

SEASONAL SCREENING OF SOME ECTOCILIATE PARASITIC DISEASES IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*) AT KAFR EL-SHEIKH AND DAKAHLIA GOVERNORATES

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Abstract

Eight hundred specimens of African catfish (*Clarias gariepinus*) from Kafr El-Sheikh and Dakahlia governorates, 100 fish per season/governorate, were investigated for seasonal incidence of some external ciliated protozoans. The results revealed the isolation of *Ichthiophtherius multifilius*, *Chilodinella* and *Trichodina*. In general, most examined fishes showed emaciation, pale or dark body coloration, excessive mucous secretion on the external body surface, scattered hemorrhagic patches, wounds and ulcers on different parts of the fish's body as well as ascitis. The highest recorded rate of infestation was 20.5% in autumn and winter and 18.75% in spring and summer season in Kafr El-Sheikh fish farms. However, in Dakahlia fish farms, it was 20% in summer and winter, 18.5% in autumn and 18% in the spring season.

A higher infection rate of *Ichthiophtherius multifilius* was observed in winter (50%) and summer (54%), *Chilodenella sp.* during autumn (18%), and autumn, winter and spring (18%) and of *Trichodina sp.* was (20%) for both in spring and summer , (22%) in winter; in Kafr El-Sheikh and Dakahlia governorates respectively. Moreover, some hematological and serum biochemical parameters were studied on the blood and sera of diseased fish. The histopathological alterations in the skin and gills of the infested fish were recorded.

Key words: Catfish, *Clarias gariepinus*, Disease, Ectociliate, Screening

INTRODUCTION

The majority of the constructed pond-based aquaculture around the Nile Delta lakes; is the main source of fish production in Egypt (GAFRD, 2016). Generally in Egypt, the fish farms' water supply is mainly from the agriculture drainage water that may be sometimes mixed with sewage wastes; which is considered as predisposing factors paving the way for parasitic disease prevalence in cultured fishes.

Eissa *et al.*, (2013) revealed that the optimum warm weather in Egypt, enables the quick reproduction of parasites; causing worse effects on fish. So, parasitic diseases constitute the largest sector (80%) of fish diseases in Egypt (Eissa, 2002).

Generally, fish parasites result in economic losses not only due to fish mortalities, growth reduction, tissue damage and loss of the opportunity to sell the fish, but also due to high expenses of drug treatment (El-Asely *et al.*, 2015).

African catfish, *Clarias gariepinus*, is one of the most economically important fish species for successful aquaculture. However, farmers are constrained with massive fry and fingerling mortalities due to parasitic infestation (Abo Esa, 2008).

Ectoparasitic ciliated protozoa constitutes one of the most dangerous threats to fish health. These parasites attack the external fish body causing massive destruction of the skin and gill epithelium (Sterud *et al.*, 2003; Enayat *et al.*, 2008). Even moderate infection of these organisms, may cause a fatal disease, because the infected fish lose their appetite and stop feeding (Meyer, 1966; Hoffman *et al.*, 1975).

The infection with the protozoan *Ichthiophtherius multifiliis*, parasitizing on the skin and gills of the fish, is world-wide and has been responsible for severe mortalities at various places in the world (El-Saftawy, 2015).

The infection with the protozoan *Chilodonella sp.*, parasitizing on the skin and gills of the fish, is world-wide on warm and cold water fish species and

has been responsible for large-scale mortalities at various places in the world. In heavy infested cases, fish appeared emaciated with darkening or dulling of the skin color (Van As and Basson, 1988 and El-Tantawy and El-Sherbiny, 2010).

Trichodina sp.; the dominant external parasite as it was extensively isolated from gills of both freshwater and marine water fishes (Xu *et al.*, 2002; Yemmen *et al.*, 2011 and Soliman *et al.*, 2013); results in severe pathological effects on fish and increasing mortality (Akoll, 2005). Most *trichodinids* are not pathogens, but under certain environmental conditions or when the fish are stressed by certain other factors, the parasite increases its rate of infestation among fish and become pathogenic resulting in gill epithelium necrosis and hyperplasia causing mass mortality of the infested fish (Abdel-Meguid, 2001).

The present study was planned to investigate the seasonal incidence of some ectociliate parasitic infestation in African catfish (*C. gariepinus*) in Kafr El-Sheikh and Dakahlia governorates as well as, evaluation of some haematological, serum biochemical parameters, and evaluation of histopathological changes induced by the detected parasites.

MATERIALS AND METHODS

Fish samples:

A total number of 800 *C. gariepinus* were collected alive from different freshwater fish farms in Kafr El-Sheikh and Dakahlia governorates along the year 2016 (400 sample/governorate; 100 sample/ season). The collected fishes were transferred alive to the wet laboratory, Fish Diseases and Management Department, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt (Hetrick, 1983 and Langdon and Jones, 2002). Collected samples were held in well-prepared glass aquaria supplied with sufficient amounts of dechlorinated water with continuous aeration (Innes, 1966).

Clinical and Postmortem examination:

The fish were subjected to full clinical examination for any color changes and any external gross lesions like wounds, hemorrhages, ulcers,

slimness or eroded skin on the external body surface (skin, gills, eye and mouth), according to the method described by Lucky (1977); Austin and Austin (1987) and Woo (1995).

Parasitological Examination:

The ectociliates parasites present on the skin, fins and/or gills of *C. gariepinus* were detected and identified.

For parasitological examination of the ciliated protozoans (*Ichthyophtherius multifiliis*, *Trichodina sp.* and *Chilodenella sp.*), skin and gills were examined immediately to avoid the escape of the external protozoa.

Direct smears from skin and fins were prepared with a drop of normal saline and covered with a clean cover slip (Wet mount preparation) and examined microscopically, using both low and high magnification powers according to Lucky (1977). Tissue scraping from the gill arch and filaments was prepared using few drops of distilled water, to obtain a uniform distribution under the entire cover slip; and examined under the microscope according to Lucky (1977).

Very thin Smears were taken and allowed to dry for 2-3 minutes and fixed with absolute methyl alcohol for 5 minutes, then stained with freshly diluted Giemsa stain for 30-45 minutes and impregnated in dense canada balsam then left to dry in the incubator at $37 \pm 1^\circ\text{C}$ for 24 hours for driving any bubbles. Examinations of both fresh and stained smears were carried out under low, high objectives and oil immersion lenses according to the methods described by Lucky (1977); Kabata (1985) and Woodland (2006).

The collected parasites were identified according to the identification keys of Paperna (1996); El-Tantawy and El-Sherbiny (2010).

Haematological investigations:

Fresh blood samples were collected without anticoagulant from the caudal posterior blood vessels. The needle is run, quite deep, as much as possible through a middle line in the middle of the anal fin in a dorso-cranial

direction till striking the vertebrate. By drawing the needle gently backward, blood is usually sucked into the syringe.

Blood samples were divided into two parts; one part was collected in heparinized micro-hematocrite tubes for hematological studies and the other part was centrifuged post collection at 3000 rpm for 10 minutes to separate serum for biochemical analysis.

The erythrocytes, leukocytes and hemoglobin concentration were determined according to the method described by Stoskopf (1993). For differential leucocytic count, blood films were prepared and stained according to Lucky (1977). The percentage and absolute value for each type of cells were calculated according to Schalm (1986).

Blood serum biochemical analysis:

Serum total proteins were determined calorimetrically according to the method described by Peters *et al.* (1982). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were determined according to Reitman and Frankel (1957).

Histopathological Examination:

Tissue specimens from the skin and gills of the infested catfish were taken, fixed immediately in 10% buffered neutral formalin, dehydrated and embedded in paraffin wax. Paraffin blocks were sectioned at 4-5 μm thickness and stained with Hematoxylin & Eosin (H&E) and examined under light microscope (Leica) using $\times 200$ and $\times 400$ magnification power according to Bancroft and Gamble (2007).

Statistical analysis:

Statistical analysis was performed using SPSS software version 16.0, Chicago, IL. Significant difference was determined at a probability level of ($P < 0.05$).

RESULTS and DISCUSSION

Aquaculture is necessary to increase fish production, through increasing the density of cultured fish population; in a trial to overcome protein shortage problems all over the world. Knowledge of fish parasites is of particular interest in relation not only to fish health, but also to understand the ecological problems (Mahfouz, 1997). The present work was applied to investigate the seasonal incidence of some ectociliate parasitic infestation in African catfish (*C. gariepinus*) in Kafr El-Sheikh and Dakahlia governorate as well as, evaluation of some haematological, serum biochemical parameters, and evaluation of histopathological changes induced by the detected parasites.

Clinical examination:

The *Clarias gariepinus* showed no pathognomic abnormalities except in the heavily naturally infected fishes. They exhibited reduced appetite, emaciation, restlessness; respiratory difficulties as well as fish became dull with loss of escape reflex. The results are similar to Atwa (2006) and Eissa *et al.*, (2011).

The external gross lesions of the examined *C. gariepinus* revealed emaciation, pale or dark body coloration, excessive mucous secretion on the external body surface, scattered hemorrhagic patches (Fig. 1), wounds and ulcers (Fig. 2 and 3) on different parts of the fish's body as well as ascitis could be observed in some cases. These clinical signs may be contributed to the continuous irritation produced by ciliated protozoa on the fish for feeding, attachment, fixation and locomotion; which may be similar to that reported by Abboud (2001); El-Khatib (2003); Eissa *et al.* (2013) and Gado *et al.* (2017). However, excessive mucous secretion may be attributed to that mucous might be released to relieve the irritating inflammatory reaction caused by continuous irritation of the ciliated ectoparasites (Marzouk, 2002; Mohammed *et al.*, 2004 and Khalil, 2010).

Postmortem examination of the examined *C. gariepinus* revealed that liver and spleen might be pale anemic in some cases and dark congested in other cases together with distended gall bladder. The intestine was containing

mucoïd secretion and abdominal ascitis could be observed in some cases; the result is similar to those reported by Rawia Adway (2000); Ibtam (2004) and Osman, (2005).



Fig. 1. Skin of *Clarias gariepinus* showing scattered hemorrhagic patches (arrows) on different parts of the skin.



Fig. 2. Skin of *Clarias gariepinus* infected with *Trichodina* sp. Showing hemorrhagic ulcer (arrow) on the skin.



Fig. 3. Skin of *Clarias gariepinus* infected with *Chilodenella* sp. showing scattered ulcerative areas (arrows) in different parts of the skin.

Parasitological examination:

Microscopic smears taken from gills, skin and fins of examined *C.gariepinus* revealed the presence of some ciliated protozoana. *Ichthyophthirius multifillii*s have the highest rate of the infestation (45.8%, 42%) followed by *Trichodina* sp. (18.25%, 17.75%), then *Chilodenella* sp. (14.5%, 16.75%) as shown in Table (1).

*Ichthyophthirius multifillii*s, has large round to oval shape ciliated parasites from 0.5- 1 mm in diameter. They have a horseshoe, crescent or C-shape macronucleus embedded in the protoplasm. The micronucleus is spherical and very small (Fig. 4).

Another protozoan appeared as large, flattened, ovoid or heart shaped ciliates with bands of cilia along the long axis of organisms. A single oval to

round macronucleus as well as round micronucleus, were easily seen. Such ciliated protozoan was identified as *Chilodonella sp.* (Fig. 5).

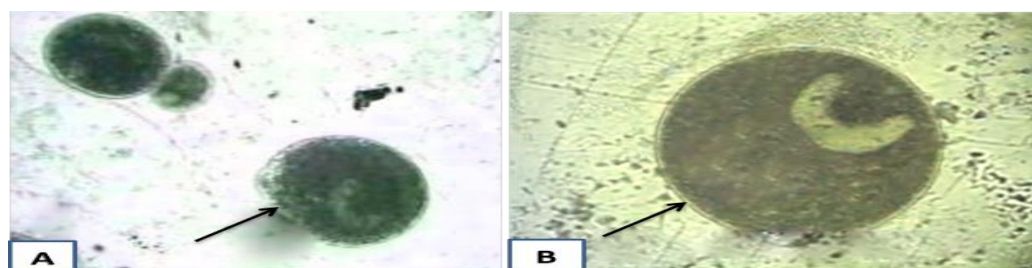


Fig.4. *Ichthyophthirius multifiliis* (arrow) isolated from skin of *C. gariepinus*; A:(X200), B: (X400).

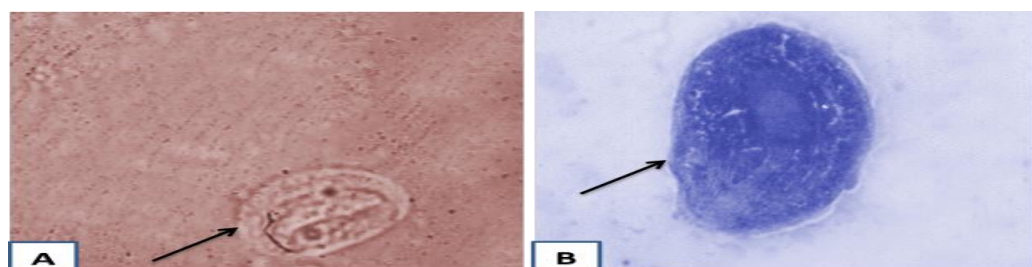


Fig. 5. *Chilodenella sp.* (arrow) isolated from the skin of *Clarias gariepinus*; A:(X200), B: Stained with Geimsa, (X400).

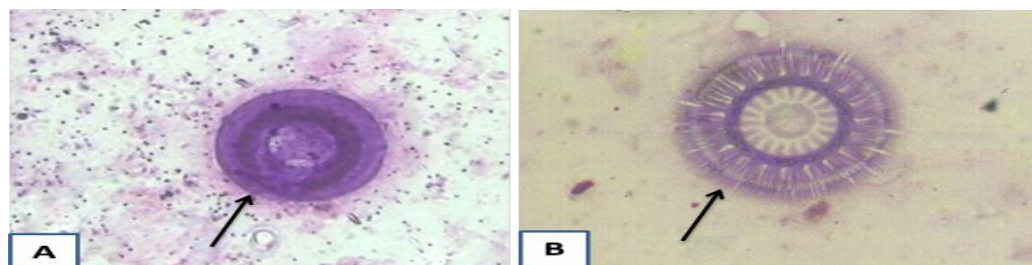


Fig. 6. *Trichodina sp.* isolated from the skin of *Clarias gariepinus*. (A) Stained with Geimsa X 200 (arrow), (B) Stained with Geimsa X 400 preparation X 400 (arrow).

Trichodina, a peritrichus ciliated protozoan, has a denticulate ring of hollow conical structures with flat lateral projections. The centrifugal projections of denticles were semicircular. The upper view is round, while the lateral view is either dish when standing or bell-shaped when free swimming in the water (Fig. 6). Such ciliated protozoans were identified as *Trichodina heterodentata* Duncan, 1977.

The ciliated protozoans (*Ichthyophthirius multifiliis*, *Chilodenella sp.* and *Trichodina sp.*) were morphologically and parasitologically identified and were nearly similar to the descriptions given by Kabata (1992).

Incidences of fish ectociliates among different fish farms and seasons:

Parasitological examination of 800 specimens *C. gariepinus* revealed the presence of different ectociliated protozoans in (314, 306) positive infested cases (78.5, 76.5%) in Kafr El-Sheikh and Dakahlia governorates, respectively. In Kafr El-Sheikh fish farms, the seasonal prevalence was 20.5% in autumn and winter and 18.75% in spring and summer season. However, in Dakahlia fish farms, it was 20% in summer and winter, 18.5% in autumn and 18% in the spring season (Table 1).

A higher infection rate of *Ichthyophtherius multifilus* was observed winter and summer (50%, 54%), followed by autumn and winter (48%, 40%), summer and autumn (45%, 38%), then spring (40%, 36%) in Kafr El-Sheikh and Dakahlia governorates, respectively. On the other hand, a higher infection rate of *Chilodenella sp.* was observed during autumn (18%), followed by winter and spring (15%), then summer (10%) in Kafr El-Sheikh farms but, it was in autumn, winter and spring (18%) then, summer (13%) in Dakahlia farms. However, a higher infection rate of *Trichodina sp.* was (20%) for both in spring and summer, (17%) in winter and (16%) in autumn in Kafr El-Sheikh farms but, it was (22%) in winter, (18%) for both in autumn and spring, (13%) in summer in Dakahlia farms.

Regarding the seasonal variation of the prevalence of *Ichthyophtherius multifilus* in the present study, the highest rate of infestation was during the winter and summer in Kafr El-Sheikh and Dakahlia farms respectively; while in *Chilodenella sp.*, the highest seasonal prevalence was in autumn; however, in *Trichodina sp.*, the highest seasonal prevalence was in spring and summer in Kafr El-Sheikh farms and in winter in Dakahlia farms.

This results partially agree with that reported by Tawfik (2005); El-Saftawy (2015) and Noor El-Deen *et al.* (2015) who recorded a higher prevalence of ciliated protozoans in winter (as observed for *Ichthyophtherius multifiliis*); and partially agree with Hoffman (1987); Hassan (1992); El-Khatib (1993); Osman (2001); Tawfik (2005); Rashed (2013); Noor El-Deen *et al.* (2015) and Gado *et al.* (2017) who reported a higher prevalence in spring (as observed for *Trichodina sp.*) and autumn (as observed for *Chilodenella sp.*). On the other side, Ibtam (2004); Awad (2007) and El-Moghazy (2008) reported that the prevalence of fish ciliated protozoans in Egypt; highly prevail mainly in the summer season, because of increased parasite and/or host activity and the promoted parasitic reproduction due to the higher temperature (Bakke *et al.*, 1991). Despite, stress (high water temperature) was accompanied with decreases of circulating lymphocytes, increase of macrophage cells, and enhanced red blood cell degradation resulting in increases the susceptibility of fish to diseases (Peters and Schwarzen, 1985).

However, Paperna and Vanas (1983) and Jerônimo *et al.* (2011) confirmed the higher incidence in cooler seasons; these differences in the prevalence rates; may be attributed to the differences in the culture system, geographic regions, environmental conditions, water sources and the type of examined fish (Eissa, 2002; Saleh & El-Nobi, 2003 and Yatabe *et al.*, 2011). While, Bassiony (2002) mentioned that the most important protozoan ciliate infections occur in autumn. Moreover, Rashed (2007) found that *Ichthyophtherius multifiliis*, reach the maximum rate of infection during winter season in Kafr El-Sheikh governorate; the result which is closely similar to the present study. But, Abd Elmegiud (1989); Eissa (2002) and Rashed (2013) recorded that *Ichthyophtherius multifiliis*, has a higher prevalence in summer and spring seasons.

Table 1. Incidence and Prevalence of different ectoparasites in the skin and gills of *Clarias gariepinus* in Kafr El-Sheikh and Dakahlia Governorates

Season	Parasite	Total No. of examined fish	positive infested cases		<i>Ichthiophtherius multifilus</i>		<i>Chilodinella sp.</i>		<i>Trichodina sp.</i>	
			No.	%	No. of infected fish	%	No. of infected fish	%	No. of infected fish	%
Kafr El-Sheikh farms	Winter	100	82	20.5	50	50	15	15	17	17
	Spring	100	75	18.75	40	40	15	15	20	20
	Summer	100	75	18.75	45	45	10	10	20	20
	Autumn	100	82	20.5	48	48	18	18	16	16
Total		400	314	78.5	183	45.8	58	14.5	73	18.25
Dakahlia farms	Winter	100	80	20	40	40	18	18	22	22
	Spring	100	72	18	36	36	18	18	18	18
	Summer	100	80	20	54	54	13	13	13	13
	Autumn	100	74	18.5	38	38	18	18	18	18
Total		400	306	76.5	168	42	67	16.75	71	17.75

Haematological investigations on protozoan infected fish:

The effect of different ectociliated protozoan on the infested *C. gariepinus* on different hematological parameters are summarized in Table 2. In spite of Azevedo *et al.* (2006) stated that the total number of erythrocytes, leucocytes haven't relation with the ectoparasites infections, the results in the current study revealed significant decrease in RBCs, non-significant decrease in Hb amount and significant increase in WBCs count; the results which coincide with that recorded by Murad and Mostafa (1988); Tavares-Dias *et al.* (2002) and Ibsam (2004) where they reported lower erythrocytic, hemoglobin levels and a higher leucocytic count especially in catfish.

In fact, all the ectociliated protozoans isolated in the current study (*Ichthiophtherius multifilus*, *Chilodenella* & *Trichodina*) showed significant increase in all values of differential leucocytis count except the heterophils count showed significant decrease in all infected cases.

Table 2. Effect of External Ciliated Protozoan infestations on haematological parameters in *C. gariepinus*

Season	Area	Treat	RBCs ($\times 10^6 / \text{mm}^3$)	Hb (g/100ml)	Pcv (%)	WBCs ($\times 10^3 / \text{mm}^3$)	
Winter	Kafr El-Sheikh farms	<i>I. multifilus</i>	2.78 \pm 0.01 ^b	7.81 \pm 0.5 ^a	23.00 \pm 0.5 ^a	73.45 \pm 0.81 ^d	
		<i>Chilodenella sp.</i>	2.81 \pm 0.2 ^{ab}	7.82 \pm 0.11 ^a	24.00 \pm 1.0 ^a	74.23 \pm 1.3 ^c	
		<i>Trichodina sp.</i>	2.68 \pm 0.08 ^c	7.65 \pm 0.2 ^a	22.00 \pm 0.5 ^a	76.07 \pm 2.1 ^b	
	Dakahlia farms	<i>I. multifilus</i>	2.78 \pm 0.1 ^b	7.70 \pm 0.24 ^a	22.00 \pm 0.9 ^a	75.86 \pm 1.12 ^b	
		<i>Chilodenella sp.</i>	2.79 \pm 0.09 ^b	7.64 \pm 0.09 ^a	22.50 \pm 0.5 ^a	72.51 \pm 0.5 ^e	
		<i>Trichodina sp.</i>	2.63 \pm 0.11 ^d	7.27 \pm 0.15 ^b	22.50 \pm 0.18 ^a	76.82 \pm 0.5 ^a	
	Non-infested			2.83 \pm 0.08 ^a	7.70 \pm 0.41 ^a	24.00 \pm 0.19 ^a	67.23 \pm 0.59 ^f
	Spring	Kafr El-Sheikh farms	<i>I. multifilus</i>	2.68 \pm 0.14 ^b	7.78 \pm 0.18 ^{ab}	23.00 \pm 1.0 ^a	75.46 \pm 1.15 ^f
			<i>Chilodenella sp.</i>	2.72 \pm 0.2 ^a	7.83 \pm 0.07 ^a	23.50 \pm 0.5 ^a	73.61 \pm 0.54 ^e
<i>Trichodina sp.</i>			2.48 \pm 0.05 ^d	7.64 \pm 0.5 ^c	22.50 \pm 0.5 ^{ab}	78.41 \pm 0.11 ^b	
Dakahlia farms		<i>I. multifilus</i>	2.63 \pm 0.4 ^c	7.62 \pm 0.42 ^d	21.50 \pm 0.5 ^{bc}	79.33 \pm 0.72 ^a	
		<i>Chilodenella sp.</i>	2.58 \pm 0.2 ^b	7.63 \pm 0.31 ^c	22.50 \pm 0.45 ^a	76.52 \pm 2.1 ^d	
		<i>Trichodina sp.</i>	2.43 \pm 0.09 ^e	7.23 \pm 0.17 ^e	21.50 \pm 0.65 ^c	79.59 \pm 0.5 ^c	
Non-infested			2.89 \pm 0.23 ^a	7.78 \pm 0.5 ^b	23.50 \pm 0.5 ^a	69.77 \pm 0.5 ^g	
Summer		Kafr El-Sheikh farms	<i>I. multifilus</i>	2.73 \pm 0.3 ^b	7.79 \pm 0.5 ^{ab}	23.50 \pm 1.0 ^a	71.29 \pm 0.2 ^c
			<i>Chilodenella sp.</i>	2.79 \pm 0.2 ^a	7.82 \pm 0.28 ^a	23.50 \pm 0.5 ^a	71.09 \pm 0.35 ^c
	<i>Trichodina sp.</i>		2.68 \pm 0.04 ^d	7.64 \pm 0.33 ^c	22.00 \pm 0.5 ^{ab}	80.03 \pm 0.25 ^a	
	Dakahlia farms	<i>I. multifilus</i>	2.71 \pm 0.2 ^c	7.62 \pm 0.41 ^c	21.50 \pm 0.5 ^{ab}	73.56 \pm 0.14 ^b	
		<i>Chilodenella sp.</i>	2.73 \pm 0.3 ^b	7.63 \pm 0.15 ^c	23.00 \pm 1.0 ^{ab}	74.06 \pm 0.89 ^b	
		<i>Trichodina sp.</i>	2.55 \pm 0.25 ^e	7.20 \pm 0.05 ^d	21.00 \pm 0.48 ^b	80.19 \pm 0.73 ^a	
	Non-infested			2.80 \pm 0.31 ^a	7.78 \pm 0.26 ^b	23.00 \pm 0.5 ^{ab}	69.47 \pm 1.25 ^d
	Autumn	Kafr El-Sheikh farms	<i>I. multifilus</i>	2.71 \pm 0.2 ^b	7.82 \pm 0.11 ^a	23.50 \pm 1.0 ^a	70.35 \pm 2.0 ^e
			<i>Chilodenella sp.</i>	2.77 \pm 0.11 ^b	7.84 \pm 0.19 ^a	23.50 \pm 1.0 ^a	71.26 \pm 0.56 ^f
<i>Trichodina sp.</i>			2.54 \pm 0.2 ^d	7.66 \pm 0.51 ^b	22.00 \pm 0.5 ^a	77.55 \pm 0.74 ^c	
Dakahlia farms		<i>I. multifilus</i>	2.60 \pm 0.16 ^c	7.61 \pm 0.07 ^b	21.00 \pm 0.5 ^a	77.67 \pm 0.58 ^b	
		<i>Chilodenella sp.</i>	2.69 \pm 0.21 ^c	7.68 \pm 0.13 ^b	22.50 \pm 0.5 ^a	72.52 \pm 0.5 ^d	
		<i>Trichodina sp.</i>	2.40 \pm 0.09 ^e	7.23 \pm 0.5 ^c	20.50 \pm 0.81 ^a	77.14 \pm 0.5 ^a	
Non-infested			2.78 \pm 0.2 ^a	7.81 \pm 0.037 ^a	23.50 \pm 0.46 ^a	69.83 \pm 0.5 ^g	

For each season: means within the same column of different letters are significantly different at (P<0.05)

The effect of different ectociliated protozoan on the infested *C. gariepinus* on differential leucocytic count values are summarized in Table 3. Ciliated parasites stimulate granulocyte synthesis that disturbs the values of differential leucocytic counts (Mahfouz, 1997). Liu and Lu (2004) reported that the ciliated protozoan; *Ichthyophthirius multifiliis*, renders the fish to be very susceptible to other fish pathogens which leading to variable fluctuations in haematogram components. However, Alvarez Pellitero (2008) stated that

ciliates may modulate inflammatory reactions in fish. On the other side, El-Seify *et al.* (2003) reported an increase in differential leucocytic counts in infected fish except for esinophils which are less significantly changed. Although, Murad and Mostafa (1988) recorded an increase in esinophils and monocytes. El-Saftawy (2015) reported increased level of lymphocytes in infected cases with ciliated protozoans.

Table 3. Effect of External Ciliated Protozoan infestations on Differential Leucocytic count in *C. gariepinus*

Season	Area	Treat	Heterophil ×10 ³ /mm ³	Lymphocyte ×10 ³ /mm ³	Monocyte ×10 ³ /mm ³	Esinophil ×10 ³ /mm ³	Basophil ×10 ³ /mm ³
Winter	Kafr El-Sheikh farms	<i>I. multifilus</i>	13.21±0.15 ^f	35.48±1.0 ^e	7.76±0.2 ^d	0.14±0.02 ^d	0.04±0.01 ^d
		<i>Chilodenella sp.</i>	13.44±0.09 ^e	35.85±0.5 ^d	7.89±0.09 ^c	0.23±0.2 ^c	0.05±0.01 ^c
		<i>Trichodina sp.</i>	14.02±0.5 ^b	41.42±0.5 ^b	9.47±0.25 ^a	0.37±0.1 ^b	0.06±0.02 ^b
	Dakahlia farms	<i>I. multifilus</i>	13.46±0.72 ^e	35.05±0.5 ^f	7.13±0.5 ^e	0.15±0.2 ^d	0.06±0.02 ^b
		<i>Chilodenella sp.</i>	13.86±0.45 ^d	38.62±0.5 ^c	7.93±0.2 ^c	0.13±0.2 ^d	0.03±0.05 ^e
		<i>Trichodina sp.</i>	13.98±0.89 ^c	41.77±0.64 ^a	9.34±0.18 ^b	0.43±0.01 ^a	0.07±0.01 ^a
Non-infested			14.10±1.01 ^a	31.68±0.14 ^g	6.55±0.36 ^f	0.02±0.2 ^e	0.01±0.02 ^f
Spring	Kafr El-Sheikh farms	<i>I. multifilus</i>	12.86±0.5 ^g	37.83±0.58 ^d	8.03±0.5 ^e	0.20±0.04 ^b	0.06±0.08 ^c
		<i>Chilodenella sp.</i>	13.78±0.5 ^d	36.93±1.25 ^f	8.18±1.0 ^c	0.22±0.2 ^b	0.05±0.01 ^c
		<i>Trichodina sp.</i>	13.44±0.33 ^b	43.42±0.77 ^b	9.89±0.25 ^a	0.42±0.1 ^b	0.06±0.01 ^a
	Dakahlia farms	<i>I. multifilus</i>	13.10±0.14 ^c	36.94±0.11 ^e	7.66±0.2 ^f	0.16±0.1 ^b	0.07±0.01 ^b
		<i>Chilodenella sp.</i>	13.57±0.11 ^e	38.52±0.35 ^c	7.94±0.27 ^d	0.14±0.2 ^b	0.04±0.01 ^c
		<i>Trichodina sp.</i>	14.47±0.58 ^a	42.84±0.5 ^a	9.87±0.22 ^b	0.42±0.2 ^a	0.11±0.02 ^a
Non-infested			14.05±1.0 ^f	30.78±0.5 ^g	6.74±0.2 ^g	0.01±0.01 ^b	0.02±0.01 ^d
Summer	Kafr El-Sheikh farms	<i>I. multifilus</i>	12.97±0.21 ^g	36.97±3.0 ^c	7.83±0.13 ^e	0.17±0.01 ^{cd}	0.05±0.01 ^c
		<i>Chilodenella sp.</i>	13.25±0.54 ^f	36.81±2.14 ^d	7.98±0.2 ^c	0.19±0.02 ^c	0.05±0.02 ^c
		<i>Trichodina sp.</i>	13.96±0.5 ^b	42.13±0.5 ^a	9.93±0.16 ^a	0.39±0.02 ^b	0.06±0.02 ^a
	Dakahlia farms	<i>I. multifilus</i>	13.87±0.5 ^c	36.53±0.81 ^e	7.44±0.28 ^f	0.12±0.2 ^e	0.06±0.03 ^{bc}
		<i>Chilodenella sp.</i>	13.76±0.49 ^d	37.95±0.64 ^b	7.90±0.2 ^d	0.15±0.2 ^{de}	0.04±0.01 ^d
		<i>Trichodina sp.</i>	14.25±0.11 ^a	42.08±0.5 ^a	9.72±0.19 ^b	0.44±0.09 ^a	0.06±0.08 ^{ab}
Non-infested			13.46±0.18 ^e	31.07±0.5 ^f	6.66±0.5 ^g	0.01±0.01 ^f	0.01±0.01 ^e
Autumn	Kafr El-Sheikh farms	<i>I. multifilus</i>	13.11±0.25 ^g	37.14±0.5 ^d	7.72±0.5 ^c	0.17±0.2 ^b	0.05±0.01 ^{cd}
		<i>Chilodenella sp.</i>	13.36±2.0 ^c	36.28±0.87 ^e	8.12±0.54 ^b	0.20±0.2 ^b	0.05±0.02 ^d
		<i>Trichodina sp.</i>	14.07±1.34 ^e	41.10±0.46 ^a	9.92±0.2 ^a	0.18±0.07 ^a	0.07±0.01 ^{bc}
	Dakahlia farms	<i>I. multifilus</i>	13.47±0.5 ^f	36.45±0.5 ^e	7.55±0.16 ^e	0.11±0.01 ^c	0.06±0.02 ^b
		<i>Chilodenella sp.</i>	13.30±0.05 ^d	37.53±2.94 ^c	7.82±0.87 ^d	0.11±0.2 ^c	0.05±0.01 ^e
		<i>Trichodina sp.</i>	14.44±0.5 ^a	41.26±0.5 ^b	9.82±0.2 ^a	0.39±0.06 ^a	0.07±0.01 ^a
Non-infested			13.23±0.35 ^b	31.12±0.53 ^f	6.72±0.17 ^f	0.01±0.01 ^d	0.02±0.03 ^f

For each season: means within the same column of different letters are significantly different at (P<0.05)

Blood serum biochemical analysis of protozoans infected fish:

Recognizing the effect of different ectociliated protozoans on blood serum components of *Clarias gariepinus*; lower level of total serum protein was recorded in all infected cases as summarized in Table 4.

Table 4. Effect of External Ciliated Protozoan infestations on Serum Biochemical Parameters in *C. gariepinus*.

Season	Area	Treat	Total protein (g/dl)	ALT (U/l)	AST (U/l)	
Winter	Kafr El-Sheikh farms	<i>I. multifilus</i>	4.78±0.41 ^b	53.05±2.1 ^f	40.75±1.1 ^d	
		<i>Chilodenella sp.</i>	4.81±0.21 ^b	54.76±0.92 ^e	40.08±2.11 ^e	
		<i>Trichodina sp.</i>	4.43±0.18 ^e	59.52±0.5 ^c	43.18±0.29 ^b	
	Dakahlia farms	<i>I. multifilus</i>	4.63±0.2 ^c	60.27±3.12 ^b	39.84±1.54 ^f	
		<i>Chilodenella sp.</i>	4.53±0.2 ^d	58.58±1.54 ^d	42.08±0.5 ^c	
		<i>Trichodina sp.</i>	4.32±0.09 ^f	64.86±0.58 ^a	45.46±0.7 ^a	
	Non-infested			5.06±0.2 ^a	49.75±0.5 ^g	32.17±1.3 ^g
	Spring	Kafr El-Sheikh farms	<i>I. multifilus</i>	4.83±0.22 ^c	52.72±0.5 ^d	40.21±0.5 ^e
			<i>Chilodenella sp.</i>	4.78±0.5 ^b	55.49±1.5 ^d	40.97±0.5 ^d
<i>Trichodina sp.</i>			4.35±0.2 ^e	61.80±0.97 ^b	45.82±0.98 ^b	
Dakahlia farms		<i>I. multifilus</i>	4.63±0.5 ^{cd}	60.16±2.4 ^c	40.55±1.25 ^e	
		<i>Chilodenella sp.</i>	4.58±0.16 ^d	59.85±1.9 ^b	42.75±0.66 ^c	
		<i>Trichodina sp.</i>	4.15±0.59 ^f	65.94±2.13 ^a	48.35±3.1 ^a	
Non-infested			5.14±0.87 ^a	51.11±1.3 ^e	31.95±2.14 ^f	
Summer		Kafr El-Sheikh farms	<i>I. multifilus</i>	4.75±0.91 ^b	54.50±0.5 ^e	39.75±1.01 ^f
			<i>Chilodenella sp.</i>	4.67±0.2 ^{cd}	53.51±0.5 ^f	40.79±0.87 ^d
	<i>Trichodina sp.</i>		4.48±1.0 ^e	60.14±0.5 ^b	44.54±0.58 ^b	
	Dakahlia farms	<i>I. multifilus</i>	4.72±0.05 ^{bc}	58.15±0.18 ^c	40.18±0.67 ^e	
		<i>Chilodenella sp.</i>	4.62±0.11 ^d	59.12±1.54 ^d	42.44±1.17 ^c	
		<i>Trichodina sp.</i>	4.24±0.2 ^f	65.37±2.0 ^a	47.29±0.59 ^a	
	Non-infested			5.62±0.12 ^a	50.89±97 ^g	32.05±0.68 ^g
	Autumn	Kafr El-Sheikh farms	<i>I. multifilus</i>	4.76±0.19 ^b	53.25±0.92 ^e	39.13±3.1 ^f
			<i>Chilodenella sp.</i>	4.82±0.2 ^c	52.68±0.57 ^d	40.14±0.88 ^d
<i>Trichodina sp.</i>			4.42±0.2 ^f	59.09±0.5 ^b	44.09±0.64 ^b	
Dakahlia farms		<i>I. multifilus</i>	4.72±0.31 ^d	57.28±0.67	39.17±0.5 ^e	
		<i>Chilodenella sp.</i>	4.69±0.2 ^e	59.30±0.38 ^c	41.44±0.29 ^c	
		<i>Trichodina sp.</i>	4.27±0.2 ^g	65.05±0.5 ^a	44.26±3.0 ^a	
Non-infested			5.31±0.57 ^a	51.28±0.15 ^f	32.50±1.25 ^g	

For each season: means within the same column of different letters are significantly different at (P<0.05)

The blood serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes activities were elevated in all cases of

infested *C. gariepinus* with external ciliated protozoans than the non infected fishes; this indicates that the external parasites stimulated the activities of ALT and AST enzymes. This agrees with Younis, (1999) and (Adham, 2002) which recorded that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed significant increase in *O. niloticus* infested with external parasites and to El-Saftawy, (2015) in *C. gariepinus* infested with external ciliated protozoan. This may be attributed to the hepatic cells injury or increased synthesis of the enzymes by the liver (Yang and Chen, 2003). Moreover, these findings may be due to the inflammatory reactions and intoxications produced by the parasite in the affected fish.

Although Awad (2007) recorded that the total serum proteins didn't show any significant changes between naturally infected and apparently healthy fish, the total proteins, globulins and albumin were decreased in ciliated infected fish (Mahfouz, 1997 and El-Seify *et al.*, 2003).

Histopathological findings:

Histopathological alterations of the skin of the infested *C. gariepinus* revealed vascular congestion, lymphatic dilatation and peri-lymphatic eosinophilic granular cells infiltration as well as presence of the parasitic cysts in the underlying degenerated muscle tissues (Fig 7; A, B) together with necrosis of some muscle fibers. The result is similar to that recorded by Aly *et al.* (1998).

While, the gills showed degenerative changes in primary and secondary gill lamellae, severe degree of fusion of gill lamellae with marked leucocytic infiltration as well as elongation and interlamellar hyperplasia (Fig 8; A, B). These alterations were evenly distributed due to the infections of *Ichthyophthirius multifiliis*, *Chilodonella* and *Trichodina* (FAO, 2013 and De Padua *et al.*, 2014). These results are similar to those reported by Osman (2001), Noor El-Deen *et al.*, (2014) and Gado *et al.*, (2017). Also, similar to that recorded by Roberts (2012) that mentioned that the most common response

of the gill to damage by protozoan parasites is hyperplasia and hypertrophy of epithelial cells. This gill damage by protozoan parasites, may be attributed to the feeding activity, attachment, fixation and locomotion and caused a massive destruction of the respiratory epithelial cells (Abd El- Hady (1998).

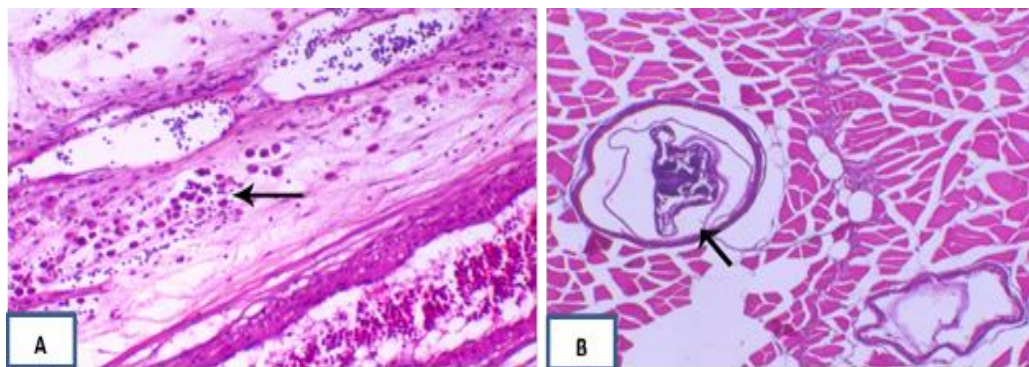


Fig. 7. Skin of affected *C. gariepinus*. A) vascular congestion, lymphatic dilatation and peri-lymphatic eosinophilic granular cells infiltration (arrow). H&E, X200. B) showing presence of encysted parasitic cyst(arrow). H&E, X200.

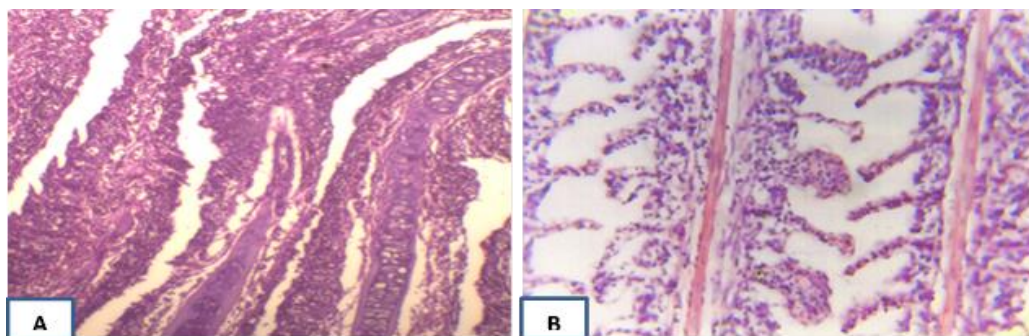


Fig. 8. Gills of affected *C. gariepinus*. A) showing severe degree of fusion of gill lamellae as a result of marked leucocytic infiltration. B) showing elongation of secondary lamellae and interlamellar hyperplasia. H&E stain. A X100; B X200.

CONCLUSION

From the current study, the highest prevalence of the parasitic infestation in *C. gariepinus* was 20.5% in autumn and winter and 18.75% in spring and summer season in Kafr El-Sheikh fish farms. However, in Dakahlia

fish farms, it was 20% in summer and winter, 18.5% in autumn and 18% in the spring season. *Ichthiophtherius multifiliis* have the highest percentage of the infection (45.8%, 42%) with highest infestation in winter and summer (50%, 54%), followed by *Trichodina sp.*, (18.25%, 17.75%) with highest infestation rate in both spring and summer (20%) and winter (22%), then *Chilodenella sp.*, (14.5%, 16.75%) with highest infestation rate in autumn (18%), and autumn, winter and spring (18%) in Kafr El-Sheikh and Dakahlia governorates, respectively.

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فحص موسمي لبعض الأمراض الطفيلية الهدبية في القرموط الأفريقي (كلارياس غاربيينوس) في محافظتي كفر الشيخ والدقهلية

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الملخص العربي

أجريت هذه الدراسة بهدف فحص موسمي لبعض الأمراض الطفيلية الهدبية في القرموط الأفريقي (كلارياس غاربيينوس) في محافظتي كفر الشيخ والدقهلية. تم فحص ثمانمائة عينة من القرموط الأفريقي (كلارياس غاربيينوس) من محافظتي كفر الشيخ والدقهلية؛ ١٠٠ سمكة لكل موسم / محافظة، لدراسة معدل الاصابه الموسمي لبعض الطفيليات الخارجية المهدبة. أسفرت النتائج عن عزل أكتيوفتاريس مالتيفيلس، كيلوددينيللا و تريكودينا. بشكل عام، أظهرت معظم الأسماك التي تم فحصها هزال، تغير في لون الجسم (اللون الشاحب أو اللون الداكن)، الإفراز المخاطي المفرط على سطح الجسم الخارجي، بقع نزفية متناثرة، والجروح والقرحة على أجزاء مختلفة من جسم الأسماك. وكان أعلى معدل تسجيل للإصابة ٢٠.٥% في موسمي الخريف والشتاء و ١٨.٧٥% في موسمي الربيع والصيف في مزارع كفر الشيخ. أما في مزارع الدقهلية السمكية فقد بلغت ٢٠% في موسمي الصيف والشتاء و ١٨.٥% في موسم الخريف و ١٨% في موسم الربيع.

وقد لوحظ اعلي ارتفاع لمعدل الإصابة بأكتيوفتاريس مالتيفيلس بمحافظه كفرالشيخ في فصل الشتاء (٥٠%) و بمحافظه الدقهليه كان الصيف (٥٤%) هو الأعلى، وكذلك كان أعلى معدل للاصابه بالكيلوددينلا في محافظه كفرالشيخ خلال الخريف (١٨%)، و بينما في محافظه الدقهليه كان الخريف والشتاء والربيع (١٨%). كان معدل الاصابه بالتريكودينا (٢٠%) لكل من الربيع والصيف بكفرالشيخ، و (٢٢%) في الشتاء بالدقهليه، وعلاوة على ذلك، أجريت بعض الاختبارات الدموية علي دماء الأسماك المصابة طبيعيا، بعض الدراسات البيوكيميائية في مصل دماء الأسماك المصابة لدراسة بعض الإنزيمات وكذلك البروتينات. وأيضا تسجيل بعض التغيرات النسيجية في الجلد والخياشيم من الأسماك المصابة.