A CONTRIBUTION ON MOTILE *AEROMONAS* SEPTICEMIA AMONG CULTURED EEL (*ANGUILLA ANGUILLA*) IN ABBASSA FISH FARM

EL-REFAEE, A. M. E., EL-ASHRAM, A. M. M. AND SAKR, S. F.

Fish Diseases Dept., Central Lab. For Aquaculture Research, Agriculture research Center, Egypt.

Abstract

Eel fish culture was newly introduce to Abbassa and it susceptible to various diseases. *Aeromonas hydrophila* was isolated from cultured eel in Abbassa fish farm suffered from heavy mortality and biochemical identified. Motile Aeromonas septicemia infected eel showed loss of balance, reduced growth, hemorrhage in fins (red fin disease) and tail rot, ulcer and enlargement of the abdomen. Internally, the organs were congested. The intraperitoneal route was more pathogenic than intramuscular one in the experimentally infected eel. Ciprofloxacin was the drug of choice for the control and prevention of MAS under laboratory condition in both in-vivo, and oxytetracyclin in-vitro. Microscopically different organs showed histopathological changes. It is recommended to have an effective fish health management during eel culture to avoid the infection by Aeromonas.

INTRODUCTION

Eel fish are likely to be important cultured fish in the 21st century due to its nutritional and medicinal values. Infectious diseases of cultured fish are among the most notable constraints on the expansion and development of aquaculture (Plumb, 1999 and Woo and Bruno, 1999). Bacterial pathogens are the most serious disease problem in fish production causing 80% of mortalities (Austin and Austin, 1993).

Motile *Aeromonas* Septicemia (MAS) is a more drastic one and distributed world-wide, affecting various fish species and shellfish, Feral as well as cultured in both freshwater and sea water (Inglis *et al.,* 1993, Austin and Austin, 1993, Plumb, 1999 and Woo and Bruno, 1999). The external clinical signs of the affected fish

make them unmarketable. Also, the causative agent of MAS, *Aeromonas hydrophila*, is also an opportunistic pathogen of humans causing illness that ranged from mild to dysentery-like diarrhea as well as meningitis and septicemia particularly when fish is eaten raw or improperly cooked (Inglis *et al.*, 1993).

During the last decade, the interest in protection against fish diseases has grown enormously. The lack of effective disease control has the potential of being the chief limiting factor of the realization of highly stable fish production (Newman, 1982). Therefore, the current investigation was planned to throw light on Motile *Aeromonas* Septicemia as a major disease agent among the cultured eel.

MATERIAL AND METHODS

Naturally Infected Fish: A total number of 60 clinically and grossly diseased eel *Anguilla anguilla* were collected from semi-intensive Abbassa fish farm suffered from a high mortalities during July 2007. The diseased fish were subjected to full clinical and postmortem examinations as described by Amlacher (1970) and Lucky (1977).

Bacteriological Examination

Samples were taken under complete aseptic condition from the affected areas of the external body (fins, tail and gills) and from the internal organs (liver, spleen and kidney) as well as ascetic fluid and inoculated into tryptic soya broth (Difco), then incubated at 25°C for 24 hours, further identification was done using standard microbiological procedures according to Schaperclaus *et al.*, (1992).

Sensitivity test

The antibiograms of the recovered pathogen were done using the disc diffusion method. The interpretations of zones of inhibition were estimated according to the limits given by Biomerieux (1984).

Experimental studies

A total number of 60 apparently healthy *Anguilla anguilla* were collected from EI-Abbassa fish ponds and transferred alive to the laboratory to be used in the artificial infection, and treatment. Fish were acclimatized to laboratory conditions for 2 weeks, maintained at 25±1 °C in glass aquaria (5 eel per aquaria) (tables 3 and 5) supplied with well-aerated, dechlorinated water. During this time and throughout the experiment, they were fed on ration 40 % protein.

A- Challenge infection

A total of 30 apparently healthy *Anguilla anguilla* were divided into 3 equal groups. 0.2 ml dose of 24 hr. broth culture from virulent previously isolated bacterial pathogen of *A. hydrophila* (5×10^5 CFU/ml) were given by intramuscular (i.m.) and intraperitoneal (i.p.) injection for the first and second groups respectively (Schaperclaus *et al.,* 1992). The third group was kept as a control and inoculated with sterile broth (5 fish i.m. and 5 fish i.p.). All the experimentally infected fish were daily noticed for any abnormal clinical signs and mortalities. The dead and clinically diseased fish were subjected to bacterial re-isolation and histopathological examination.

B- Treatment experiment

A total number of 30 apparently healthy *A. anguilla* were divided into 3 equal groups. The first group was treated by I.M. injection with Ciprofloxacin at a dose of 10 mg/kg fish, then challenged by 0.2 ml of pathogenic strain (isolated strain) of *A. hydrophila* (5×10^5 CFU/ml) (Bowser *et al.,* 1994). The second group received same dose of Ciprofloxacin only. The third group was injected with *A. hydrophila* only. During 15-d postmedication, any adverse clinical signs and mortalities were recorded. All fish were examined for the presence of *A. hydrophila.* And for the ponds the eel treated by using of oxytetracycline hydrocholoride 75mg/kg fish for 10 days.

Histopathological Examination

Samples from affected organs and tissues of naturally infected eel were fixed in 10% phosphate buffered formalin. Paraffin sections (5 µ thick) were prepared and stained with hematoxylin and eosin (H&E) and examined microscopically (Roberts, 1989).

RESULTS

MAS infected eel showed loss of appetite, dullness, loss of equilibrium, sluggish swimming at the water surface, skin erosion and ulcer. The ulcers were usually shallow. Some of the infected eel had exophthalmia accompanied by hemorrhage or opaqueness of the eye. Hemorrhage on the fins (red fin disease), fin and tail rot, enlarged abdomen with ascites and vent were seen prolapsed. Gills might be congested or pale and anemic and covered with excessive mucus. The anal and genital regions were also swollen, hyperemic and sometimes small ulcer was noticed near them, other cases showed reddish mouth (Fig. 1 & 2). Internally, the organs were friable and showed a generalized hyperemic appearance (Fig. 3). The liver varied in color from yellow to dark brown with necrotic foci in some cases. The gall bladder was over distended with bile. The body cavity contained a bloody fluid. The intestine was flaccid, hyperemic, contained yellowish mucus and voided of food. The kidney, gonads and spleen were swollen and congested.

A. hydrophila appeared to be G-ve, of short motile rods, and cytochrome oxidase positive (Table, 1).

The distribution of *A. hydrophila* in various tissues and organs of diseased eel was demonstrated in Table (2), where a higher percentage was reported from tail and fins (29.86%) followed by gills (21.53%). The lowest was recorded from the ascitic fluid (4.86%).

Regarding to the sensitivity of *A. hydrophila* to different antimicrobials, the bacterial isolates were sensitive to Ciprofloxacin (which showed highly efficacy),

Oxytetracycline, Chloramephenicol, Erythromycin, Nalidixic acid, Tetracycline, Trimethoprim + sulfamethoxazole (Table, 3). On the other hand, it was noticed that *A. hydrophila* was resistant to Penicillin G, Amoxicillin and Ampicillin.

Experimentally infected eel showed nearly similar clinical signs, with postmortem and histopathological changes to those observed in naturally infected ones. Table (4) showed mortalities pattern among the artificially inoculated fish with previously isolated *A. hydrophila* from naturally infected fish. The i.p. route infection produced a higher mortality than the i.m. route. Re-isolation of *A. hydrophila* was succeeded from all dead and clinically diseased fish. On the contrary, the control group showed neither clinical signs nor mortalities. Furthermore, no *A. hydrophila* was isolated from the control group.

The laboratory trial for the treatment of infected fish indicated that the Ciprofloxacin was quite effective against MAS (Table 5). No *A. hydrophila* was detected from any fish that survived to the end of the trial in both medicated challenged (group1) and non-challenged (group2) groups. On the other hand, *A. hydrophila* was isolated from fish that have died during the trial from challenged non-medicated group (group3).

In the pond treated with oxytetracycline hydrochloride give a good result with en 3 days.

Histopathologically, the infected eel showed focal sloughing in the epidermis with hyalinization of the underlying dermal tissue which was infiltrated with melanin carrying cells. Edematous fluid separated the dermis from underlying muscular layer which suffered Zenker's necrosis (Figure 4). Gills, showed desquamation of the epithelial covering of secondary lamellae (Figure 5). The liver showed degenerative changes mainly, vacuolar degeneration beside dissociation of the hepatocytes (Figure 6). Kidney showed focal degeneration of hematopoietic elements (Figure 7). The eye showed focal degeneration at most inner layer of the retina which showed focal detachment (Figure 8).

DISCUSSION

Aquaculture is the most growing sector in agriculture allover the world. The causative agent of MAS, *A. hydrophila* has a worldwide distribution, infecting fishes, birds as well as human (Austin and Austin, 1993 and Plumb, 1999). Moreover, MAS is a serious problem for the fish farming industry in Egypt, causing heavy economic losses.

The high prevalence of *A. hydrophila* could be attributed to its presence as a part of intestinal flora of healthy freshwater and marine fish (Newman, 1982, Austin and Austin, 1993 and Plumb, 1999).

Regarding to the clinical signs, it was revealed that fish infected with *A. hydrophila* showed loss of equilibrium, fin and tail rot, ulcer and enlargement of abdomen. These results went hand in hand with those recorded by Kabata (1985), Post (1987), Austin and Austin (1993), Inglis *et al.*, (1993), Newman (1982), Stoskoph (1993), Ahmed *et al.*, (1995), Plumb (1999), Woo and Bruno (1999), Toranzo *et al.*, (2005) and Yavuzcan *et al.*, (2005) who mentioned that the sluggish movement associated with *A. hydrophila* infection was probably the result of frayed and sloughed tail, beside hemorrhagic edematous and ulcerated fins, in addition to anorexia which affected the vital activities of the diseased fish.

The common gross lesions observed in the diseased fish were septicemic in nature as they revealed congestion of all internal organs with abdominal distension and yellowish ascitic fluid. The postmortem lesions were in accordance with the findings of Newman (1982), Kabata (1985), Post (1987), Roberts (1989), Stoskoph (1993), Inglis *et al.*, (1993), Plumb (1999), Woo and Bruno (1999), Toranzo *et al.*, (2005) and Yavuzcan *et al.*, (2005) who mentioned that the overdistended gall bladder could be attributed to enteritis and constriction of the common bile duct. Also, the gills were severely congested and the fins were congested and hemorrhagic. However, many other bacterial infections in eel cause the same or similar clinical signs and post-mortem lesions (Plumb, 1999 and Toranzo *et al.*,

2005), it was therefore considered prudent to make isolates from various tissues of the moribund fish.

The morphological and biochemical investigations revealed that *A. hydrophila* was Gram negative, with short motile rods and comparable to that recorded by Kabata (1985), Post (1987), Austin and Austin (1993), Inglis *et al,* (1993), Plumb (1999) and Woo Bruno (1999).

Less of balance could be attributed to lesions in the labyrinth which is associated with maintenance of equilibrium (Roberts 1989).

The sluggish movements were probably the result of frayed tail beside hemorrhagic, edematous and ulcerated fin in addition to anorexia. The observed swimming of the affected fish near the surface of the water suggests their extraordinary need for oxygen which could be attributed to coating of the gills with profuse mucus together with congestion and desquamation of epithelial cell covering.

The highest recovery rate of *A. hydrophila* suggested that tail and fins could be the primary entrance for systemic infection as suggested by Woo and Bruno, (1999).

The artificial infection, in the present study showed that the i.p. route was more pathogenic than i.m. one. Also, the same clinical signs, postmortem and microscopic findings were similar to that of naturally infected eel as mentioned by Newman (1982), Kabata (1985), Post (1987), Roberts (1989), Stoskoph (1993), Plumb (1999), Toranzo *et al.,* (2005) and Yavuzcan *et al.,* (2005).

Moreover, similar histopathological changes in the infected fish were observed by Amlacher (1970), Kabata (1985), Post (1987), Roberts (1989), Inglis *et al.,* (1993), Ahmed *et al.,* (1995), Plumb (1999) and Aly *et al.,* (2000).

It was shown also that Ciprofloxacin was the drug of choice for the treatment of infected eel from MAS, both in-vitro and invivo and under the laboratory conditions. Similar results were recorded by Bowser *et al.,* (1994), Abd El-Rahman and El-Ashram, (2005) and El-Refaee, (2005) who mentioned that

Ciprofloxacin has proved a world-wide efficacy to control or treat MAS infection in fish.

Outbreaks of diseases are usually accompanied with stress factors as overcrowding, sudden change in temperature, pollution, handling and nutritional imbalance (Plumb, 1999, Woo and Bruno, 1999 and Yavuzcan *et al.*, 2005). According to Austin and Austin (1993), proper management is essential to success of aquaculture operations, as the inadequate management is the principal factor in triggering disease outbreaks.

The high prevalence of *A. hydrophila* could be attributed to its presence as a part of the intestinal flora (Newman 1982) the higher recovery rate of *A. hydrophila* suggests that the tail and fins could be the primary entrance for *A. hydrophila* to spread throughout the body (Inany *et al.,* 1995). The septicemic lesions in viscera other places may be come from lesion on the intestinal mucosa that favors the passage of bacteria into the blood stream from the intestinal contents due to the hunger during winter season (Kopp 1951). Ulcerations are produced by synergistic effect of other parasites and *A. hydrophila* transported by blood and lymph (Kopp 1951). However, this view does not agree with our finding, or with others (Tomaesec 1965), who mentioned that the entrance through the skin is the real path of infection.

Such ascites could be explained on the basis of renal explained on the bases of the renal portal hypertension, induced by hepatic renal lesions or decreased osmotic pressure of the blood beside congestion and edema. It is evident that such hepatic lesions interfere with the hepatic circulation. The kidney in the present study showed focal depletion and necrosis of hemopoietic elements, and proliferation of epithelia lining of renal tubules may be attributed to that organ is a target in many diseases. A reason for this may be the affinity of the organ for circulating particulate antigen. 70% of bacteria inoculated into the dorsal aorta are initially trapped by phagocytes lining the renal sinusoids (Ferguson 1989). The depletion of hemepoietic elements may be attributed to cytolytic and fibrolytic capacities of *A. hydrophila*.

The external lesion (erosion, hemorrhage, edema and ulcers) and focal sloughing of the epidermis could be primarily induced by the release of powerful bacterial proteolytic enzymes which leads to electrolyte and protein loss together with disturbed circulation (Morita 1975). The aggregation of melanin carrying cells may be of help in initiating immunity against the infection (Easa and Mahady 1984). Hyalinization of underlying tissues occurred due to prolonged sodium pump failure which frequently results in changes in the proteins of the cells due to alteration in electrolyte change which can give the cytoplasm a shiny appearance, this is knew hyaline degeneration.

Table 1. Morphological and biochemical characters of the isolated *A. hydrophila.*

Test	Reaction
Characteristics of colony on TSA media	Circular convex white colored colonies
Characteristics of colony on R.S. media	Small, smooth and yellow colonies
Gram stain	Gram –ve short rods
Motility	+
Gram staining	-
Gelatin I liquefaction	+
Oxidase	+
O/F	F
Growth on 5% NaCl	-
Indol	+
V.P	+
Methyl red	+
H2S production	-
Catalase	+
Nitrate reduction	+
Citrate utilization	+
Arginin hydrolysis	+
Fermentation of	
Glucose	+
Sucrose	+
Maltose	+
Lactose	-
Galactose	+
Trehalose	+
Fructose	+

Table .2. *A. hydrophila* distribution in various tissues and organs of the naturally infected eels.

Bacteria	Tail & fins		Gills		liver		kidney		spleen		Ascetic fluid	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A. hydrophila	43	29.86	31	21.53	23	15.97	21	14.85	19	13.19	7	4.86

Table 3. Sensitivity of the naturally isolated *A. hydrophila* to different antibiograms.

Antimicrobial agent	Disk potency	Zone diameter (mm)			Zone result	Result
		R	I	S		
Amoxicillin	25 µg	≤22	23-30	≥31	16	R
Ampicillin	10 µg	≤22	23-30	≥31	0	R
Erythromycin	15 µg	≤13	14-17	≥18	25	S
Penicillin	10 µg	≤11	12-21	≥22	10	r
Oxytetracyclin		≤13	14-20	≥21	37	S
Nalidixic acid	30 µg	≤13	14-18	≥19	35	S
Tetracycline	30 µg	≤14	15-18	≥19	32	S
Chloramephenicol	30 µg	≤12	13-17	≥18	35	S
Ciprofloxacin	5 µg	≤15	16-20	≥21	46	S
Kanamycin	30 µg	≤13	14-17	≥18	16	I
Trimethoprim + sulfamethoxazole	1.25 µg	≤10	11-15	≥16	25	S

Table 4. The mortalities pattern among the artificially injected eel with *A. hydrophila*.

Bacterial isolate	A. hydrophila		Control	
Route of injection	I.P.	I.M.	I.P.	I.M.
Dose	$0.2X\ 10^6$	$0.2X\ 10^6$	0.2 ml SB	0.2 SB
No. of injected fish	10	10	5	5
Mortality rate	100	80	0	0
No. of died fish	10	8	0	0

Table 5. Mortality rate of laboratory trial for the use of Ciprofloxacin in the treatment of motile Aeromonas septicemia.

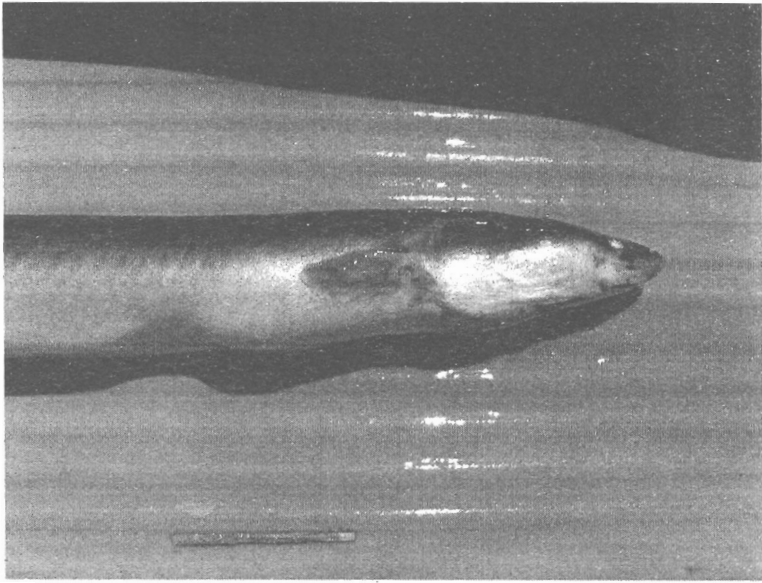| Fish group	No. of fish	No. of dead fish	% of dead fish
1	10	2	20
2	10	0	0
3	10	10	100

Fig. 1. Naturally infected eel with *A. hydrophila* showing hemorrhage and reddens of fin (red fin disease)
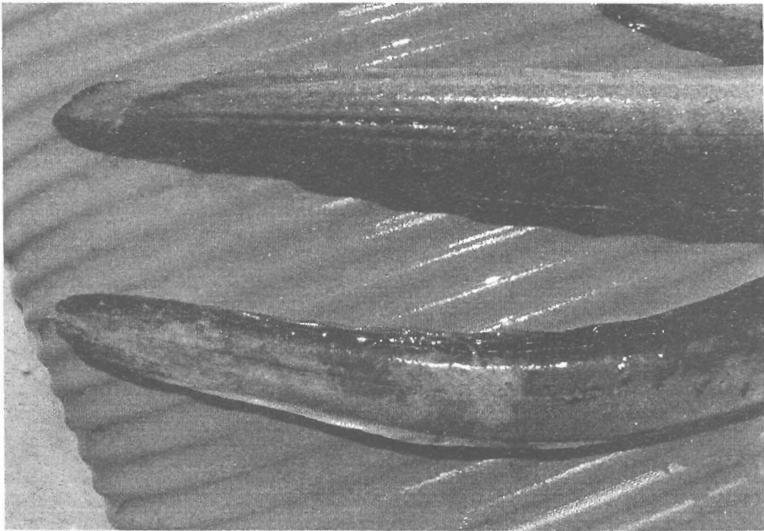

Fig. 2. Naturally infected eel with *A. hydrophila* showing hemorrhage, skin erosion and ulcer.
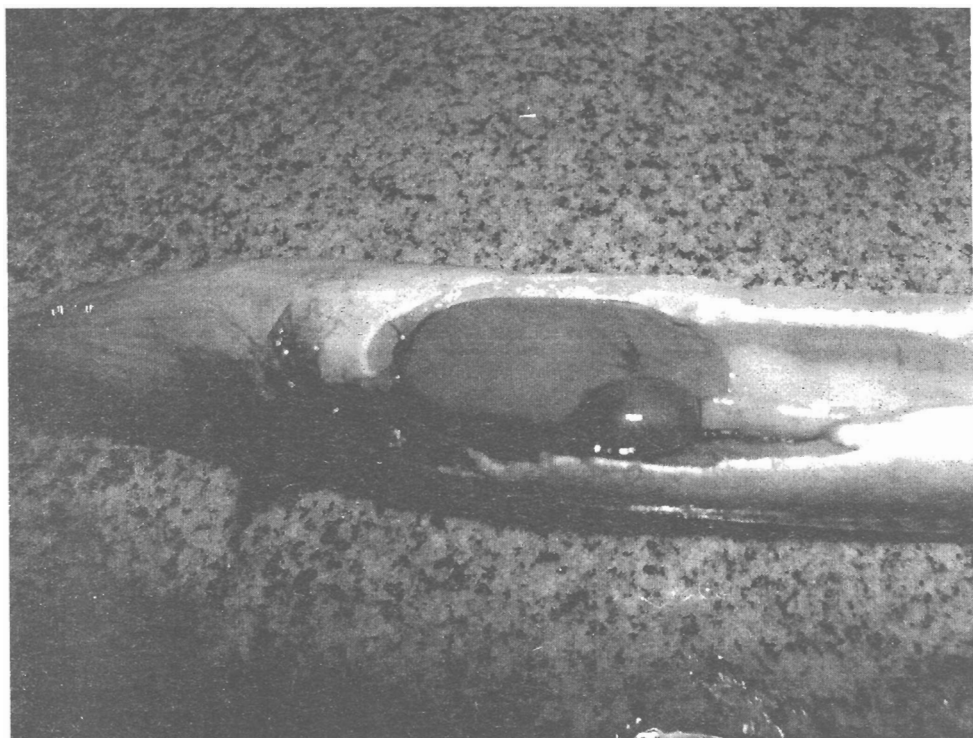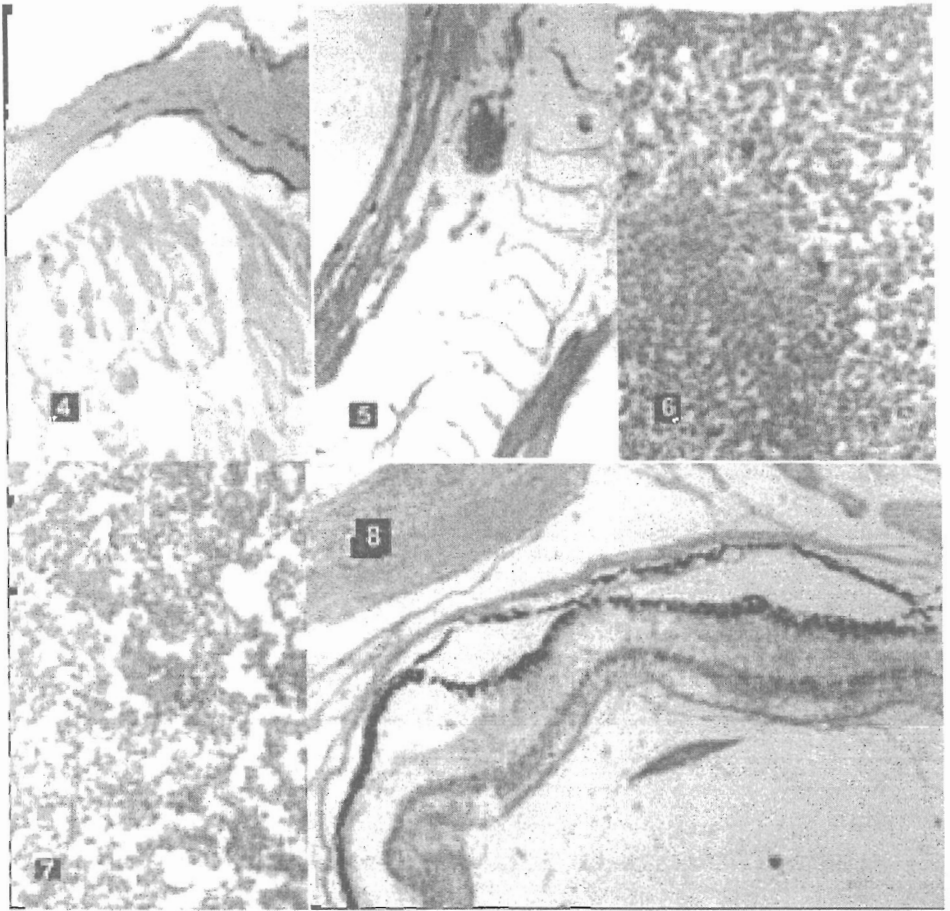
Fig. 3. Postmortem changes associated with *A. hydrophila*, showing necrotic foci and enlargement of the gallbladder.

Fig. 4. Skin of *Anguilla anguilla* infected by *A. hydrophila,* showing focal sloughing of epidermis, hyalinization of the underlying dermal tissue which was infiltrated with melanin carrying cells, separation of dermis from underlying muscular layer edematous fluid and zenker's necrosis of some muscular bondles (H&E X 300).

Fig. 5. Gills of *Anguilla anguilla* infected by *A. hydrophila,* showing desquamination of the epithelial covering of secondary lamellae. (H&E X 150).

Fig. 6. Liver of *Anguilla anguilla* infected by *A. hydrophila,* showing degenerative changes. (H&E X 300).

Fig. 7. Kidney of *Anguilla anguilla* infected by A. hydrophila, showing degenerative and focal depletion of hemepoietic elements. (H&E X 300).

Fig. 8. Eye of *Anguilla anguilla* infected by A. hydrophila, showing degeneration and detachment of retina. (H&E X 300).

REFERENCES

1. Abd El-Rahman, A. M. M. and A. M. M El-Ashram. 2005. Some studies on vibriosis caused by Vibrio vulnificus in cultured *Oreochromis niloticus.* 2nd International conference Vet. Res. Div., NRC, Cairo, Egypt. pp. 185-203.

2. Ahmed, S. A, M. S. Marzouk, R. M. A Megid, M. Moustafa and M. Gado. 1995. Histopathological and immunological studies on *Aeromonas hydrophila* infection in common carp. Vet. Med. 1., Giza, 43 (4): 389 - 396.

3. Aly, S. M., H. M. Tantawy, A. F. Badran and M. A. EI-Baz. 2000. Histopathologic and immunologic response of *Clarias lazera* to the injection of *Aeromonas hydrophila* vaccine. Suez Canal Vet. Med. 1., 111 (1): 133 - 144.

4. Amlacher, E. 1970. Text book of fish diseases. Gustav Fisher Verlag, Tena DDR.

5. Austin, B. and D. A. Austin. 1993. Bacterial Fish Pathogens. In Disease in Farmed ancl wild fish. Ellis Horwood Ltd. Publisher, Chichester, England.

6. Bio-merieux. 1984. Laboratory reagents and products. Bacterial. Barcy-L. Etiole 69260 charbonmieres- Les- Bains. France.

7. Bowser, P. R., G. A. Wooster and H. Hsu. 1994. Laboratory efficacy of enrofloxacin for the control *Aeromonas solmanicida* infection in rainbow trout. 1. Aq. Anim. Health, 6: 288 - 291.

8. Easa, M. E, M. M. Mahady. 1984. Repair of skin wounds in *Clarias lazera* (Armout catfish) a preliminary study. Veterinary and Medical Journal 32 (3): 35-43.

9. El-Refaee, A. M. E. 2005. Streptococcus infection in freshwater fish. Ph.D., Microbiology Dept., Faculty Vet. Med., Alexandria Univ.

10. Ferguson H. W. 1989. Systematic Fish Pathology. Iowa State University Press/Ames.).

11. Inglis V., R. S. Roberts and R. Bromage. 1993. Bacterial diseases of fish. 151 ed. Halsted Press New York.

12. Kabata, Z. 1985. Parasites and diseases of fish cultured in the tropics. (ed. By Taylor and Francis) London and Philadelphia, Int. Dev. Res. Co. 15t Ed.

13. Lucky, Z. 1977. Methods of the diagnosis of Fish diseases. Amerind Pub!. Co. PVT ltd., New York, 15t Ed.

14. Miyazaki, T. and N. Kaige. 1985. A histopathological study on motile aeromonal disease of crucian carp. Fish Pathology, 21:181-185.

15. Morita, R.Y. 1975. Psychrophilic bacteria. Bacteriol. Rev. 39, 144-167.

16. Newman, S. G. 1982. *Aeromonas hydrophila*: A review with emphasis on its role in fish disease. In: D. P. Anderson, M. Dorsonnand, D. H. Dubourget eds. Antigens of fish pathogens: development and production for vaccines and serodiagnostics. Collection Foundation Marcel Mérieux, Lyon, France, 87-118

17. Plumb, J. A. 1999. Health maintenance and principal microbial diseases of cultured fishes. Iowa state Press. U.S.A.

18. Post, G. 1987. Textbook of fish health T.F.H. publ., Inc Ltd., U.S.A.

19. Roberts, R. J. 1989. Fish Pathology. Sailliere Tindal, London.

20. Schaperclaus W., H. Kulow and K. Shreckenbach. 1992. Fish Diseases, Vol. I. A. A. Balkema Rotterdam.

21. Stoskoph M. 1993. Fish Medicine. W. B. Saunders Co., U. K.

22. Toranzo, A.E., B. Magarinos and J. L. Romalde. 2005. A review of main bacterial fish diseases in mariculture systems. Aquaculture 246: 37-61.

23. Vol. 3, Viral, Bacterial and Fungal infections. CAB! Publishing, London, U. K.

24. Woo, P. T. K. and D. W. Bruno. 1999. Fish diseases and disorders.

25. Yavuzcan Y. H., S. Bekcan, A. C. Karasu Benli and M. Akan. 2005. Some blood parameters in the eel (*Anguilla anguilla*) spontaneously infected with *Aeromonas hydrophila*. Vet. Med. Association J. 60(3) 91-93.

A CONTRIBUTION ON MOTILE *AEROMONAS* SEPTICEMIA
AMONG CULTURED EEL (*ANGUILLA ANGUILLA*) IN ABBASSAFISH FARM

الإصابة بالتسمم الدموى لأسماك الثعابين فى مزرعة العباسة

أحمد محمد عزت الرفاعى، أحمد محمد محمد محمود الأشرم ، صالح فتحى صقر

قسم أمراض الأسماك – المعمل المركزى لبحوث الثروة السمكية بالعباسة

تم عزل ميكروب الإيرومونـاس هيدروفيلا وتم تعريفه بواسطة الإختبارات البيوكيميائيــة من أسماك ثعابين مستزرعة بمزرعة المعمل بالعباسة ظهرت بها وفيات بأعداد كبيرة.   أسماك الثعابين المصابة ظهر عليها أعراض مختلفة منها اضطراب فى التوازن، قلة النمو، تآكـل فـى الزعانف والذيل، إحمرار فى الزعانف (مرض الزعنفة الحمراء) نتيجة للأنزفة مع نقرحات وكبر حجم البطن. من الداخل كان هناك إحتقان فى الأعضاء الداخلية مع وجود تكرنز فى الكبد و كبر حجم الحويصلة المرارية. وجد أن الحقن عن طريق الغشاء البريتونى أكثر تأثير مرضـى مــن الحقن فى العضلات. وقد وجد أن أحسن العقارات المستخدمة فى العلاج هو السبروفلوكساسـين تحت الظروف المعملية و الأوكسى تتراسيكلين تحت الظروف الحقلية. بالفحص الهتوبـاثولوجى وجدت تغيرات مرضية فى الأعضاء المختلفة.