Fish Diseases, Dept. Animal Med.,
Faculty of Vet. Med., Assiut University.

BACTERIAL HAEMORRHAGIC SEPTICAEMIA IN OREOCHROMIS NILOTICUS AT ASWAN FISH HATCHERIES

(With 6 Tables and 8 Figures)

By

Sh.M. AHMED and A.A.M.SHOREIT*

*: Dept. of Botany, Faculty of Science, Assiut Univ.

(Received at 17/3/2001)

مرض التسمم الدموي البكتيري في أسماك البلطي النيلي بمفرخات الأسمـــاك في محافظة أسوان.

شعبان محمد أحمد ، أحمد شريت

استهدف البحث توضيح ظاهرة الأنزفة الدموية التي ظهرت على أسـماك البلطـي النيلـي بمفرخات الأسمـاك في محافظة أسـوان. أشتبه في مرض التسمم الدمـوي البكتـيري بناءا على الأعراض الإكلينيكية والصفات التشريحية لبعض الأسـماك المريضـة. أظـهر الفحص البكتيري لعينات الأسماك المختبرة (٥٠ عينة) عن إصابة الأسماك بعترات مختلفـة من بكتريا الايروموناس والسدوموناس. أظهر الفحص الطفيلـي إصابـة الأسـماك ببعـض الطفيليات الداخلية والخارجية. كان أوليات الاكثيوفثـيريس وديـدان الدكتيلوحـيرس هـما السائدان، بالإضافة إلى وجود قمل السمك و بعض البرقات المتحوصلة. تم دراسـة بعـض العوامل المهيأة لحدوث الإصابة بالمرض المذكور. باجراء العدوى التجريبية بالميكروبـات المعزولة اتضح أن عترات مجموعة الايروموناس أشد ضراوة في إحـداث المـرض مـن مجموعة السدوموناس. واتضح أيضا من نتائج العـدوى التجريبيـة أن عـترة الاريمونـاس هيدروفيلا كانت أشد ضراوة من العترات الأخرى من نفس المجموعة لأنها أدت إلى نفـوق كل الأسماك المعدية خلال خمسة أيام من الحقن. وكذلك أسفرت العدوى التجريبية أن عـترة السدوموناس فلوروسنس كانت أكثر ضراوة (٦٠ % نفوق) من عترات السدوموناس الأخرى. أشارت نتائج اختبار الحساسـية بالمضـادات الحيويـة للعـترات المعزولـة أن مركبـي الأوكسيتيتراسيكلين والنيوميسين ذاتا تأثير مميت لجميع البكتريا المعزولة.

SUMMARY

Oreochromis, niloticus obtained from fish hatcheries in Aswan Governorate showed signs of septicemia. Bacterial haemorrhagic septicemia was suspected according to the clinical screening and necropsy findings. The bacteriological examination of the tested samples (n = 50 fish) yielded two groups of bacteria viz., aeromonads and pseudomonads. Parasitological examinations revealed that the examined fishes were infested by different types of parasites, in which *Ichthyophthirius multifiliis and Dactylogyrus spp.* were predominant. Also, *Argulus spp.* and metacercariae were found. Predisposing factors that enhance the bacterial infection were also monitored. Experimental infection with the isolated bacterial strains in the apparently healthy fishes concluded that the motile aeromonads were more virulent than pseudomonads. *Aeromonas hydrophila* was the highest virulent strain (100 % mortalities within 5 days post inoculation) than the other strains of aeromonads, whereas, *Pseudomonas fluorescens* (60 % mortalities within 8 days post inoculation) was more virulent than other pseudomonads. In-vitro sensitivity test cleared that oxytetracycline and neomycin had strong inhibitory effect on all tested strains.

Key words: *Haemorrhagic septicaemia, Oreochromis niloticus.*

INTRODUCTION

Fish diseases caused by aeromonads and pseudomonads considered to be the major bacterial problems facing the aquaculture development causing mass mortalities, reduced production and low quality of aquatic organisms (Ghittino, 1976).

Motile aeromonads of the *Aeromonas hydrophila* complex cause a hemorrhagic septicaemia in fish (Bullock et al., 1971; Egusa, 1978; Cipriano et al., 1984 and Schäperclaus, 1992). This bacterium has been observed in numerous species of freshwater fish throughout the world (Aoki, 1999).

Aeromonas and *Pseudomonas species* are distributed widely in freshwater and bottom sediments containing organic materials, as well as the intestinal tract of fish (Aoki, 1974; Egusa, 1978; Hazen et al., 1978; Seidler et al., 1980; Kaper et al., 1981 and Sugita et al., 1994).

Pseudomonas fluorescens, *Pseudomonas chlororaphis*, *Pseudomonas putida* and *Pseudomonas anguilliseptica* were recognized as the causative agents of bacterial haemorrhagic septicaemia in different species of fish (Post, 1983; Ausin and Austin, 1987; Roberts, 1989 and Schaperclaus, 1992).

In Egypt *Aeromonas hydrophila* isolated from *Oreochromis niloticus, Cyprinus carpio, Mugil cephalus and Mormyrus kannume* which were naturally infected with hamorrhagic septicaemia (Amin and Abd-El Kerim, 1976; Eissa et al., 1990; Marzouk and Nawal, 1991; Badran and Eissa, 1991 and Ahmed et al., 1991). While *Pseudomonas fluorescens* was isolated from *Clarias lazera* and *O. niloticus* (Marzouk et al., 1989; Badran and Eissa, 1991).

Environmental stresses including overcrowding, high water temperature, rough handling, transportation of fish, low dissolved oxygen, nutritional deficiency and the ectocommansal fungal or parasitic organisms contributed to physiological changes and increased susceptibility of the fish to infection with motile aeromonads and pseudomonads (Wedemeyer, 1970; Post, 1983; Noga, 1996 and Aoki, 1999).

Reviewing the available literature declared that there is no record about bacterial haemorrhagic septicaemia among fish's hatcheries at Aswan Governorate-Egypt. Consequently, the present work was carried out to describe the clinical signs and gross lesions of that disease in *Oreochromis niloticus'* hatcheries, isolation and identification of the causative agents, experimental trial for induction of the disease in such fish and in-vitro sensitivity tests of the isolated bacteria against different members of antibiotics were also achieved.

MATERIAL and METHODS

Fish:

a- Naturally infected fish:

Fifty clinically diseased *Oreochromis niloticus* samples with body weight ranged from 500 to 1000 g were collected from fish hatcheries at Aswan Governorate. The samples were brought to the laboratory in ice box and the following examinations were carried out:

b- Experimental fish:

Oreochromis niloticus with an average body weight of (30±3 g) were collected from River Nile tributaries, at Assiut and brought to the laboratory. Fish were kept in glass aquaria for three weeks for

acclimatization and for further pathogenicity tests. Random samples were subjected to bacteriological and parasitological examinations to exclude their natural infection.

1-Clinical and postmortem findings:

The infected fish were thoroughly examined. The external and internal gross lesions were recorded according to the methods described by Schäperclaus (1992) and Stoskopf (1993).

2-Bacteriological examination:

Swabs from skin lesions; gills, liver, spleen and kidney were taken aseptically and inoculated into brain heart infusion broth. The inoculated tubes were incubated at 22°C for 48 hours. Each tube with a positive growth was streaked onto blood agar medium and incubated at 22°C for 48 hours. Two or three isolates from each agar plates were subsequently purified by subculturing and identified biochemically according to Allen et al. (1983) and Popoff (1984). Purified isolates were maintained at –30 C with 15% (v/v) glycerol.

3-Parasitological examination:

Skin, gills, internal organs and intestinal tract of infected fish were examined for parasitic infestation by naked eye and microscopically by direct smear according to Kabata (1985).

Experimental infection:

Seventy fish of apparently healthy *Oreochromis niloticus* were divided into seven equal groups (each group have 10 fish). Six groups were inoculated intraperitonealy, each one of the isolated strains (*A. hydrophila*, *A. caviae*, *A. sobria*, *P. fluorescens*, *P.aeruginosa* and *P. putida*). These isolates were isolated from internal organs of the naturally infected fish. The inoculum was composed of 0.5 ml broth culture containing 2 x 10^{10} colony forming units. The last group was inoculated with 0.5 ml sterile broth as a control group. The inoculated fish used in the experiments were kept under observation for two weeks in glass aquaria at 19 ± 1 C. Air pumps were employed for aeration.

5-In-vitro drug sensitivity test:

Drug sensitivity test for the isolated *Aeromonas* and *Pseudomonas spp.* were applied by different types of antibiotics and sulpha discs according to Amtsberg *et al.* (1973). The interpretation of inhibition zone was estimated according to the limits given by Finegold and Martin (1982).

RESULTS

Clinical findings and postmortem lesions:

The clinical sings, postmortem lesions and their prevalence in naturally infected fish were summarized in Table (1).

Bacteriological examination:

Primary isolation revealed that 48 isolates were recovered from skin, gills, liver and kidney of the naturally diseased fish. The complete morphological and biochemical identification proved the isolation of 8 *Aeromonas hydrophila*, 6 *Aeromonas caviae*, 6 *Aeromonas sobria*, 4 *Pseudomonas aeruginosa*, 16 *Pseudomonas fluorescens* and 8 *Pseudomonas putida*. Tables (2 and 3).

Parasitological examinations:

Examinations of the skin, gills, fins and abdominal cavities of the naturally infected fish for parasitic infestations by naked-eye and direct smear revealed that the diseased fish were infested with *Ichthyophthirius multifiliis*, *Dactylogyrus spp.*, *Argulus spp.* (Photo 8) and encysted metacercaria. The percentage of the infested fish and parasitic habitats in the fish body were illustrated in Table (4).

Pathogenicity test:

The results of experimental inoculation of *Oreochromis niloticus* with different bacterial strains isolated from naturally infected fish indicated that the mortality rate of fish ranged from 40% to 100% in different groups within 8 days postinoculation. However the time elapsed for death in each group was varied (Table 5). The clinical signs and postmortem lesions of the hemorrahgic character were remarkable in fish experimentally infected by *Aeromonas* isolates. The inoculated isolates were re-isolated from the internal organs of the experimentally infected fishes.

In-vitro antibiotic sensitivity test:

Drug sensitivity test revealed that the tested isolates were in-vitro sensitive to oxytetracycline, neomycin, netilmicin and chloramphenicol (Table 6).

DISCUSSION

The presence of obvious diffuse hemorrhages on the body surface of the fish in association with congestion of gills, petechial hemorrhages on visceral organs and marked bilateral ocular cloudiness (Table 1) of the clinically examined fishes (*O. niloticus*) suggested the presence of bacterial haemorrhagic septicemia. Such suggestion was

confirmed by the bacteriological isolation of different *Aeromonas* and *Pseudomonas species*.

Austin and Austin (1987), Shepherd and Bromage (1988), Roberts (1989), Schaperclaus (1992), Plumb (1994) and Aoki (1999) concluded that aeromonads and pseudomonads bacteria were frequently incriminated as principle etiologic pathogens responsible for septicemia picture in different species of fish including *O. niloticus*.

Results of the experimental inoculation (Table 5) proved that the isolated *Aeromonas species* were comparatively more virulent than *Pseudomonas species*. Within motile aeromonads group, *A. hydrophila* was more virulent (100% mortality of the inoculated fish). On the other hand, *P. fluorescens* (60% mortality) was found to be more virulent than other tested pseudomonads strains. Wakabayashi et al. (1981), Nieto et al. (1984) and Santos et al. (1987) revealed that there were significant variations in virulence within the aeromonads and pseudomonads groups based on the mortality rate of inoculated fish. Such variations mainly associated with difference in the cellular surface characteristics of inoculated isolate (Mittal et al., 1980). Moreover, Marzouk and Nawal (1991) reported that extra-cellular substances produced by *A. hydrophila* played an outstanding role in virulence and the pathogenesis of the disease.

The frequent isolation of *Aeromonas* and *Pseudomonas species* from diseased fish may suggest that the respective fish in hatcheries were probably under stresses. The obtained results listed in table (4) revealed that 100% and 80% of the examined fish were parasitically infested with the ciliated protozoa, *Ichthyophthirius multifiliis* and the monogentic trematode, *Dactylogyrus species* respectively. Such parasites were encountered as a good predisposing factor for aeromonad and pseudomonad infections. On the other hand, the case history of rough handling during catching and transportation of the fishes from Lake Nasser to fish hatcheries ponds should not also be neglected as assisting factors for bacterial infections.

Overcrowding, transportation and heavy infestation with parasites increased the susceptibility of the fish to infection with motile aeromonads and pseudomonads (Bullock et al., 1971; Post, 1983; Austin and Austin, 1987 and Aoki, 1999). Moreover, Badran and Eissa (1991) concluded that the fish should be tested for *A. hydrophila* infection before long transportation.

Results of drug senstivity test revealed that the isolated *Aeromonas* and *Pseudomonas species* were highly sensitive to

oxytetracycline, neomycin, netilmicin and chloramphenicol (Table 6). Such results coincided with the results obtained by Eissa et al. (1990) who reported that oxytetracycline was the most effective compound on the *A. hydrophila*. On the other hand, *P. aeruginosa* and *P. fluorescens* were sensitive to tetracycline and chloramphenicol (Fernandez et al., 1990). Consequently, it is suggested that the aforementioned drugs can be used as treatment of the aeromonads and pseudomomads infection.

From the result of the present work, it can conclude that, once a septicemia picture in *O. niloticus* appeared, aeromonads and / or pseudomonads infections should primarily be suspected. However, from clinical point of view, differentiation between both infections seems to be difficult and the definitive diagnosis depends mainly on the bacteriological examination. Proper handling of fish during transportation and treatment of parasitic infestations are necessary before introducing the fish coming from natural resources for spawning to hatcheries ponds.

REFERENCES

Ahmed, Sh.M.; Zaitoun, A.M. and Ali, H.S.(1991): Motile Aeromonas septicemia (MAS) in *Morymrus kannume* at Assiut Governorate. Assiut Veterinary Medical Journal V.25, 49, 145-151.

Allen, D.A.; Austin, B. and Colwell, R.R. (1983): Numerical taxonomy of bacterial isolates associated with a freshwater fishery. J. Of general Microbiology, 129: 2043-2062.

Amin, N.E. and Abd-El Kerim, S.E. (1976): Studies on the pathogenicity of *Aeromonas punctata* to the Common carp (*Cyprinus carpio L.*). 13th Arab Vet. Med. Congress. Cairo 13-18 Nov Pp43-48.

Amtsberg, G.; Krabisch, P. and Mattiesen, I. (1973): Ubersicht uber die Ergebnisse von Resistenzprufungen verschieder Bakterienatten bzw Gruppen in der veterinarmedizinischen bakterioloische Diagnostik.Tierart.Umsch.28:495-500.

Aoki, T. (1974): Studies of drug resistant bacteria isolated from water of carp-ponds and intestinal tracts of carp (in Japanese). Bulletin of Japanese Society of Scientific Fisheries, 40:247-254.

Aoki, T. (1999): Motile Aeromonads (*Aeromonas hydrophila*). In: Woo, P.T.K. and Bruno, D.W. (Eds.) Fish Diseases and Disorders, Vol.3: Viral, Bacterial and Fungal infections. CAB International.U.K. USA.pp.427-453.

Austin, B. and Austin, D.A. (1987): Bacterial Fish: Pathogens: Disease in Farmed and Wild fish. Ellis HORWOOD.Limited-England pp.250-260.

Badran, A.F. and Eissa, I.A.M.(1991): Studies on bacterial diseases among cultured freshwater fish (*Oreochromis nilolicus*) in relation to the incidence of bacterial pathogens at Ismailia Governorate. J. Egypt. Med.Ass. 51,4,837-847.

Bullock, G.L., Conroy, D.A. and SnieszKo, S.F. (1971): Septicemic diseases caused by motile aeromonads and pseudomonads. In: Snieszko, S.F. and Axelrod, H.R. (eds.) Diseases of Fishes. Book2A: Bacterial Diseases of fishes. TfH publications Neptune, New Jersy, pp. 21-41.

Cipriano, R.C.; Bullock, G.L. and Pyle, S.W. (1984): Aeromonas hydrophila and motile Aeromonas Septicemia of fish. Disease Leaflet68 United States. Department of the interior fish and Wildlife Service. Division of Fishery Research Washington, D.C.20240.

Eissa, I.A.M.: Badran, A.F. and Moustafa, M. (1990): An outbreak of redmouth disease among culture freshwater fishes in Ismailia Governorate. Alex.J.Vet.Sci.6, 2,109-116.

Egusa, S. (1978): Infectious Diseases of Fish (in Japanese). Kousisha Kouseikaku, Tokyo, pp.554.

Fernandez, A.I.G., Rodriguez, L.A. and Nieto, T.P. (1990): Characterizations of pseudomonas strains producing septicemia in rainbow trout cultured in the North-West of Spain. Bull. Eur. Ass. Fish Pathol. 10, 5, 133 – 137.

Finegold, S.M. and Martin, W.J. (1982): Diagnostic Microbiology. 6th Ed .The C.V.Mos by Company, U.S.A.

Ghittino, P. (1976): International aspects of disease control in aquaculture. FAO. Technical conference on aquaculture. Kyoto, Japan. 26 May-2 June.

Hazen, T.C., Flirmans, C.B., Hirsch, R.P. and Esch, G.W. (1978): Prevalence and distribution of *Aeromonas hydrophila* in the United States. Applied and Environmental Microbiology, 36: 731-738.

Kabata, Z. (1985): Parasites and disease of fish cultured in the tropics. 1st Ed. Tylor and Francis. London and Philadelphia.

Kaper, J.B.; Lockman, H.; Colwell, R. and Joseph, S.W. (1981): *Aeromonas hydrophila*, ecology and toxigenicity of isolates from an estuary. J. Of Applied Bacteriology. 50: 359-377.

Marzouk, M.S.; Eissa, I.A.M. and Moustafa, M. (1989): Contribution to tail and finrot disease in catfish (*Clarias lazera*). Zagazig Vet. Journal. 17,3,244-256.

Marzouk, M.S.M. and Nawal, M.A.Y. (1991): Some investigations on the pathogenic properties of *Aeromonas hydrophila* infecting cultured fish in Egypt. J. Egypt. Vet. Med. Ass.51, 1&2,137-152.

Mittal, K.R.; Lolonade, G.; Leblanc, D.; Oliver, G. and Lallier, R. (1980): *Aeromonas hydrophila* in rainbow trout: Relation between virulence and surface characteristics. Can.J.Microbiol. 26:1501-1503.

Nieto, T.P.; Toranzo, A.E.; Barja, J.L. (1984): Comparison between the bacterial flora associated with fingerling rainbow trout culture in two different hatcheries in the Northwest of span. Aquaculture, 42:193-206.

Noga, E.I. (1996): Fish Diseases. Diagnosis and Treatment. Mosby.Boston, Chicago.Newyork. London, Sydney, Tokyo.Pp141-146.

Plumb, J.A. (1994): Health maintenance of cultured fishes, Principal microbial Diseases. CRC. Boca Raton, Ann Arbor, London Tokyo. PP. 148 – 155.

Popoff, M. (1984): Genus III. *Aeromonas* kluyver and vanNiel 1936 In: Krieg, N.R (ed.) Bergey's manual of systemic bacteriology, Vol. 1. Williams and Wilkins, Baltimore, pp. 545-548.

Post, G.W. (1983): Textbook of fish health. T. F.H. publications, Inc. Ltd. PP.34-44.

Roberts, R.J. (1989): Fish pathology 2nd Ed. Bailliere Tindall. London, Philadelphia, Sydney Tokyo, Toronto pp. 300-301.

Santos, Y.; Toranzo, A.E.; Dopazo, C.P.; Nieto, T.P. and Barjo, J.I. (1987): Relationships among virulence for fish, enterotoxigenicity, and phenotypic characteristics of motile aeromonas. Aquaculture, 67:29-39.

Schaperclaus, W. (1992): Fish Diseases Vol.1. Akademic Verlag, Berlin pp.498-503.

Seidler, R.J.; Allen, D.A.; Lockman, H.; Colwell, R.R.; Joseph, S.W. and Daily, O.P. (1980): Isolation, enumeration and characterization of *Aeromonas* from polluted waters encountered in diving operations. Applied and Environmental Microbiology, 21: 864-868.

Shepherd, G.J. and Bromage, N.R. (1988): Intensive fish farming. BSP. Professional Books, Oxford, London, Edinburgh, Boston, Paloalto, Melbourne.

Stoskopf, K.M. (1993): Fish Medicine. W. B. saunders company. Harcoutr Brace. Jovaovish, Inc.

Sugita, H.; Nakamura, T.; Tanaka, K.; and Deguchi, Y. (1994): Identification of *Aeromonas species* isolated from freshwater fish with the micropate hybridization method. Applied and Environmental Microbiology, 66: 3036-3038.

Wakabayashi, H.; Kanai, K.; Hsu, T. and Egusa, S. (1981): Pathogenic activities of *Aeromonas hydrophila biovar hydrophila* (Chester) Popoff and Veron, 1976, to fishes. Fish Pathology, 15:319-325.

Wedemeyer, G. (1970): The role of stress in disease resistance of Fishes. In A Symposium on Diseases of fishes & Shellfish (Sneiszko, S.F., Editor) American Fisheries Society, Bethesdo, Maryland, pp30-33.

PHOTOGRAPHIC LEGENDS

Photo1: Diffuse hemorrhages on the skin surface of ***O. niloticus*** naturally infected by bacterial haemorrhagic septicemia.

Photo2 &3: Intensive hemorrhages at the head region and on the tail fins (***Photo 3***) of the infected fish, with remarkable ocular cloudiness.

Photo 4: Severe infestation with encysted metacercaria in the abdominal cavity of the examined fish.

Photo 5: The superficial blood vessels of the liver are engorged with blood.

Photo 6: Gall bladder is greatly distended.

Photo 7: Congestion of the intestinal tract and the intestinal lumen is filled with reddish fluid.

Photo 8: External parasite - Argulus spp (X 250).

Table (1): Clinical and post-mortem findings (n = 50). And their prevalence lesions in naturally infected *O. niloticus*

Organs	Clinical signs and Postmortem	Prevalence %
Skin	Diffuse haemorrhages on skin surface and more concentrated at mouth regions (Photo 1)	100%
Fins	All fins were congested and have finrot. Haemorrhages were obvious at the base of fins and also covered with mucus (Photo 3)	80%
Eyes	Cloudy and have complete opacity (Photo 2)	30%
Anal orifice	Inflammed and protruded	15%
Gills	Congested and covered with mucus. Pale or white patches on gill lamellae	70%
Abdominal cavity	Yellowish or reddish ascitic fluid. Filled with encysted metacercariae (Photo 4)	35%
		30%
Liver	Congested and swollen. Superficial blood vessels engorged with blood (Photo 5)	90%
Gall bladder	Distended with bile (Photo 6)	
Intestinal tract	Congested and filled with reddish fluid (Photo 7)	80%
		50%

Table (2) Biochemical characters of motile aeromonas group

Features	*A. hydrophila*	*A. caviae*	*A. sobria*
No. of isolates	8	6	6
Gram stain	-	-	-
Motility	+	+	+
Oxidase	+	+	+
O/F test	O/F	O/F	O/F
Catalase	+	+	+
Hemolysis on sheep blood agar 5%	+	+	+
Growth on Tryptone water	+	+	+
With 4% Nacl	+	+	+
With 5% Nacl	-	-	-
Growth on MacConkey	+	+	+
Growth at 5 to 37 C	+	+	+
Asculin hydrolysis	+	+	-
Gelatin hydrolysis	+	+	+
Starch	-	-	-
Urease	-	-	-
Indole	+	-	-
H_2S-production	+	-	+
Ariginine dihydrolase	+	+	-
Voges-Proskauer reaction	+	-	+
Gas from glucose	+	-	+
Production of acid from Glucose	+	+	+
Salicin	+	+	-
Trehalose	+	+	+
Mannitol	+	+	+
Arabinose	+	+	-
Lactose	-	-	-
Inositol	-	-	-

Table (3) Biochemical characters of Pseudomonas group.

Features	*P.aeroginosa*	*P.fluorescens*	*P.putida*
No. of isolates	4	16	8
Gram stain	-	-	-
Motility	+	+	+
Oxidase	+	+	+
Catalase	+	+	+
O/F test	O/-	O/-	O/-
Fluorescent pigments			
King`s A	+	-	-
King`B	+	+	+
Denitrification	+	+	-
Arginine dihydrolase	+	+	+
Starch hydrolysis	-	-	-
Gelatin hydrolysis	+	+	-
Lipase	+	+	-
Urease	+	+	-
Indole	-	-	-
Growth at 4°C	-	+	+
Growth at 41°C	+	-	-
Utilization of carbohydrates			
Glucose	+	-	+
Trehalose	-	+	-
Salicin	+	-	+
Maltose	+	-	-

Table (4) Parasitological examination of the examined fishes (n = 50).

Parasite	Site	% of infestation
Ichthyophthirus multifillis	Skin	100
	Fins	100
	Gills	100
Dactylogyrus spp	Skin	10
	Gills	70
Argulus spp.	Gills	6
	At the base of fins	20
Encysted metacercaria	Abdominal cavity	30

Table (5): Pathogenicity test

Isolated strains	No. of dead fish after inoculation			% of mortality rate
	1-2 days	3-5 days	6-8 days	
A. hydorphila	9	1	-	100
A. caviae	5	3	-	80
A. sobria	-	3	2	50
P. fluorescens	4	2	1	60
P. aeroginosa	1	3	-	40
P. putida	-	3	2	50
Sterile broth	No death occurred			-

Table (6) Drug sensitivity test of the tested isolates.

Antibiotic	*A. hydrophila*	*A. caviae*	*A. sobria*	*P. aeruginosa*	*P. fluorescens*	*P. putida*
Oxytetracycline 30 µg	HS	HS	HS	HS	HS	HS
Neomycin 10 µg	MS	HS	MS	HS	HS	HS
Netilmicin 10 µg	MS	HS	MS	HS	HS	HS
Chloramphenicol 30 µg	HS	SS	SS	SS	SS	SS
Streptomycin 10 µg	SS	MS	SS	MS	R	MS
Ampicillin 25 µg	R	R	R	R	R	R
Erythromycin 15 µg	MS	R	SS	R	R	R
Penicillin 10 IU	R	R	R	R	R	R
Sulfamethoxazole-trimethoprim 25 µg	MS	R	R	R	R	R

HS: Highly sensitive (+++) MS: Moderately sensitive (++)
SS: Slightly sensitive (+) R: resistant .

Photo (1)

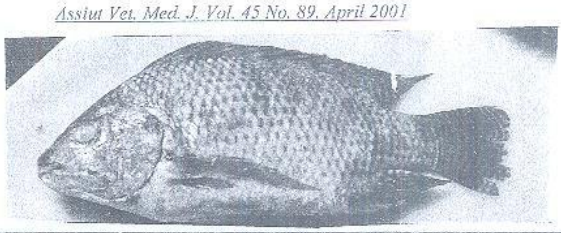

Photo (2)

Photo (3)

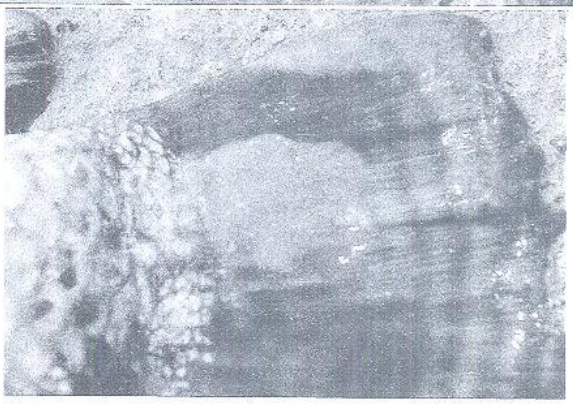

Photo (4)

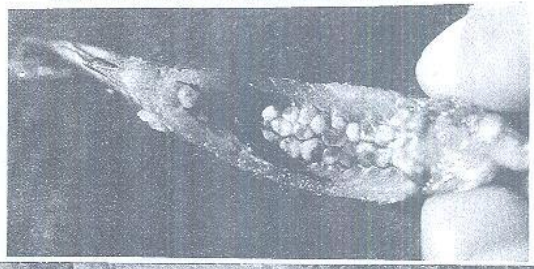

Photo (5)

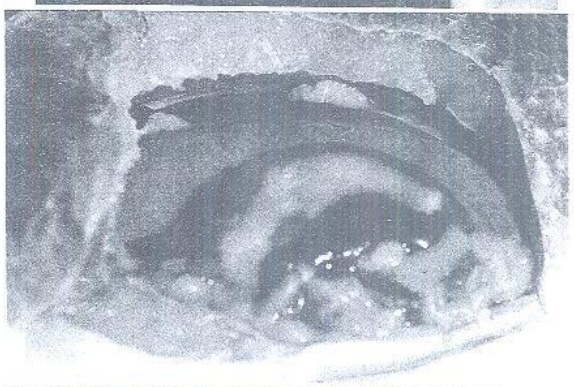

Photo (6)

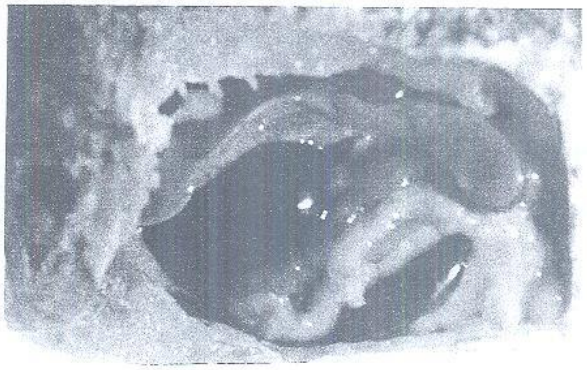

Photo (7)

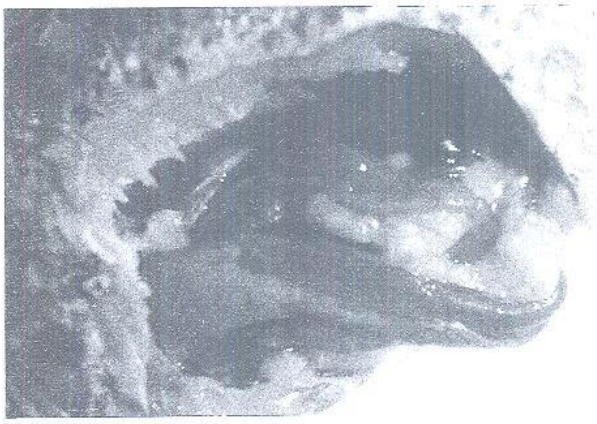

Photo (8)