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Prevalence of some ectoparasitic diseases in African catfish (*Clarias gariepinus*) at Kafr El-Sheikh governorate

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Abstract
Four hundred specimens of African catfish (*Clarias gariepinus*), 100 fish per each season throughout the year; with different sizes and body weight were investigated for seasonal incidence of some external parasites. The results revealed isolation of *Dactylogyrus, Gyrodactylus, Trichodina and Chilodinella*. The clinical signs, in general, of most examined fishes showed emaciation, dark or pale body coloration, excessive amounts of mucous on the external body surface, scattered hemorrhagic patches, wounds and ulcers on different parts of the body, ascitis as well as congestion of the gills. The highest rate of the infestations was recorded in autumn and winter (15.5%) spring and summer (15%) season; respectively. A higher infection rate of *Dactylogyrus sp.* was observed during spring and summer (27%), *Gyrodactylus sp.* during autumn and winter (19%), *Trichodina sp*. during autumn and summer (12%), and of *Chilodenella sp*. was during autumn and winter (8%). In addition, the histopathological alterations in the skin and gills of the infested fish were recorded.

Keywords: Catfish, *Clarias gariepinus*, disease, ectoparasite, prevalence

1. Introduction
Aquaculture in Egypt is the main source of fish production; (77%) of the total fish production of which 85% produced by the constructed pond-based aquaculture around the Nile Delta lakes [1]. Kafr El-Sheikh Governorate has the highest fish production rate from either owned or temporarily private fish farms; 109,725 and 214,900 MT per 31,350 and 61,400 acres, respectively [2].

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 [3].

In Egypt, the optimum warm weather, enable the outburst of parasites spread; causing worse effects on fish [4]. So, parasitic diseases constitute the largest sector of fish diseases in Egypt; reaching 80% [5].

Generally, fish parasites result in economic losses not only due to mortalities, tissue damage and growth reduction, but also due to high expenses of drug treatment [6].

The protozoan *Trichodina sp.* and the monogenean trematodes were dominant external parasites which result in severe pathological effects on fish and increasing mortality [7]. Gyrodactylids are viviparous monogenean ectoparasites, parasitized skin and gills resulting in mortalities especially among young fishes [5, 8]. Most trichodinids are not pathogens, but under certain environmental conditions or when the fish are stressed by certain other factors, the parasite increases greatly its rate of infestation among fish and become pathogenic resulting in gill epithelium hyperplasia and necrosis and cause mass mortality of the infested fish [9]. *Trichodina sp.* was extensively isolated from gills of both freshwater and marine water fishes [10, 11, 12].

The protozoan *Chilodonella sp*. parasitize the skin and gills of fish. The infection is world-wide on warm and cold water fish species and has been responsible for large-scale mortalities. The heavy infested fish appeared emaciated with darkening or dulling of the skin colour [13, 14].

The present study aimed to investigate the seasonal incidence of some external parasites isolated from African catfish (*C. gariepinus*) as well as, evaluation of histopathological

changes induced by such detected parasites; which weren't checked before.

2. Materials and Methods

2.1 Fish samples

A total number of 400 *C. gariepinus* with different sizes and weights were collected alive from different freshwater fish farms and markets in Kafr El-Sheikh governorate along the four seasons of the year 2016. The collected fishes were transferred alive to the wet lab., Fish Diseases and Management Department, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt [15, 16]. Collected samples were held in well-prepared glass aquaria supplied with sufficient amounts of dechlorinated water with continuous aeriation [17].

2.2 Clinical and Postmortem examination

The fish were subjected to full clinical examination for the changes in colour 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 some authors [18, 19, 20].

2.3 Parasitological Examination

The ectoparasites present on the skin, gills and pseudo bronchial organs of *C. gariepinus* were detected and identified.

Tissue scrapings from skin and gill filaments were prepared with a drop of normal saline and covered with a clean cover slip (Wet mount preparation) and examined microscopically [18].

Microscopic parasites were collected by a brush, special needle or dropper, then washed for several times in warm saline solution and left in the refrigerator until the specimens has been died and completely relaxed [18].

Management ectoparasites were collected using binocular dissecting microscope with a small pipette and transferred into small petri-dish and cleared several times with water to remove the debris and mucus. The worms were then left in refrigerator at 4 °C for complete relaxation, fixed in 5% formalin for permanent preparation, washed carefully in water to get rid of formalin traces and finally stained with acetocarmine for 5-10 minutes. Specimens were passed through ascending grades of ethyl alcohol (30, 50, 70, 90% and absolute) for dehydration and then cleared in clove oil, xylene and mounted in Canada Balsam [21].

Parasitological examination of ciliated protozoans (*Trichodina sp*. and *Chilodenella sp*.), skin and gills were examined immediately to avoid the escape of the external protozoa. Smears were taken very thin 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± 1°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 lens**es** according to the methods described by some authors [18, 22, 23].

2.4 Histopathological Examination

Tissue specimens from the skin, gills and pseudobronchial organs of the infested catfish were taken. Specimens were 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 Hematoxilin & Eosin (H&E) and examined under light microscope (Leica) using ×200 and ×400 magnification power according to Bancroft and Gamble [24].

3. Results and Discussion

The present work studied the seasonal incidence of different external parasitic diseases among naturally infested *C. gariepinus* in Kafr El- Sheikh governorate.

3.1 Clinical examination

The external gross lesions of the examined *C. gariepinus* revealed emaciation, dark or pale body coloration, excessive amounts of mucous 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, ascitis as well as congested gills could be observed in some cases (Fig. 4). Excessive amount of mucous might be released to relieve the irritating inflammatory reaction caused by continuous irritation of ciliated ectoparasites [25, 26, 27].

Infested fish appeared asphyxiated and exhausted; this may be due to low oxygen intake of destructed gill epithelium, which caused by feeding activity, attachment, fixation and locomotion of trichodina and monogenea causing massive destruction of the respiratory epithelial cells which may be similar to that reported by Eissa *et al.* [28]**;** where he reported the clinical picture of infested freshwater fishes induced by the parasites on the skin, fins and gills; as abnormal swimming, flashing, rubbing the body against the sides of aquaria to get rid of the irritation and fish gathered at the water surface, gulping the atmospheric air, appeared asphyxiated and exhausted.

Regarding the scattered hemorrhagic patches with small wounds, or ulcers on the body surface together with darkening of skin of some fishes infected with *Gyrodactylus*; this might be attributed to that *Gyrodactylus* (skin fluke); is 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. These results nearly similar to that recorded by other authors [29, 30].

Congestion of gills may be attributed to destruction of the efferent vessels by monogenea; where the blood pressure is low causing extensive hemorrhage and clotting of blood leading to rapid occlusion of the vessel, ischemia and necrosis in some areas; which may progress into pale gills giving the Marbling appearance [31, 32].

3.2 Parasitological examination

Microscopic smears taken from gills, skin and fins of examined *C.gariepinus* revealed the presence of some ectoparasites. *Dactylogyrus sp*. have the highest rate of the infestation (25.8%) followed by *Gyrodactylus sp*. (17.8%), *Trichodina sp*. (10.8%) then *Chilodenella sp*. (6.8%) as shown in Table (1).

Adult worms isolated from the gills of infested catfish; were flat and elliptical in shape. Their anterior end (prohaptor) was divided into four cephalic lobed heads, with sticky and adhesive organs (cephalic glands), in addition to four black eye spots. The posterior end, appeared a dome shape and composed of one pair of connecting bars (V-shaped) and

seven pairs of small marginal booklets. The intestinal limbs were connected, the ovary located in front to the testes. Such adult worms are related to the phylum *Platyhelminthes*, class *Trematoda*, order *Mongenea* family *Dactylogyridae* and genus *Dactylogyrus claridii* (Fig. 5).

However, the adult worms isolated from the skin of infested catfish**;** were flat and elliptical in shape and 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. Such adult worms are related to the phylum *Platyhelminthes*, class *Trematoda*, order *Mongenea*, family Gyrodactylidae and genus *Gyrodactylus claridii* (Fig. 6). Regarding monogenean trematodes (*Gyrodactylus sp.* and *Dactylogyrus sp*.), they were morphologically and parasitologically described and were nearly similar to the descriptions given by Yamaguti [33].

Microscopic smears 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 heterodentata Duncan, 1977* (Fig. 7). 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. 8). The ciliated protozoans (*Trichodina sp.* and *Chilodenella sp.* were morphologically and parasitologically identified and were nearly similar to the descriptions given by Kabata [34].

3.3 Incidences of fish ectoparasites among different seasons

Parasitological examination of 400 *C. gariepinus* revealed the presence of different ectoparasites in 244 positive infested cases (61%) with a seasonal prevalence of 15.5% in autumn and winter and 15% in spring and summer season.

A higher infection rate of *Dactylogyrus claridii* was observed during spring and summer (27%) for both, followed by winter (26%) then autumn (23%) as shown in table (1) and histogram (1). While, a higher infestation rate of *Gyrodactylus claridii* was observed during autumn and winter (19%) for both, followed by spring (18%) then summer (15%) as shown in table (1) and histogram (2). On the other hand, a higher infection rate of *Trichodina sp*. was observed during autumn and summer (12%) for both, followed by spring (10%) then winter (9%) as shown in table (1) and histogram (3). However, a higher infection rate of *Chilodenella sp*. was (8%) for both in autumn and winter, (6%) in summer and (5%) in spring as shown in table (1) and histogram (4).

Concerning the seasonal variation of the prevalence of *Dactylogyrus sp*. in the present study, the highest rate of infestation was during Spring and summer; while in *Gyrodactylus sp*, the highest seasonal prevalence was in autumn and winter. This result partially agrees with that reported by Osman [30] where the highest prevalence in summer. However, the result is closely similar to that reported by Noor El Deen [35]. These differences in the prevalence rates; may be attributed to the differences in environmental conditions and the type of examined fish. On the other hand, the seasonal prevalence of *Trichodina sp*. in the present study, has the highest rate during autumn and summer; while in *Chilodenella sp.*, was in autumn and winter. The result is partially similar to that obtained by some authors [31, 36].

A number of researchers [37, 38, 39, 40, 41] have reported that the incidence of fish ciliated protozoans in Egypt; highly prevail mainly in the summer season, because of the higher temperature which is suitable for parasitic reproduction. On the other side, neither Paperna and Vanas [42] nor Jerônimo *et al*. [43] mentioned that the highest incidence was recorded in summer but, it was confirmed to be in cooler seasons; these differences may be attributed to different geographic regions, water sources and fish species [5, 44]. While, Bassiony [45] mentioned that the most important protozoan ciliate infections occur in autumn.

3.4 Histopathological examination:

Histopathological alterations of the skin of the infested *C. gariepinus* revealed presence of the parasitic cysts in the underlying degenerated muscle tissues (Fig 9; A, B and C) together with necrosis of some muscle fibers ((Fig 9; D).

While, the gills showed congestion and hemorrhages (Fig 10; A) as well as proliferation of the cartilaginous tissues inside the gill filament tissues with degenerated lamellae (Fig. 10; B). The induced gill damage by protozoan parasites, may be due to the feeding activity, attachment, fixation and locomotion and caused a massive destruction of the respiratory epithelial cells; these agree with that reported by Abd El- Hady [46] where the histopathological examination of tilapias for trichodina affections showed hemorrhage, congestion. Also, similar to that recorded by Roberts [47] that mentioned that the most common response of the gill to damage by protozoan parasites is hyperplasia and hypertrophy of epithelial cells.

Table 1: Incidence & Prevalence of different ectoparasites in the skin and gills of *Clarias gariepinus*

Season / Parasite	Total No. of examined fish	positive infested cases		*Dactylogyrus sp.*		*Gyrodactylus sp.*		*Trichodina sp.*		*Chilodinella sp.*	
		No.	%	No. of infected fish	%	No. of infected fish	%	No. of infected fish	%	No. of infected fish	%
Winter	100	62	15.5%	26	26%	19	19%	9	9%	8	8%
Spring	100	60	15%	27	27%	18	18%	10	10%	5	5%
Summer	100	60	15%	27	27%	15	15%	12	12%	6	6%
Autumn	100	62	15.5%	23	23%	19	19%	12	12%	8	8%
Total	400	244	61%	103	25.8%	71	17.8%	43	10.8%	27	6.8%

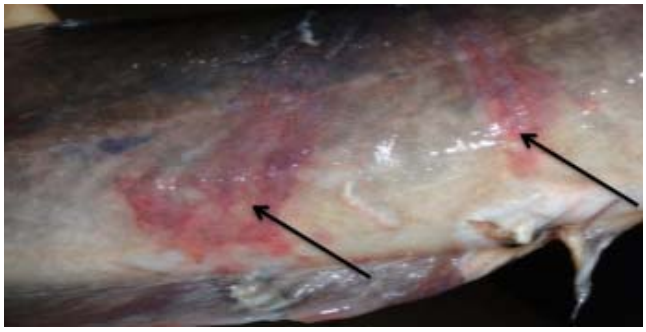


Fig 1: Skin of *Clarias gariepinus* infected with *Dactylogyrus sp*. showing scattered hemorrhagic patches (arrows) on different parts of the skin

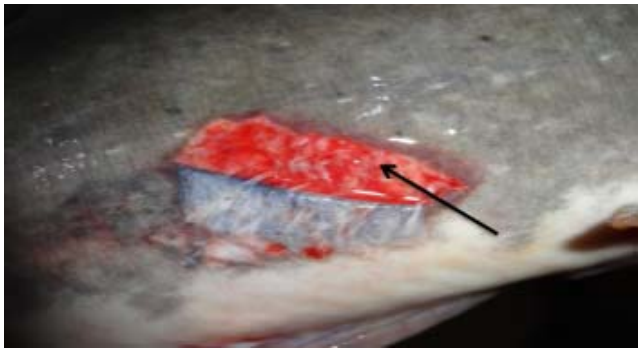


Fig 2: Skin of *Clarias gariepinus* infected with *Trichodina sp*. showing hemorrhagic ulcer (arrow) on the skin.

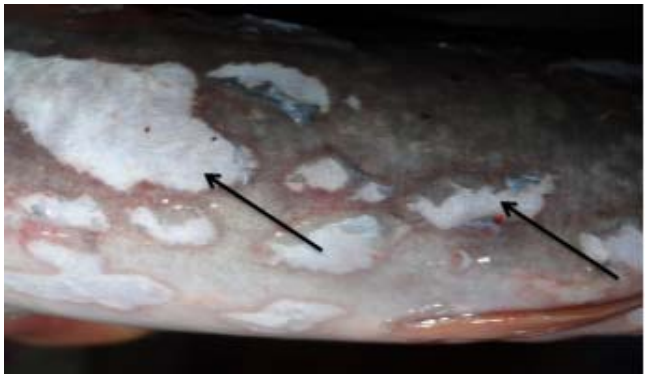


Fig 3: Skin of *Clarias gariepinus* infected with *Gyrodactylus sp*. showing scattered ulcerative areas (arrows) in different parts of the skin**.**

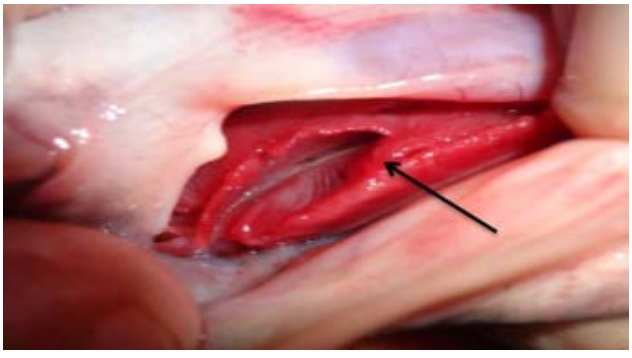


Fig 4: Gills of *Clarias gariepinus* infected with *Dactylogyrus sp*. showing severe congestion (arrow).

Fig 5: Wet mount from gills of *Clarias gariepinus* infected with *Dactylogyrus claridii* (arrow).

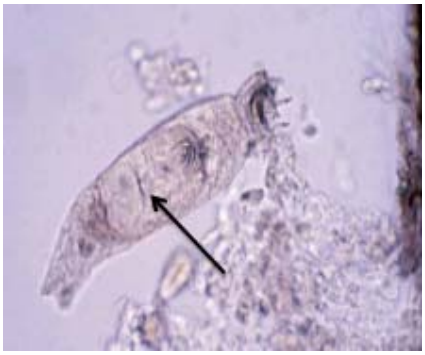


Fig 6: Wet mount from the skin of *Clarias gariepinus* showing *Gyrodactylus claridii* (arrow).

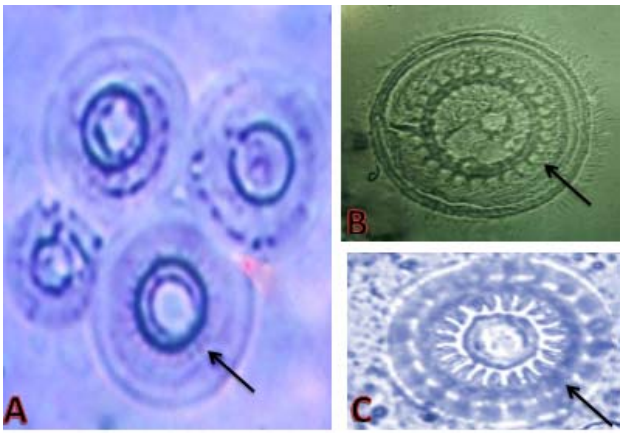


Fig 7: *Trichodina sp*. isolated from the skin of *Clarias gariepinus*. (A) Stained with Geimsa X 200 (arrow), (B) Wet mount preparation X 400 (arrow) (C) Geimsa stained X 400 (arrow)

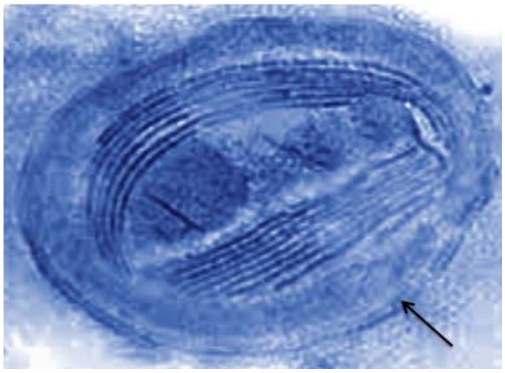


Fig 8: *Chilodenella sp*. isolated from the skin of *Clarias gariepinus* Stained with Geimsa, X 400 (arrow)

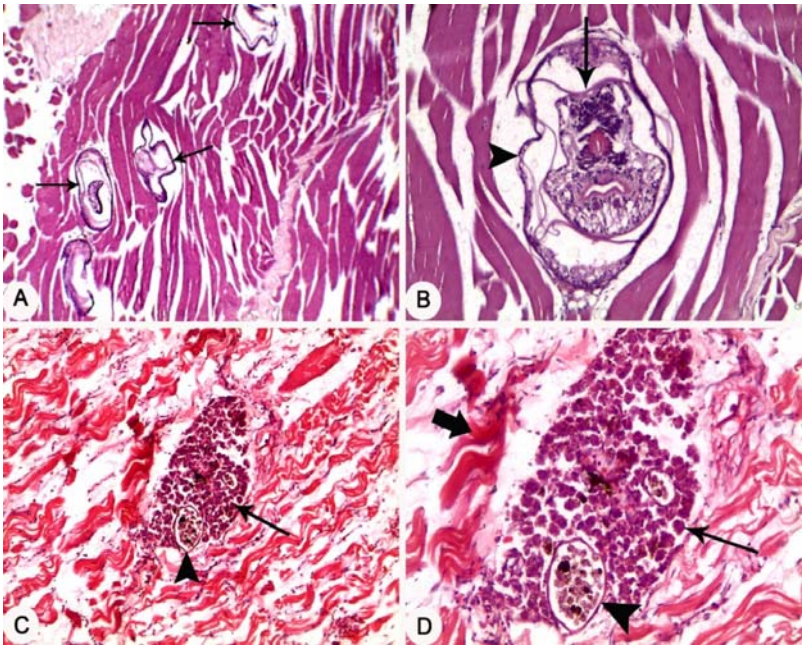


Fig 9: Muscles of African catfish (*Clarias gariepinus*). A & B) showing parasitic cyst in muscle tissues without any inflammatory reaction. Arrow indicating the parasite and arrowhead indicating the cyst capsule. C) showing parasitic cysts in degenerated muscular tissues. Arrow indicating the parasites in ruptured cyst; and the arrowhead indicating unruptured parasitic cyst. D). showing parasitic cysts in degenerated muscular tissues. Thin arrow indicating the parasites in ruptured cyst; the arrowhead indicating unruptured parasitic cyst; and the thick arrow indicating necrosis in muscle fibers. H&E stain. A X100; B X200; C X100 & D X200

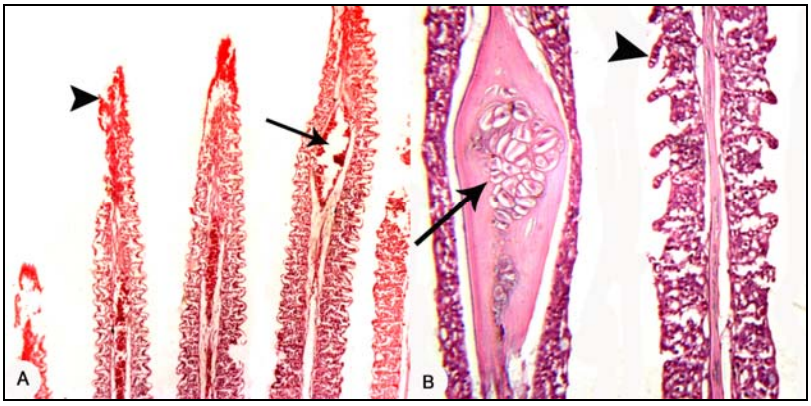
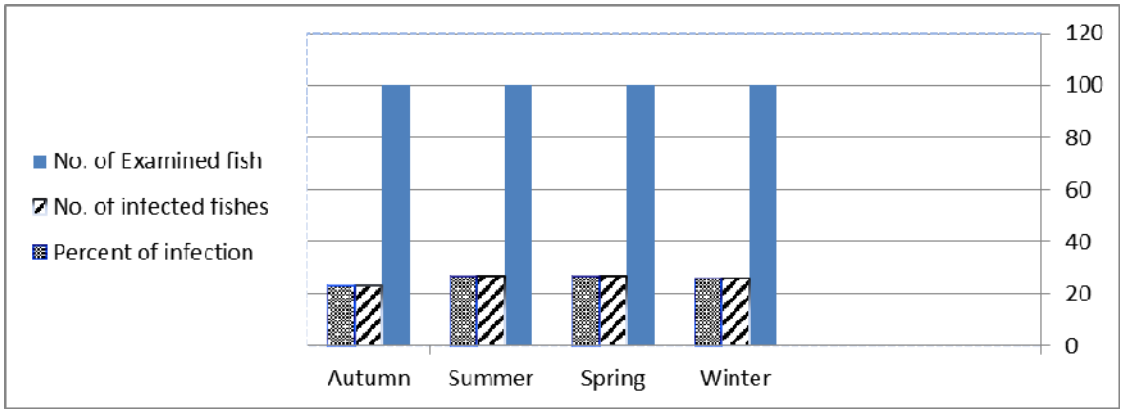
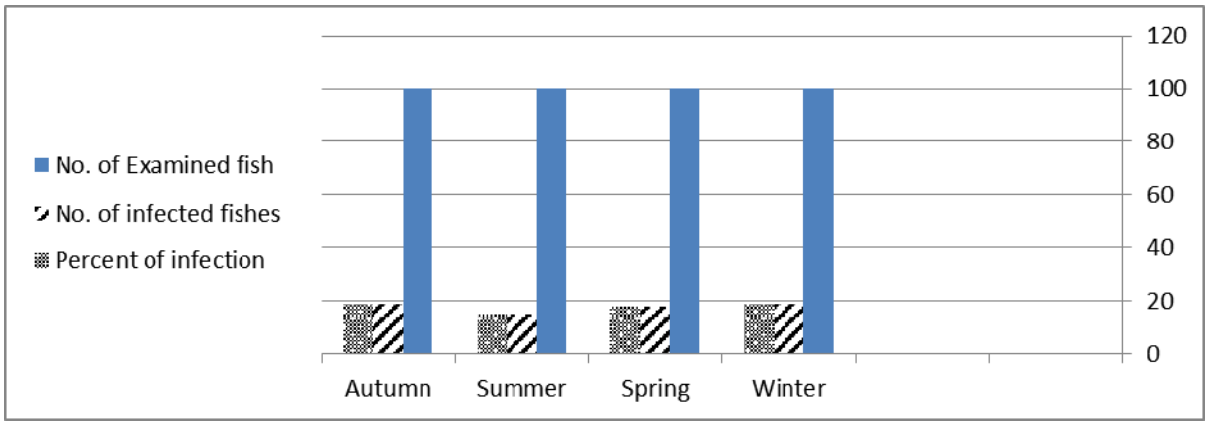


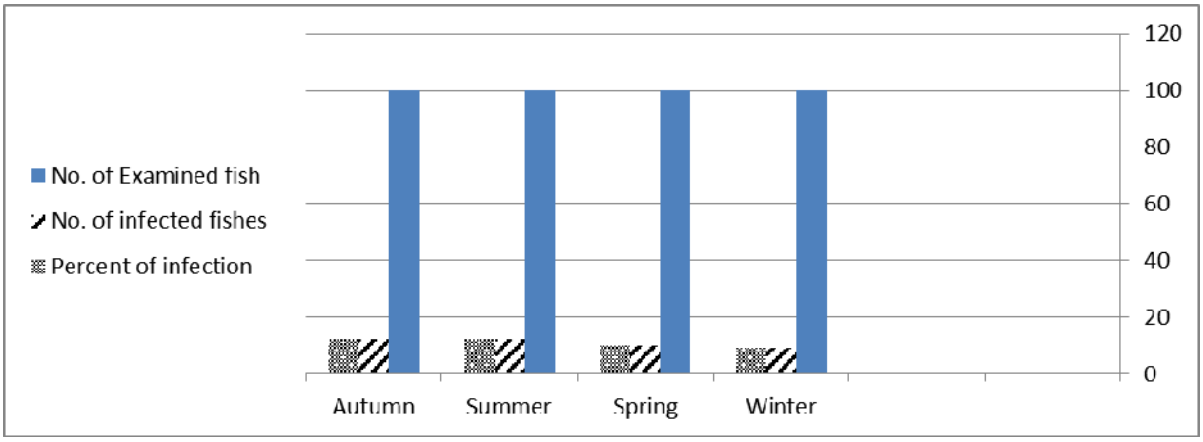
Fig 10: Gills of African catfish (*Clarias gariepinus*). **A)** Showing congestion (arrow) and hemorrhage (arrowhead) in the gills. **B)** showing proliferation of cartilaginous tissues inside the gill filament (arrow) and degeneration of gill lamellae (arrowhead). H&E stain, A X100; B X400



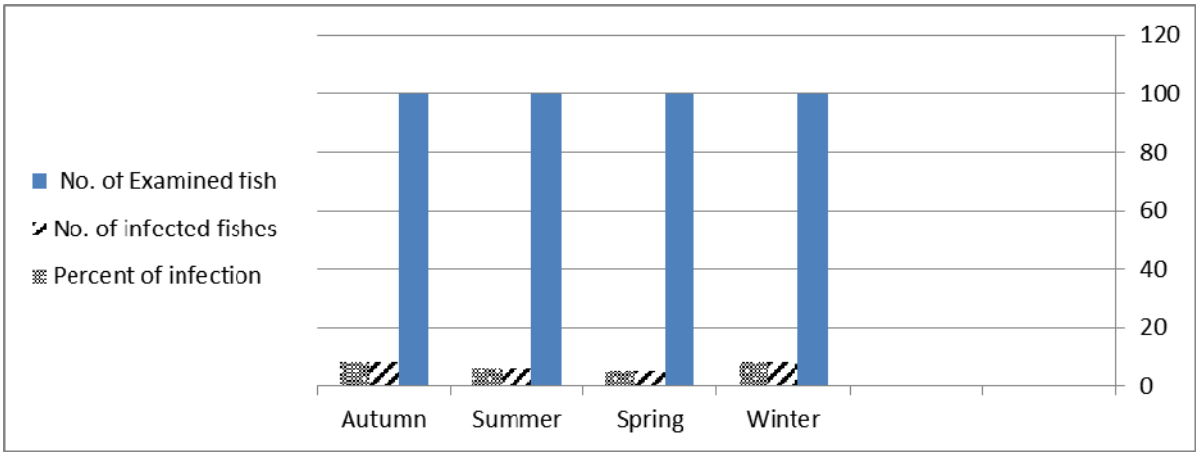
Histogram 1: The prevalence of *Dactylogyrus* sp. in the skin and gills of *Clarias gariepinus*



Histogram 2: The prevalence of *Gyrodactylus sp.* in the skin of *Clarias gariepinus*



Histogram 3: The prevalence of *Trichodina sp.* in the skin of *Clarias gariepinus*



Histogram 4: The prevalence of *Chilodenella sp.* in the skin of *Clarias gariepinus*

4. Conclusion

From the current study, the African catfish (*Clarias gariepinus*) which is one of the most economically important fish species for successful aquaculture, is suffering from highly obvious problems due to parasitic infestation. The highest prevalence of the ectoparasitic infestation was (15.5%) in both autumn and winter, and (15%) in spring and summer season. Many ectoparasites could be isolated. *Dactylogyrus sp*., is the most dominant isolated ectoparasite (25.8%) with highest infestation rate in spring and summer, followed by *Gyrodactylus sp*., (17.8%) with highest infestation rate in autumn and winter, *Trichodina sp*., (10.8%) with highest infestation rate in autumn and summer, then *Chilodenella sp*., (6.8%) with highest infestation rate in autumn and winter.

5. Conflict of interests

The authors declare that there is no conflict of interests.

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