Vet.Med.J.,Giza. Vol.44,No.1.(1996):11 - 22 .

STUDIES ON MOTILE AEROMONAS SEPTICAEMIA INFECTION IN CULTURED CARP IN EGYPT

SAMIA A. AHMED*, ROUFAYDAH M. A. MEGID**, M. S. MARZOUK*, M. MOUSTAFA* and M. GADO***,

* Dept. Int. Med., Infec. Dis. and Fish, Fac. Vet. Med. Cairo University, Cairo, Egypt.
**Dept. Microb. Fac. Pharma. Kasr El-Eini, Cairo University, Cairo, Egypt.
*** Dept. of fish Dis, Fac. Vet. Med Kafr El-Sheikh, Tanta University.

SUMMARY

The problem of Motile Aeromonas septicaemia (MAS) among common carp (Cyprinus carpio L.) and the toxicological characters of the extracellular products (ECPs) produced by Aeromonas hydrophila (A.hydrophila) organism were studied. Bacteriological examination revealed the isolation of ten A. hydrophila isolates which were identified into three virulent isolates to common carp while the rest of isolates were less virulent.

INTRODUCTION

A. hydrophila is one of the major important and serious opportunistic fish pathogens causing septicaemic conditions among a wide variety of aquatic animals (Trust and Sparrow 1974 and kaper et al., 1981).

Concerning the microbiological aspect, A. hydrophila has a wide variety of strains with different virulence (Lallier et al., 1980) and antigenicity which constitutes a problem for aquaculture in tropical countries.

In Egypt, the widly spread fish culturing facilities for production of high quality and/or of low cost animal protein for expanding human population neccessitate strict control measures of the most drastic bacterial infections namely, Moltile Aeromonas Septicaemia (MAS). The present work was planned to investigate the most common causes of septicaemic conditions among the cultured common carp (Cyprinus carpio L.) in Kafr El-Sheikh Governorate.

MATERIAL, AND METHODS

1. Fish:

a-Naturally infected fish

A total number of (765) fish; 365 adult, 200 fingerlings and 200 fry of common carp with external signs of septicaemia were collected alive from different fish farms and fish hatcheries at Kafr El-Sheikh Governerate. The fish were kept in full glass aquaria supplied with chlorine free tap water at 20°C (Innes. 1966).

b- Experimental fish:

A total number of 110 clinically normal common carp with body weight of 90g ± 5/fish to be used for experimental infection, were collected from fish farm at Kafr El-Skeikh Governerate. The fishes were acclimatized to a water temperature of 20 ± 2°C in the fish diseases laboratory of, Faculty of Vet. Medicine, Cairo University.

2. Clinical examination of clinically infected fish:

For the clinical examination, the collected moribund fish were examined for any external abnormalities and internal lesions according to the method described by Plumb and Bowser (1982).

3. Bacteriological examination

The collected moribund common carp were subjected to a full bacteriological examination as follows;

a- Primary bacterial isolation and purification

Bacterial swabs from heart, blood, liver, spleen, kidneys, ascitic fluids, skin and muscles of moribund common carp were streaked onto nutrient agar and trypticase soy agar plates and incubated at 28°C for 24-48 hours.

The suspected A. hydrophila colonies were recultivated on the diferential, selective Rimler-Shotts (R-S) agar media, trypticase soy agar and 5% blood agar and incubated at 30°C for 18-24 hours.

The suspected yellow-orange colonies of A. hydrophila were restreaked on new R-S plates for purifcation and identification by means of colony typing criteria as described by Popoff and Vernon (1976).

The pure identifled A. hydrophila colonies were inoculated into a trypticase soy agar slant tubes as stock for further identification.

b- Identification of bacterial isolates

i- Morphological and cultural identification

Identification of isolated bacteria by studying the colonial growth criteria, morphological and/or motility characteristics of the bacteria were carried out as described by Cruickshank et al., (1982).

ii- Biochemical identification

The biochemical activities of bacterial isolates were finally studied according to the schedule of biochemical reactions provided by Popoff and Vernon (1976), Cruickshank et al. (1982) and Janda and Battone (1984).

4- Preparation of extracellular products of bacterial isolates

For production of extracellular products (ECPs) of suspected A. hydrophila isolates; Trypticase soy broth (TSB) dialysate was prepared byt dissolving 60g of TSA (2X) in one litre of distilled water. The prepared TSA was dialysed against one litre of distilled water at 4°C for 24 hours. The distilled water containing the fine particles of the media (dialysate broth) was dispensed into 250ml capacity conical flasks; each with 100ml dialysate broth. The flasks were autoclaved at 121°C for 15 minutes.

The autoclaved flasks were inoculated with A.hydrophila isolates and incubated at 28°C for 24 hours with periodical shaking. The grown bacterial cells were removed by centrifugation at 7000 Xg for 5 minutes at 4°C. The supernatant fluids were collected and screened for the presence of ECPs after which, it was redialysed against distilled water for 24 hours; during which at least, for to five times; water changes were applied. The collected ECPs in the cellulose dialysis bags (Spectra/Por) were concentrated using polyethylene glycol.

5- Toxigenicity of concentrated crude ECPs of A.hydrophila isolates to common carp fish

In this experiment a total of 110 fish were grouped into eleven groups, each of ten fish with average body weight 90±5g/fish. The fish in each group of the first ten groups were inoculated intramuscularly (I/M) with 0.5 ml/fish with one of the ten crude ECPs of A. hydrophila isoaltes respectively. The fish in the eleventh group were inoculated with 0.5 ml/fish of sterile dialysed broth and left as control. All inoculated fish were observed for ten days post-inoculation.

6- Pathogenicity test:

The pathogenicity test of isolated and identified A.hydrophila was carried out as described by Plumb & Bowser (1982) through inoculation of 0.2 ml of 24 hour broth culture (1.5 X 10^9 cells / ml) from each isolate I/M in ten common carp. A group of 10 fishes inoculated with 0.2 ml/fish of sterile broth was left as control. All inoculated and control fishes were observed for 10 days during which the clinical signs and mortalities were recorded.

RESULTS

1- Results of seasonal incidence:

The results of incidence of MAS among naturally infected adult common carp denoted a seasonal occurance of the disease with a peak of infection during summer season (41.4%), while the incidence of infection in spring and autumn were almost the same (37.0% and 36.6%, respectivley). On the other hand, the incidence of infection during winter season was the least (22.8%), (Table 1 and Fig. 1).

The incidence of MAS among common carp at the fingeling stage (April-May) indicated the highest disease epizootics (32%) during July month while, the least infection rate was in April (25%) (Table 2 and Fig. 2).

The results of MAS incidence among common carp fry revealed an incidence of 16% during June while, the least incidence was reported at March (12%) (Table 3 and Fig. 3).

Table 1: Seasonal incidence of Motile *Aeromonas specticaemia* among adult common carp.

Season	No. of estimated fish	Diseased fish	
		No.	%
Winter	105	23	22.8
Spring	70	26	37.0
Summer	70	29	41.4
Autumn	120	44	36.4

Fig. 1: Seasonal incidence of Motile Aeromonas septicaemia among adult common carp.

Table 2: Monthly incidence of Motile *Aeromonas septicaemia* among common carp fingerlings.

Month	No. of examined fish	Diseased fish	
		No.	%
April	50	15	25
May	50	23	46
June	50	27	54
July	50	16	32

Fig. 2: Monthly incidence of Motile Aeromonas septicaemia among common carpfingerlings

Table 3: Monthly incidence of Motile *Aeromonas septicaemia* among common carp fry.

Month	No. of examined fish	Diseased fish	
		No.	%
March	50	6	12
April	50	8	16
May	50	12	24
June	50	11	16

Fig. 3: Monthly incidence of Motile Aeromonas septicaemia among common carp fry

Table 4: Biochemical identification of the suspected *A.hydrophila* isolates.

	Biochemical test	Bacterial isolates									
		1	2	3	4	5	6	7	8	9	10
1	Indole production	+	+	+	+	+	+	+	+	+	+
2	Methyl red	+	+	+	+	+	+	+	+	+	+
3	Voges-proskauer	-	-	-	-	-	-	-	-	-	-
4	Citrate utilization	+	+	+	+	-	-	+	+	-	+
5	Cytochrome oxidase	+	+	+	+	+	+	+	+	+	+
6	Catalase test	+	+	+	+	+	+	+	+	+	+
7	H_2S on TSI	-	-	-	-	-	-	-	-	-	-
8	Urease test	-	-	-	-	-	-	-	-	-	-
9	Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+
10	Gas from glucose	+	+	+	+	+	+	+	+	+	+
11	Acid from glucose	+	+	+	+	+	+	+	+	+	+
12	Acid from lactose	-	-	-	-	-	-	-	-	-	-
13	Acid from sucrose	+	+	+	+	+	+	+	+	+	+
14	Acid from galactose	+	+	+	+	+	+	+	+	+	+
15	Acid from maltose	+	+	+	+	+	+	+	+	+	+
16	Acid from mannose	+	+	+	+	+	+	+	+	+	+
17	Acid from dulcitol	-	+	-	-	+	-	-	-	-	-
18	Acid from inositol	-	-	-	-	-	-	-	-	-	-
19	Acid from arabinose	+	+	+	+	+	+	+	+	+	+

Table 5: Results of pathogenicity test of **A.hydrophila** isolates to common carp.

No. of isolates	No. of fish inoculated	Days post inoculation			Fish mortality	
		1-3 days	4-7 days	8-10 days	No.	%
1	10	-	6	1	7	70
2	10	-	5	1	6	60
3	10	-	1	4	5	50
4	10	7	2	-	9	90
5	10	1	5	1	7	70
6	10	-	-	4	4	40
7	10	6	3	-	9	90
8	10	-	3	2	5	50
9	10	7	2	-	9	90
10	10	-	-	4	4	40

Table 6: Toxogenic effect of the ECPs of **A.hydrophila** isolates to common carp.

No. of isolates	No. of fish inoculated	Days post inoculation			Fish mortality	
		1-3 days	4-7 days	8-10 days	No.	%
1	10	1	5	-	6	60
2	10	1	4	-	5	50
3	10	-	-	4	4	40
4	10	7	1	-	8	80
5	10	2	4	-	6	60
6	10	-	-	4	4	40
7	10	6	2	-	8	80
8	10	-	2	2	4	40
9	10	8	-	-	8	80
10	10	-	1	2	3	30

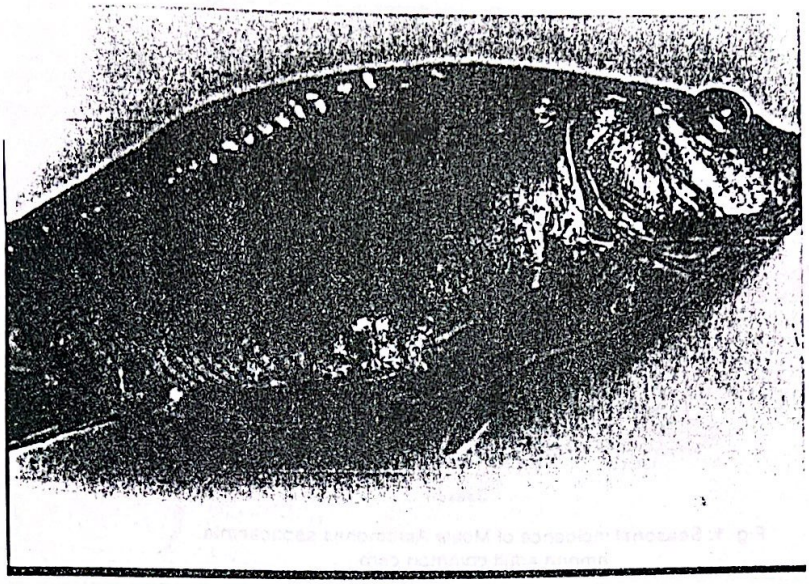

Fig. 4: Naturally infected common carp showing exopthalmic and prutrusion of anal orifice.

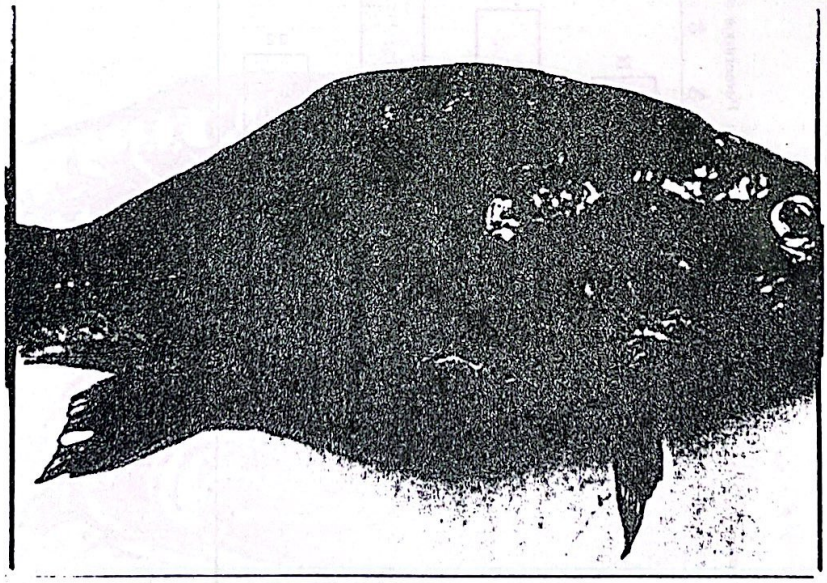

Fig. 5: Naturally infected common carp showing ulceration of the skin and underlying musculature, congestion of anal orifice and loss of scales.

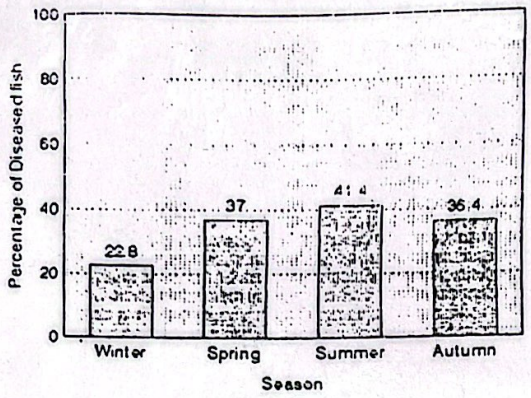

Fig. 1: Seasonal incidence of Motile Aeromonas septicaemia among adult common carp

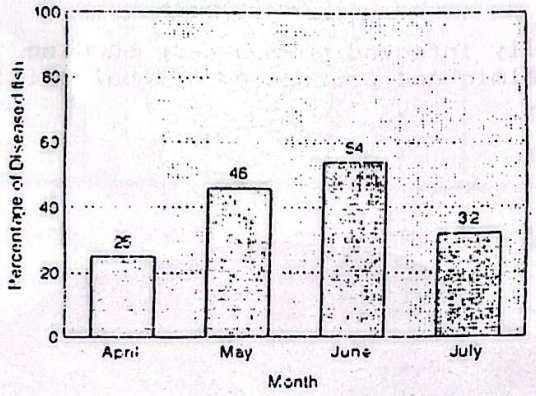

Fig. 2: Monthly incidence of Motile Aeromonas septicaemia among common carp fingerlings

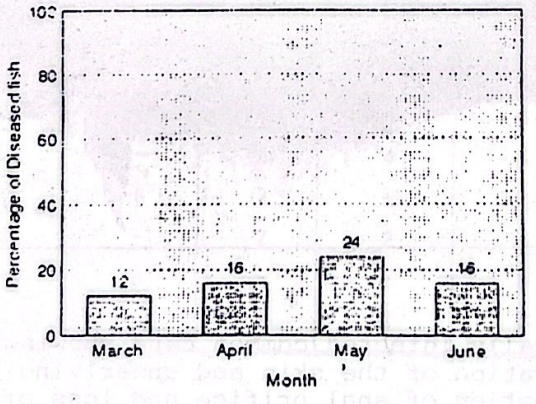

Fig. 3: Monthly incidence of Motile Aeromonas septicaemia among common carp fry

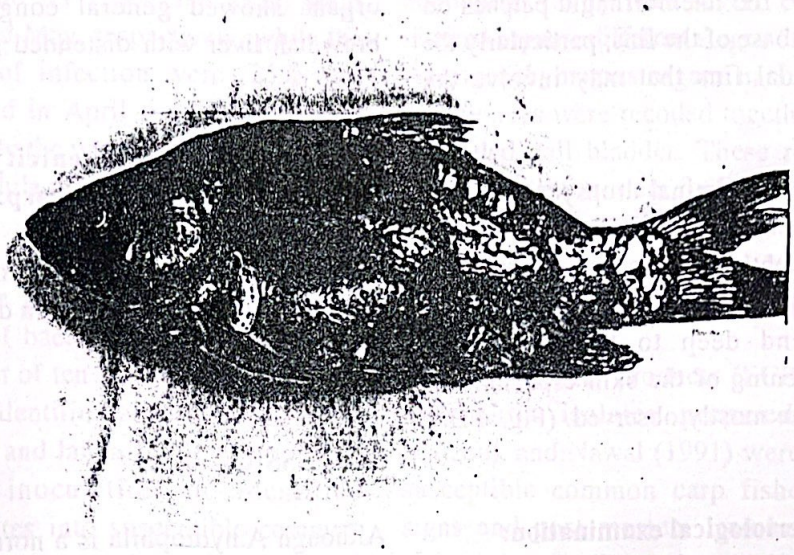

Fig. 6: Naturally infected common carp showing congestion and haemorrhagic patches allover the body and fins.

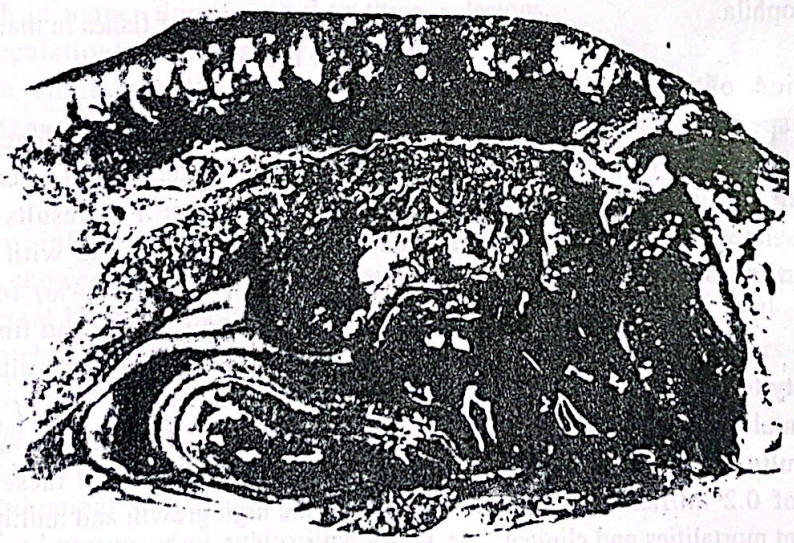

Fig. 7: Naturally infected common carp showing congestion of visceral organs and distended gall bladder.

2- Results of clinical signs and lesions:
The examined naturally infected common carp showed externally; red haemorrhagic patches on the skin and at the base of the fins; particularly the pectoral, and caudal fins that may involve the candal peduncle.

Various degrees of abdominal dropsy, protrusion of the anal orifice were observed. Most oftenly uni/or bilateral exophthalmia were seen. Detached scales with skin inflammation and ulceration which may extend deep to the underlying musculative, darkening of the skin especially at the dorsal part were mostly observed. (Fig. 4 and 5).

3- Results of bacteriological examination:
Bacteriological examination of the naturally diseased common carp resulted in isolation of ten bacterial isolates, that were identified and characterized acording to the Shotts and Rimler (1973) as well as Popoff and Vernon (1976). The produced colonies were suspected to be characteristic for A.hydrophila.

Microscopic examination of Gram's stained smears form these suspected A. hydrophila colonies appeared as Gram negative short rods.

All these suspected ten isolates, were subjected to biochemical identification and were indentified as A.hydrophila (Table 4).

4- Result of pathogenicity test:
The results of experimental I/M inoculated carp with 24 hours broth culture of A.hydrophila isolates at a dose rate of 0.2 ml/fish ($1.5X10^9$ cells/ml) revealed different mortalities and clinical signs at different times post-inoculation. The results indicated the high pathogenicity of isolated strains number 4,7 and 9 in comparison to other A.hydrophila isolates (Table 5).

The most observed clinical signs were skin haemorrhages and darkening (Fig. 6), togeth with frying and sloughing of fins. The intr organs showed general congestion, enlarg brownish liver with distended gall bladder (Fi 7).

5- Results of toxogenicity of ECPs A.hydrophila to common carp:

The results of inoculation of common carp wi concentrated crude ECP with a dose of 0.5 ml/fis I/M are shown in table 6.

DISCUSSION

Although A.hydrophila is a normal inhabitant water (Schubert, 1967), alimentary tract of fres water salmonoids (Trust and Sparrow, 1974 intestinal tract of Tilapia nilotica (Akelah, 197 and frequently isolated from apparently health carp (Heushmann, 1978); the organism wa responsible for a great epizootic; namely Motil Aeromonas septicemia of fishes in many countrie (Kaper et al. 1981).

Concerning the results of incidence of MA among naturally infected common carp in Kaf El-Sheikh Governorate; The results denoted seasonal occurence of disease with a peak o infection during summer (14,4%) followed b spring (37%), autumn (36.6%) and finally winte (22.8%) (Table, 1, Fig. 1). These results supporte those of Tysset et al. (1970); Meyer (1970) an Rippy and Cabelli (1980) who got more or les the same results and attributed these pattern o incidence to the high growth and multiplication o A.hydrophila with the decrease of oxygen conten of water during summer and spring which in turn make the fishes more susceptible to infection.

Regarding the monthly incidence of MAS among carp fingerlings and fry during the period o March-July (developmental period of commor

carp fry and fingerlings in Egypt). It was clear that the highest disease incidence among common carp fingerlings and fry (54% and 24%) were reported in June and May, respectively, while the lowest incidence of infection were (25% and 12%) were reported in April and March. These could be attributed to the water temperature which enchance A.hydrophila infection.

Concerning the microorganisms isolated and identified from the naturally infected common carp. The results of bacteriological examination proved the isolation of ten A. hydrophila isolates which were fully identified according to Popoff and Vernon (1976) and Janda and Bottone (1984) Table 4). The inoculation of identified A.hydrophila isolates into susceptible common carp denoted the presence of a wide variety of virulence among the ten isolates namely; 3 virulent strains with 90 % mortality of inoculated susceptible common carp within the first three days post-inoculation and the other seven isolates were less virulent with 40-70% mortality of inoculated fish in longer time (more than three days post-inoculation). These bacteriological findings were similar to those recorded by Soliman (1984) and (1988) and Austin and Austin (1987).

The naturally infected common carp with A.hydrophila showed a wide variety of signs including external haemorrhage of varying sizes (petechal or patches) distributed allover the sking of infected fish specially at the root of pectoral, anal, and caudal fins, Abdominal dropsy was also a prominent sign in some fishes although various degrees of abdominal dropsy were recorded. Inflammation and protrusion of anal orifice were also noticed in some infected fishes. Uni or bilateral exophthalmia of most naturally infected commom carp were observed. Severe skin ulceration with detached scales were observed in most of naturally infected fishes.

The post-mortem findings of naturally infected fishes revealed a yellowish, bloody exudate in the abdominal cavity with severe congestion of all internal organs, different stages of degeneration of the parenchymatous organs namely, liver, spleen and kidneys were recoded together with a highly distended gall bladder. These recorded clinical signs and post-mortem findings were recorded by many authors, Amin and Abdel Kerim (1976), Amlacher (1981) and Enany (1983) as the cause of septicaemic character of the organism.

The extracellular products (ECPs) of the ten A. hydrophila isolates prepared according to Marzouk and Nawal (1991) were inoculated into susceptible common carp fishes. The clinical signs and post mortem lesions observed were more or less similar to that produced by the whole A.hydrophila organism in which skin erythema and haemorrhages, abdominal dropsy, inflammation and protrusion of vent with severe congestion of the abdominal viscera and distended gall bladder were the most obvious signs of lesions.

In conclusion, Motile Aeromonas Septicaemia appeared as one of the most important and drastic fish outbreaks which has an economical importance in both fish hatcheries and semi-intensive and intensive fish farms. The matter of which neccessitate strict control and preventive measures in the form of removal of enviromental stress factors that predispose to the disease occurrance.

REFERENCES

Akelah, M. A. (1978): Some studies on intestinal flora of the genus Tilapia in the Manzalah fish farm. M. V. Sc. Thesis, Fac. Vet. Med. Zagazig University.

Amlacher, E. (1981): Taschenbuch der fischkrankeiten. Gustav Fischer Verlag. Jenda, p: 115-130.

Amin, N. E. and Abd-Elkerim, S. E. (1976): Studies on the

pathogenicity of Aeromonas punctata to common carp (Cyprinus carpio). Proc. 13th Arab. Vet. Cong., Cairo, p. 43-49.

Austin, B. and Austin, D. (1987): Bacterial Fish Pathogens. Pub. Ellis Horwood Limited, Chichester, West Sussex, England . pp. 364.

Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. (1982): Medical Microbiology, 12th Ed. Vol. II: The Practice of Medical Micrbiology, Churchill Livingston, Edinburgh, England.

Enany, M. M. (1983): Biochemical, Serological and biological properties of Aermononas spp. isolated from fish. Ph. D Thesis, Fac. Vet. Med., Zagazig University.

Heuschmann, B. G. (1978): Aeromonads of the hydrophil punctata group in fresh water fishes. Arch. Hydrobiol., 83(1): 99-125.

Innes, W. T. (1966): Exotic Aquarium Fishes. 19th Ed., Aquarium Inc., New Jersy.U. S. A., p. 530-533.

Janda, F. M. and Bottone, E. J. (1984): Biotyping of Aeromonas isolates as a correlate to delineating a species-associated disease spectrum. J. Clin. Microbiol., 19: 44-48.

Kaper, J. B.; Lockman, H.; Colwell, R. R. and Joseph, S. W. (1981): Aeromonas hydrophila: Ecology and Toxigenicity of isolates from an estuary. J. Appl. Bacteriol., 50 : 359-377.

Lallier, R. Boulanger, Y. and Olivier, G. (1980): Difference in virulence of Aeromonas hydrophila and Aeromonas sobria in rainbow trout. Prog. Fish. Cult., 42 (4): 199-200.

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

Meyer, F. P. (1970): Seasonal fluctuaion in the incidence o disease on fish farm. Symposium on disease of fish and shell fishes. Am. Fish Soc. Pub. No. 5:526.

Plumb, J. A. and Bowser, R. R. (1982): A laborato Manual of Microbial Fsih Diseases. Auburn University Auburn, Alabama, P. 77.

Popoff, M. and Vernon, M. (1976): A taxonomic study o the Aeromonas hydrophila-Aeromonas punctata group J. Gen. Microbiol., 94: 11:22.

Rippy, S. R. and Cabelli, V. J. (1980): Occurence o Aeromonas hydrophila in Limnetic enviroments relatioship to the organism to tropic state. J. Microb Ecol., 61: 45.54.

Schubert, R. H. W. (1967): The taxonomy and nomenclature of the anaerogenic aeromonad. Int. J. Syst. Bacteriol. 17: 273-279.

Shotts, E. B.;and Rimler, R. B. (1973): Media for isolation of Aeromonas hydrophila. Appl. Microbiol., 26 (2): 550-553.

Soliman, M. K. (1984): Some ecological factors and thire relations of the development and elimination o aeromonasis in fish. M. V. Sc. Thesis, Fact. Vet. Med., Alex. University.

Soliman, M. K. (1988): The pathogenesis of Aeromonas hydrophila isolates in fish with special emphasis on their control. Ph. D-Thesis, Fac. Vet. Med. Alex. University.

Trust, T. J. and Sparrow, R. A. (1974): The bacterial flora in the alimentary tract of fresh water salmoid fishes. Can. J. Microbiol, 20 (6): 1219-1229.

Tysset, C.: Brisou, J. and Durand, C. (1970): A disease of fish in an artificial lake caused by phytobacterium (Aeromonas liquefaciens). Recl. Med. Vet. 146: 1031-1043.