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STUDIES ON SOME PROBLEMS FACING CULTURED AFRICAN CATFISH (*CLARIAS GARIEPINUS*) IN ABBASSA FISH FARMS

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Abstract

A number of 440 diseased cultured African catfish *Clarias gariepinus* ranging from 50-250 g. were subjected to parasitological, bacteriological examination and correlation between some environmental factors and the incidence of *C. gariepinus* pathogens. Beside trials of treatment of naturally infected fish with *monogenea. Aeromonas hydrophila* and *Edwardseilla tarda*. The prevalence of obtained parasites reached up to 79%; monognetic trematode *(Gyrodactylus sp.*) was isolated with a prevalence of 25%, dignetic trematodes (*Oreintochridum batrachoidus)* 40%, cestodes *(Monobothrium chalmersius)* 32% and nematodes *(Procamallanus laeviconchus)* 33%. *A. hydrophila* was isolated from *C. gariepinus* with prevalence 6.5% and *E. tarda* 5.5%. The total mortality rates among injected *C. gariepinus* were 90%, 50%, 0 % and 0% respectively in *A. hydrophila, E. tarda* and control groups. The histopathological changes of parasitic diseases showed mucinous degeneration, desquamation and necrosis of intestinal epithelium and bacterial infection showed congestion and hemorrhages in kidney, liver and spleen. Enrofloxacin application according to antibiogram sensitivity test, showed good recovery of infected *C. gariepinus* with *A. hydrophila* and *E. tarda*. Monogenea were found dead in all treated *C. gariepinus* after two successive days with salt 0.5 %.There was a direct effect of low oxygen and high pH values as well as high ammonia levels on abundance of monogenea, digenea and incidence of *A. hydrophila* and *E. tarda*

infection in cultured *C. gariepinus* at earthen ponds and in drainage canals.

INTRODUCTION

Severe mortalities of fingerlings due to monogenean infestations have been reported in cultured *C. gariepinus*. Trematodes, cestode and nematode infestations in *C. gariepinus* under natural conditions are very common (Mashego 1977 and Mashego and Saayman, 1980). Fish diseases caused by Aeromonads considered being the major bacterial problems facing the aquaculture development causing mass mortalities, reduced production and low quality of aquatic organisms (Gamal *et al.*, 2002). Numerous reports describing the isolation of *Edwardsiella* from mammals, birds, reptiles, amphibians, marine and freshwater life. (Janda and Abbott, 1998). The successful treatment of diseased fish is one of the most important aspects influencing the success of any aquaculture enterprise. Thus, the aim of the present study was to address the following objectives: - Studying the seasonal prevalence of parasitic and bacterial diseases of cultured *C. gariepinus*, Studying the possible using of chemical and pharmatherapeutical in treatment of obtained parasitic and bacterial diseases of cultured *C. gariepinus*.

MATERIAL AND METHODS

A fish total number of 440 diseased African catfish *C. gariepinus* ranging from 50-250 g. were collected during the period from December 2006 to December 2007 from different localities in Abbassa fish farms. Fish were subjected to full clinical and laboratory examination for infectious parasitic and bacterial diseases.

Water samples:

All water quality analysis was carried out according to A.P.H.A. (1985)**,** to detect different water parameters.

Clinical and postmortem examinations:

Clinical and p re performed usi method described by Schaperclaus *et al*. (1992).

Parasitological examination:

Skin, gill and gut examination of fish was carried out according to Langdon and Jones (2002).

Bacteriological examination:

Under aseptic condition loopfuls from diseased fish were incubated over night at 27 °C into nutrient broth and Selenite-F broth for 24 hours. Loopfuls from broth were subcultured again onto (R-S agar for *Aeromonas hydrophila* and S-S agar for *Edwardseilla tarda*) for purity and maintained for further investigation according to Austin and Austin (1999). Gram stained films from purified isolates were made and examined microscopically for detecting their stain reaction and morphological characters.

Biochemical identification: Suspected purified isolates were identified according to Barbara *et al.* (1993).

Pathogenicity test for the isolated bacteria (Experimental infection):

It was performed according to Austin and Austin (1999). The reisolation of bacteria was performed according to Austin and Austin (1999). Samples for histopathological examination were performed according to El-Bouhy (1986).

Antibiogram sensitivity test:

Drug sensitivity of the bacterial isolates was carried out according to the criteria given by Bio-Merieux (1984), using the disc diffusion method.

Histopathological examination:

Parts of kidney, liver, intestine, spleen and other affected organs of natural infected *C. gariepinus* were examined according to Alagappan *et al.* (2009).

Treatment of *hydrophila* and *E. tarda* infection using the medical ration according to Josphas Fouric (2006):

Enrofloxacin (10 mg/kg fish body weight) were added to ration of *C. gariepinus* infected with *A. hydrophila,*and *E. tarda* and fed to the fish for 10 consecutive days.

Treatment of monognea infestation using Sodium chloride (Natural salt) according to Josphas Fouric (2006):

This was used in rate of 0.5% as an environmentally safe treatment for *C. gariepinus* kept in small water bodies such as aquaria &fiber glasses.

RESULTS AND DISCUSSION

Parasitological examinations:

Clinical and postmortem examination:

Clinical manifestation of infested *C. gariepinus* with *Gyrodactylus sp*. revealed skin erosion with local hemorrhagic lesions (Fig. 1). These signs were similar to that reported by Abo-Esa (2008). The internal organs of naturally infested *C. gariepinus* with enteric parasites revealed pale or hemorrhagic internal organs. These signs were similar to that reported by Bassiony (2002) and Heba (2005).

Microscopical examination:

The general morphological characteristics of monogenetic *Gyrodactylus* showed flatworm with one pair of projection at its anterior pole (Fig.2). This morphological identification was typical to that reported by Paperna (1996) and Abo-Esa (2008). The general morphological characteristics of *Orientocreadium batrachoides* showed

flattened parasite characterized by presence of two suckers, oral (anterior) sucker and ventral on ings agreed with described by El-Naggar (1995); Gihan (1999) and Heba (2005). Microscopic smears intestine showed an elongated body of one segment, fusiform in shape. This parasite was identified as *Monobothriodes Chalmersius* (Fig. 3). The same parasite was recorded by Rawia (2000) and Bassiony (2002). Microscopic smears from intestine showed a small larviparous worm with cuticle. The male was usually smaller than female. The female tail was conical in shape and the uterus of full mature was filled with larvae (Fig. 4). This nematode was belonging to family: Camallauidae, *species: Procammalanus laeviconchus*. Description of *Procamallanus laeviconchus* was in close agreement with those reported by Eman (2001) and Heba (2005).

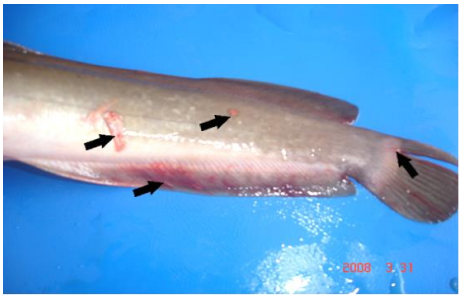
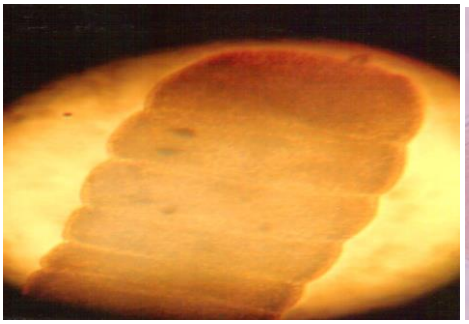


Fig. 1. Showed *C. gariepinus* infested with *gyrodactyllus sp*. in which skin erosion with local hemorrhagic lesions.

Fig.2. Showed *Macrogyrodactyllus Clarii* (direct mount) from skin and gill × 100.



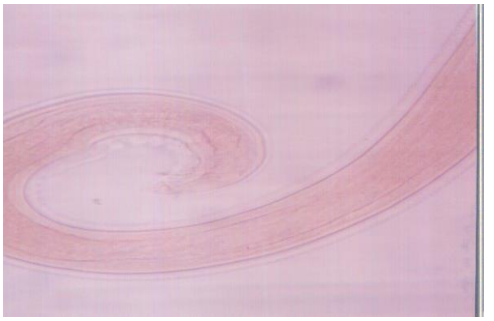


Fig. 3. Showed Monobothriodes chalmersius (Anteriorend (direct mount) × 150

Fig. 4. Showed male Procamallanus laeviconchus × 400.

Prevalence of parasitic infestations:

As shown in (Table 1), the infestation rate of helminth parasites for the examined *C. gariepinus* reached up to 79% and showed significant incidence with trematode infestation (monogenea 25%& Digenea 40%), cestodes (32%) and nematodes (33%). The highest peak was during spring (86%), followed by summer (82%), autumn (78%) and the lowest (70%) was recorded in winter. These results nearly agreed with that given by Gihan (1999) and Heba (2005). On the other hand, these results disagreed with that reported by Hassan (1992) and El-Seify *et al.* (1997).

The infestation rate of the examined *C. gariepinus* with monogenean *Gyrodactylus sp*. reached to 25% and showed a significant seasonal incidence ($p \leq 0.05$). This result agreed with that reported by Abo-Esa (2008). Also, a higher average (100%) was recorded by Paperna (1996) in Nile catfish. These variations in results might be attributed to the inhibitive quality of physical (depth, current, temperature) and chemical (Oxygen, salinities) factors of the environment and fish species. Digntic trematode *(Oreintochridum batrachoides)* infestation showed a significant seasonal incidence ($p \leq 0.05$). These results were in partial agreement with those reported by Negm El-Din *et al.* (1988); and Gihan (1999) and were similar to those recorded by Heba (2005). While disagreed with those recorded by El-Seify *et al.* (1997), he mentioned that the highest infestation of digenea was in summer while the lowest rate was in spring or autumn. Cestodes (*Monobothroides Chalmersius)* infestation showed a significant seasonal incidence ($p \leq 0.05$). This result agreed partially with that reported by Gihan (1999) and Bassiony (2002) and was similar to those recorded by Heba (2005) while disagreed with

that reported by El-Seify *et al.* (1997), who recorded that the highest infestation was in winter followed by spring, summer and autumn. Nematodes *(Procamallanus laeviconchus)* infestation showed a significant seasonal incidence ($p \leq 0.05$). These results nearly agreed with those recorded by Abd E1-Aal (1996) and was similar to those recorded by Heba (2005) While disagree with those recorded by El-Seify *et al.* (1997) and Gihan (1999).

Table (5): Seasonal incidence of detected helminth parasites among examined African catfish *Clarias gariepinus*.

Season	Total no. of examined fish	Total no. of infected fish	% of infestation	Parasitic isolates							
				Trematodes				Cestode		Nematode	
				Gyrodactyus clarias		*Orientocreadium baracholdes*		*Monobothroides Chalmersius*		*Procammalanus laeviconchus*	
				No	%	No	%	No	%	No	%
Winter	100	70	70	0	0	17	17	46	46	12	12
Spring	100	86	86	20	20	67	67	27	27	42	42
Summer	100	82	82	80	80	41	41	17	17	58	58
Autumn	100	78	78	0	0	35	35	38	38	20	20
Total	400	316	79	100	25	160	40	128	32	132	33
χ^2 values.				229.33**		53.5**		22.15**		59.52**	

**There is highly significant differences at p ≤ 0.05.

Bacteriological examination:

Clinical signs and postmortem lesions:

C. gariepinus with *A. hydrophila* infection showed darkness & hyperemic skin (Fig.5). Ulcers on skin varied from shallow to deep necrotizing ulcers, fin erosions, inflamed vent, exophthalmia, abdominal distension and fin & tail rot. The postmortem findings ranged from congested to pale liver, spleen, kidney and gall bladder. The observed clinical signs and post mortem changes in examined *C. gariepinus* suffering from Motile Aeromonas Septicemia (MAS) were previously reported by Abdel-Hadi, (2004) and Alagappan *et al.* (2009). The clinical signs of infected *C. gariepinus* with *E. tarda* showed congestion, hemorrhage allover fish body especially at base of fin, operculum and belly and fin and tail rot. Boil like on the top of the head, (old ulceration) in posterolateral skin that lead to abscess or ulcer after several weeks or months following infection (Fig.6). Similar clinical signs and postmortem lesions were described by Newton *et al.* (1988); Meyer and Bullock (1973); Maha (2000) and Heidy and Mai (2009).

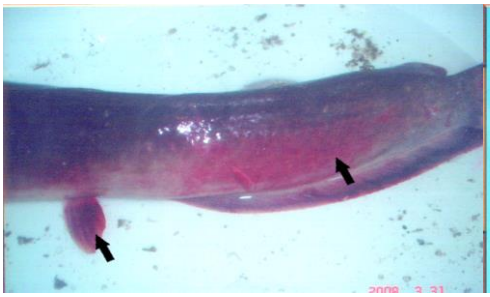


Figure (5): Showed *C. gariepinus* infected with *A. hydrophila* in which skin hyperemia.

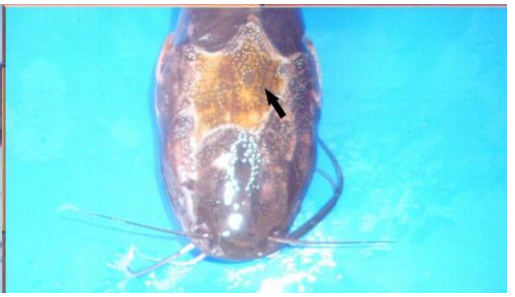


Figure (6): Showed *C. Gariepinus* infected with *E. tard*a in which old ulceration.

Prevalence of the isolated bacteria:

As shown in (Table 2), *A. Hydrophila* was isolated with a prevalence of (6.5%) and showed significant seasonal incidence ($p \leq 0.05$). These results were in agreement with those of Meyer (1970) who found high densities of motile aeromonads within the environment during midsummer when sedimentary chlorophyll and water temperature were highest. However, this finding disagreed with those of Plumb, (1994) who reported that *A. hydrophila* was common in spring and autumn where stress occurs due to low oxygen, sudden changes in water temperature, and handling. The results also disagreed with that of Cipriano (2001) and Abdel-Hadi (2004) who reported that outbreaks of MAS occurred mainly during winter in cultured fish. This could be attributed to the suppressed immunity of the cultured fishes caused by cold weather and low water temperature, which most warm water fishes couldn't tolerate, rendering fishes more vulnerable for different disease agents. Higher prevalence of *A. hydrophila* was recorded by Enany et al. (1995); 43.38 % and Diab *et al.* (2006 b); 45%.Concerning *E. tarda*, was isolated with a prevalence of (5.5%) and showed significant seasonal incidence ($p \leq 0.05$). Similar seasonal prevalence was recorded by Meyer and Bullock (1973) and Heidy and Mai (2009). Higher incidence of *E. tarda* was recorded by Wyatt *et al.* (1979); 64%& VanDamme and Vandepitte (1980); 59% & Eissa and Yassien (1994); 21.87% to 30.70% with an average of 24.93%. Lower incidence of *E. tarda* was recorded by Heidy and Mai (2009), with a recovery rate of 0.42%.

Pathogenicity test:

The total mortality rates among the injected *C. gariepinus* were 90%, 50%, 0 % and 0% in *A. hydrophila, E. tarda* and control groups (1&2) respectively. The clinical behavioral abnormalities of injected *C. gariepinus* with *A. hydrophila,* and *E. tarda*, occurred 24 hours up to 15day post-inoculation included; poor appetite, dullness, sluggish

swimming and loss of reflexes prior to death. The most common clinical signs of injected *C. gariepinus* with *A. hydrophila* were hemorrhages allover fish body and at the site of injection, skin darkening and hemorrhagic protruded vent. As the disease progress, there was exophthalmia as well as abdominal dropsy. The postmortem finding showed red or hemorrhagic patches at the opercula & base of pectoral fins, congested livers, kidneys, spleens and gills. Similar results were recorded by Abo El-Attah (2003); Abdel- Hadi (2004) and Heidy and Mai (2009). Concerning clinical signs of injected *C. gariepinus* with *E. tarda*, were congestion of the fins and petechial hemorrhages all over the body surfaces. The postmortem finding showed hemorrhagic enteritis while the abdominal wall and muscle showed sever hemorrhage and inflammation. Yellowish ascetic exudates filled with abdominal cavity which discharged at abdominal incision with characteristic putrid odeur. Similar results were recorded by Maha (2000) and Heidy and Mai (2009).

Antibiogram sensitivity test:

As shown in table (3), susceptibility patterns of *A. hydrophila* isolates were found to be sensitive to enrofloxacin, gentamicin, ciprofloxacin, neomycin and trimethoprimsulphamethoxazole and resistant to ampicillin, amoxicillin, oxytetracycline and streptomycin.

Table (2): Seasonal incidence of bacterial diseases among examined African catfish *(Clarias gariepinus)*.

Season	Total no. of examined *C. gariepinus*	No. of infected *C. gariepinus* with *A. hydrophila*	No. of infected *C. gariepinus* with *E. tarda*
Winter	100	0	0
Spring	100	8	7
Summr	100	13	12
Autmn	100	5	3
Total	400	26	22
Percent in relation to the total examined number		6.5	5.5
$\chi 2$ values.		14.64**	15.58**

** : There is highly significant differences at $p \leq 0.05$.

Table (3): Results of antibiogram sensitivity test.

Antibiotic common name	*A. hydrophilla*	*E. tarda*
Amoxicillin (AMX)	-	+ + +
Ampicillin (AM)	-	+ + +
Chloramphenicol (C)	+ +	+ + +
Enrofloxacin (ENR)	+ + +	+ + +
Gentamicin (GE)	+ +	+ + +
Nalidixic acid (NA)	+ + + +	+ + +
Neomycin (N)	+	+ + +
Oxytetracycline (OT)	-	-
Streptomycin (S)	+ +	+ + +
Sulphamethoxazole+trimethoprim, (SXT)	+ +	+ + +

-/+/+ + = Resistant + + + = Intermediate + + + + = Sensitive

Biochemical identification:

Table (4): Biochemical identification of isolated *A. hydrophila* and *Edwardseilla tarda*.

Test	Reaction	
	Aeromones hydrophila	***Edwardseilla tarda***
Motility test	+	+
Gram stainig	-	-
Gelatin liquefaction	+	-
Oxidase	+	-
O/F	F	-
Growth on 5% Na Cl	-	-
Indol	+	+
V.P	+	-
Methyl red	+	+
H2S	-	+
Catalase	+	+
Citrate reduction	+	-
citrate utilization	+	-
Arginin hydrolysis	+	-
Fermentation of glucose	+	+
Fermentation of sacrose	+	-
Fermentation of lactose	-	-
Fermentation of maltose	+	+
Fermentation of galactose	+	-
Fermentation of faractose	+	-
Fermentation of trehlose	+	-

Similar results were recorded by Cipriano (2001) and Abdel -Hadi (2004).

The susceptibility patterns of *Edwardsiella tarda* isolates were found to be resistant to oxytetracycline although they were found sensitive to the remaining antibiotics test. This result is in accordance to that of Shotts and Waltman (1990) and Maha (2000). In contrast, Meyer and BulIok (1973) recorded that, oxytetracycline controlled *Edwardsiellosis.*

Histopathological alterations:

Histopathology of parasitic infestations:

Microscopical examination of intestine of *C. gariepinus* infested with *Orientocreadium batrachoides* showed mucinous degeneration, desquamation and necrosis of intestinal epithelium (Fig. 7). Regarding intestine of *C. gariepinus*, infested with *Monobothroides Chalmersius* showed longitudinal section of parasites between intestinal folds together with desquamation, erosion and necrosis of the epithelial cells lining intestinal villi (Fig. 8). Intestine of *C. gariepinus* infested with *Procamallanus laeviconchus* revealed catarrhal enteritis. This result was in aggrement with Rawia (2000) and Heba (2005).

Histopathology of bacterial infection:

The histopathological changes of *C. gariepinus* infected with *A. hydrophila* revealed congestion and hemorrhages in liver (Fig. 9), depletion of spleen. These results were supported by those recorded by Cipriano (2001) and Alagappan *et al.* (2009). Concerning *Edwardseillosis* characterized by condensation of glomoruli and odema of bowmans' capsule of kidny (Fig. 10) and degenerative changes of muscle (necrotic muscle and myofibers) over the posterior half of the body. These results were supported by those recorded by Rashid *et al.* (1997) and Jin *et al.* (2009).

Treatment trials:

Treatment trials of bacterial infections using the medical ration containing the Antibiotic of choice according to the sensitivity test:- Enrofloxacin showed good recovery of treated *C. gariepinus* when applied as soon as possible. Similar results were recorded by Abdel – Hadi *et al.* (2004) and Josephes (2006).

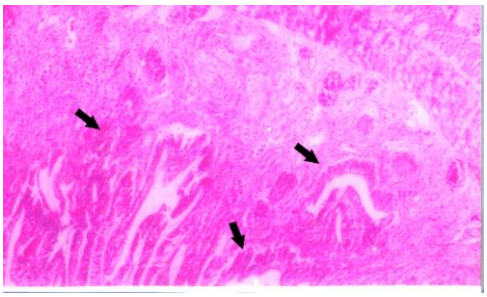


Figure (7): Showed intestine of *C. Gariepinus* infested with digenetic trematode in which mucinous degeneration, desquamation and necrosis of the intestinal epithelium (H&E) ×100.

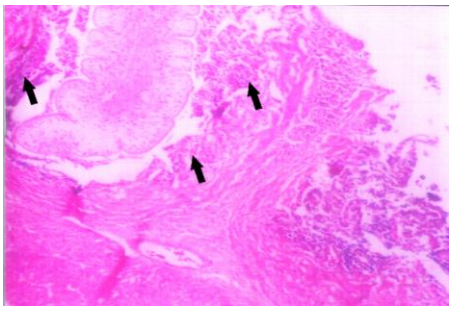


Figure (8): Showed intestine of *C. gariepinus* infested with *Monobothroides chalmercius* in which necrosis of intestinal epithelium (H&E) ×100

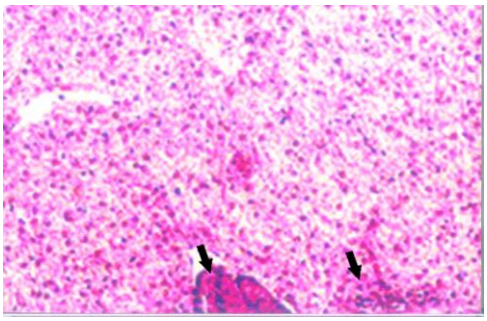


Figure (9): Showed liver of *C. gariepinus* infected with *A. hydrophila*, in which congestion, engorged blood vessels (H&E) ×20.

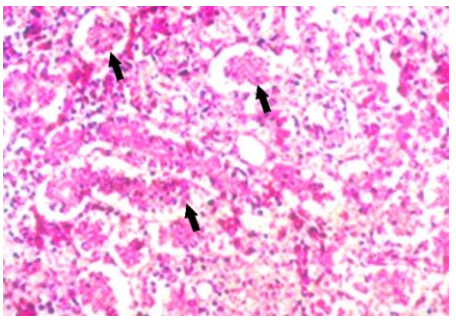


Figure (10): Showed kidny of *C. gariepinus* infected with *E. tarda* in which condensation of glomoruli and odema of bowmans' capsule (H&E) ×20.

Treatment with salt 0.5 % (5 kg/m3 of water) as indefinite treatment: results showed that, monogenea were found dead in all treated fish after twice treatment (for 2 successive days or day after day). Similar results were recorded by Abdel –Hadi (2004) and Josephes (2006).

Water quality examination & the correlation between some environmental factors and the prevalence of some fish pathogens:

The most important recorded water quality parameterswere water temperature; (26.92 ± 0.55), dissolved oxygen (3.77 ± 0.22 mg/l), pH value (7.71 ± 0.16), ammonia (0.81 ± 0.10 mg/l), and nitrite (0.05 ± 0.004 mg/l0) (Table 10). There was a direct effect low oxygen and high pH values as well as high ammonia levels on abundance of monogenea sp. and digenea in the cultured *C. gariepinus* at earthen ponds and in drainage canals. These results were supported with those recorded by Noga (1996). A similar correlation was also found between low oxygen levels, high pH values together with high ammonia levels as well as excessive stocking densities and incidence of *A. hydrophila* and *E. tarda* of *C. gariepinus sp*. This oxygen depletion acted as a severe stress factor on the fish leading to exaggerating the pathogenic effect of the bacterial isolates. These results were supported by Greenlees *et al.* (1998) and Abdel -Hadi (2004).

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دراسات على بعض المشكلات التي تواجه استزراع القرموط الأفريقي المستزرع في مصر

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تم فحص عدد (440) سمكة من القرموط الأفريقي المستزرع (كلاريـاس جاريبنس) في مزارع العباسة في الفترة من ديسمبر عام ٢٠٠٦ إلى ديسمبر عام٢٠٠٧ . وقد خضعت هذه الأسماك للفحص الإكلينيكي والمعملي لتحديد الأمراض الطفيلية والبكتيرية خلال مختلف فصول العام وقد تلخصت نتائج هذا الفحص الإكلينيكي والمعملي في الاتى:

١ – كانت معدل الإصابة بالطفيليات الخارجية والداخلية ٧٩ %. وكانت نسبة الإصابة بطفيل الجيروداكتيلس كلاريـاس ٢٥%، طفيـل الاورينتكريـدايوم باتراكويـدس ٤٠%، مونوبوثرويـدس كلاميرسياس ٣٢% وطفيل البروكملانس ليفينكو نكاس ٣٣% .

٢– الأمراض البكتيرية التي تم تشخيصها هي الايروموناس هيدرو فيلا والادوار دسيلا تاردا، وكانت نسبة الاصابة بالايروموناس هيدرو فيلا ٦.٥% والادوار دسيلا تاردا ٥.٥%.

٣– اشتملت التغيرات الباثولوجية للأمراض الطفيلية على زيادة فى الافرازات المخاطية مع تساقط وموت خلوى للخلايا الطلائية لجدار الامعاء. بالنسبة للأمراض البكتيرية فقد اشتملت على احتقان وانزفة في أنسجة الطحال والكبد والكلية.

٤– أوضحت النتائج أن هناك علاقة وثيقة بين وجود خلل فى مقاييس جودة الماء (انخفاض نسبة الأكسجين الذائب في الماء ، ارتفاع نسبة الامونيا والنيريت والأس الهيدروجيني) و حدوث كل من الأمراض الطفيلية والبكتيرية.