreochromus niloticus*.

Locations	No, of exam. fish	No. of infested Tilapia	%of infestation	Clinically diseased Tilapia		External parasites						Internal parasites			
						Protozoa		M.G.T*		Crustacea		D. G. T**		M.G.T*	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO	%
Alshakaya fish farms	200	90	22.5	24	6	30	7.5	24	6	6	1.5	24	6	6	1.5
Kafr El Sheikh fish farms	200	120	30	48	12	60	15	24	6	6	1.5	24	6	6	1.5
Total	400	210	52.5	72	18	90	22.5	48	12	12	3	48	12	12	3

Results of water parameter:

There was a direct proportion between present of infestations the amount of (ammonia "0.2-0.5" mg/l, pH "6.2-7.2" and sulphate "41-110 ppt"). On the other hand, inverse proportion between the infestations and water salinity (1.2-2 ppt) as well as, dissolved oxygen (6-10) and alkalinity (151-210 ppt).

Histopathological examination:

The examined gills of *O. niloticus* were congested, oedematus with trichodina revealed active proliferation of epithelial of the secondary lamellar cells were hyperplastic fused. Moreover in severe infested, there is sloughing of the epithelial cells of the secondary lamellae **(Plate,4 (a)** and severe hyperplasia of the gill filament and lamellae with aggregation of large number of mononuclear cells with fusion and adhesion between the gill lamellae showing oedema.Where the inter lamellar spaces revealed congestion of branchial blood vessels with monogenia **(Plate,4 (b&c)**.

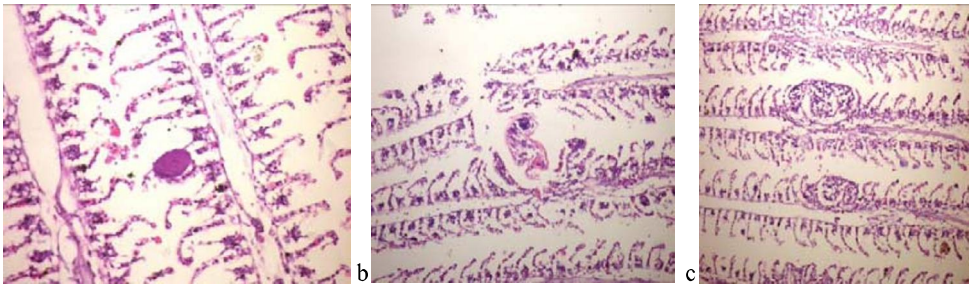


Plate 4, The secondary gills lamellae of *Oreochromis niloticus* showing protozoa embedded (arrow) (1), a parts of monogenian parasite embedded in between and surrounded by C.T. with few leucocytic infiltration (arrow)(2) and Encysted metacercariae embedded in the primary gill lamellae and surrounded with severe leucocytic infiltrations (arrows) (3). Stain: (H& E) X 125.

4. Discussion

In Egypt, more attention is being focused nowadays to improveme the fish in aquaculture to solving the shortage of animal protein. For increasing fish production in aquaculture, it is necessary to increase fish productivity; this often need increasing of cultured fish populations to compensate the shortage of animal protein in Egypt. Parasitic diseases are considered the most important factor causes decrease in aquaculture. Heavily parasitic fish infestations lead to high morbidity and mortality rates while in moderate parasitic infestation, clinical signs usually not appear but these act as stress factor which produce a decrease in the body weight **(Osman,2005)**.

Clinical signs in *O.niloticus* infested with external parasites (*Trichodena* and *Chilodenilla* sp) appeared as excessive dirty mucus covering the skin and showed signs of skin irritation manifested by rapid swimming behavior and aggregation of fish around the water inlet and at the water surface to gulp the atmospheric air, respiratory manifestations. In addition, severe emaciation with erosions and ulcerations on trunk area of the skin and sloughing of scales were observed. The same clinical signs were previously obtained by **Noor El Deen *et al* (2010).**

Concerning the results of parasitological examinations of *O.niloticus*, isolation of *Trichodina* sp. was provided with several rows of cilia at to circular periphery and a circle of more centrally lying hooklets. The morphological characters of these parasites were nearly similar to descriptions given by **Kabata (1992)**, Another ciliated protozoon, *Chilodonella* species were appeared as oval to bear-shaped. Rows of cilia are located at both sides of the

protozoal celli; the morphological characters of these parasites were nearly similar to descriptions given by **Eissa *et al* (2010)**.

The present study revealed the clinical picture of infested fish as abnormal, swimming, flashing, rubbing the body against the sides of aquaria to get rid the irritation induced by the parasites on the skin, fins and gills. Fish gathered at the water surface (surface breathing) with gulping the atmospheric air. They appeared asphyxiated and exhausted this may be due to low respirated oxygen of destructed gill epith which caused by feeding activity, attachment, fixation and locomotion of monogenea causing massive destruction of the respiratory epithelial cells Eissa *et al* (2012).

The present study showed that skin appeared with localized multifocal hemorrhagic areas with small wounds, abrasions or ulcers on the body surface with frayed and ragged appearing fins with darkening of skin of some fish. This may be attributed to *Cchhlidgurus* are provided with a pair of too long and strong anchors in the opisthaptor and 7 pairs of small strong hook lets used for fixation firmly on the external body surface of its host to resist the external water currents as well as continuous regularly locomotion and relocation from side to side and around the fin margin and frequently cross over the body surface to another fin, the caudal, pectoral and pelvic ones (Eissa, 2006). These results nearly agree with that met by Osman (2005) who reported similar findings. They added that the infested fish swam near the water surface gasping air and increase breathing frequency while the opercula were stretched open.

Regarding the congested or pale gills *(Marbling appearance)* it may be caused due to destruction of the efferent vessels by monogenea where the blood pressure is low and extensive hemorrhage are caused the very hard clotting of blood brings about rapid occlusion of the vessel, thrombus is formed resulting in ischemia which in turn leads to necrosis in some areas occured due to the inflammation and congestion of some areas with progressive degeneration of the other parts of the gill filaments giving the appearance of such phenomenon (Eissa, 2006). All stages of inflamation, degeneration and necrosis of the epith. Cells of the gill filament and gills become pale, anemic and the tips become grayish in colour. The over mucous secretion by fish may be to dilute the irritation as defense mechanism against these pathogens.

The examination of internal organs of naturally infested fish appeared pale, anemic with congestion of spleen and liver. Also, infested fish appeared emaciated with petechial haemorrhage on the surface of abdomen and slight bulging of stomach. This clinical picture nearly similar to that recorded by **Eissa *et al.* (20011).** This picture may be explained due to the presence of Enterogyrus which cause harmful effect as they embedded themselves between the curvature of stomach causing local damage to the stomach mucosa **(Woo, 2006).**

Regarding the internal monogenea, *Enterogyrus cichlidarum* was morphologically and parasitologically described and was nearly similar to the descriptions given by **Osman (2005).**

Finally, morphological and parasitological examinations of Tilapia fish revealed isolation and identification of *Acanthostomum absconditum* and *Orientocreadium batracoides* whose descriptions are nearly similar to those of original description by **Yamaguti,(1985).**

In the present study a total prevalence of protozoan in *O.niloticus* was 20% of *Trichodina fultoni* and *Chilodenella hexastica*. While, the prevalence of monogena was 11.5%.

Regarding the prevalence of Enterogyrosis in adult tilapia was 1%. Such results are lower than recorded by **Eid and Negm (1987)** who reported that the prevalence of *Enterogyrus cichlidarum* from *O.niloticus* collected from (Bahr Mouise) was 13.3% and **Ibtsam (2004)** who recorded 60% of *Entergyrosis.* Also, disagree with that recorded by **Osman (2005)** who found a prevalence of *Entergyrosis* as 67.2%. These variations may be attributed to the water quality criteria and age of fish as such worms are stomach flukes need aged fish have well developed stomach and its wall was thicker for adaptation and fixation.

Regarding the total prevalence of adult flukes in tilapia was 8 %. These results disagreed with the finding of **Hassan (1992).**

Regarding the simple correlation coefficient between water quality parameters and the prevalance of the parasitic infestations (Protozoa and monogenea) of fish,it was found that phosphate positively correlated with the prevalance of *Trichodena* and *Chilodonella* sp due to a limiting factor affecting algal growth **(Elewa 1988)**.

There was a positive correlation between the pH and alkalinity in water and the prevalence of each of Protozoa and Monogenea and negative correlation with prevalence of internal parasites. These findings may be attributed to the fact that increasing the pH and calcium carbonate in water of fish ponds represent a stress factor on respiration process of fish, especially the gills and may be facilitate such parasites to infect fish. The negative correlation between the DO in water and the prevalence of each of Protozoa and Monogenea may be attributed to the fact that low DO represent a stress factor on respiration process fish, leading to asphyxia and loss of escape reflex leading to entry of fish parasites.

The positive correlation between the concentrations of sulphate, ammonia, nitrate, nitrite could be related to the presence of nitrifying and sulphur reducer bacteria **(Elewa 1999),** which one of the most important factors in determine the presence and abundance of protozoan populations.

The negative correlation between the DO in water and the prevalance of each of Protozoa, and Monogenea may be attributed to the fact that low do represent a stress factor on respiratory organ of fish, especially the gills. This may facilitate Protozoa and Monogenea to infect fish **(Omoregie et al., 1996).** These results may be nearly similar to that recorded with **Eissa *et al* (2011)**.

Histopathological alterations of gills of *O.niloticus* infested with parasites revealed multiple forms of deformities due to hyperplasia of epithelial cells of secondary lamellae accompanied with hyperplasic of epithelial cells proliferation oedema was found in between the epithelial cells of secondary gill lamellae degenerative changes was well as necrosis were recorded in both primary and secondary gill lamellae. Numerous inflammatory cells mainly of eosinophils, lymphocytes and mononuclear leukocytes. The histopathological examination of *O.niloticus* infested gills with Trichodina sp showed congestion and numerous esinophilic granular leukocytes in the gill arch and gill lamellae also hyper plastic proliferation. This results to gill damage by protozoan parasites which caused by feeding activity, attachment, fixation and locomotion causing massive destruction of the respiratory epithelial cells. These results agreed with **Abd EL- Hady (1998)** who recorded that the histopathological examination of tilapias for trichodina affections showed hemorrhage, congestion, oedoma and mononuclear leukocyte infiltration in the gill arch and lamellae in *O.niloticus*. *Chilodnella spp* revealed oedema and congestion in the central venous sinus of primary lamellae. The secondary lamellae cells were hyperplasic fused sloughing of epith cells with marked oedema of gill lamellae. This results was similar to that recorded by **Roberts (2012)** who reported that the most common response of the gill to damage by protozoan parasites is hyperplasia and hypertrophy of epithelial cells. On the other hand the presence of monogenetic Trematodes on the gill filament in some cases or the marbled gills in other cases gill damage was manifested clinically by signs of asphyxia this may be attributed to poor quality which minimize the defense and the immunogenic status of Tilapia species these results were agree with results recorded by **Abd El-Megiuid (1989).** *O. niloticus* infested with monogenetic trematodes, showed the gill lamellae and subrabranchial organs were severely congested also the pathological lesions stared in the free ends of the gill lamellae in the form of hypertrophy and hyperplasia and diffuse lymphocytic infiltration oedema noticed in the middle of lamellae while the distal parts as well the gill arch still having apparently normal in case of heavy monogenetic infestation the epithelial hyperplasia was access to the distal parts of the gill lamellae. This agreed with the finding recorded by **Noor El Deen, *et al* (2013)**. These may be attributed to the mechanical injury induced the central hooks and marginal hooks insert into the gill epithelial tissues that leads to swelling, fusion and deformation of gill lamellae with collapse of capillaries.

Conclusion:

O. cniloticus fingerlings are less exposed to the prevailing external and internal parasites than adult ones and the infestation was higher found in Kafr El Sheihk than Sharkia distract.

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