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Pathological Evaluation of Experimental Pseudomonas Fluorescens Infection in Nile Tilapia

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Abstract: This study was carried-out on 30 of apparently healthy Nile tilapia (55 ± 10 g. B.W and 12 ± 2 cm in length) were collected from commercial fish farms in Behera Province. Fish were acclimated for two weeks in the laboratory of National Institute of Oceanography and Fisheries (NIOF) before any experimentation was started. Fish were divided into two groups (15 fish per each) inoculated intrapritoneally using 0.2ml of saline and *Pseudomonas fluorescens* at concentration $2.5x10^5$ CFU/ml. The inoculated fish were observed for 30 days. The clinical signs in the infected fishes were observed at the second day post injection. Fish showed loss of balance, excessive mucus secretions on skin and gills, ascites with slightly protruded reddish vent and hemorrhages all over the body surface, frayed and rotten tail and fins. This study, showed that Pseudomonas infection in freshwater fish causes 53.3% mortality, with severe changes in blood parameters as RBCs and WBCs as well as the serum enzymes as AST, ALT and Alkaline phosphates, also the changes extended to include the total serum proteins, albumin, urea and creatinine with severe histopathological changes in gills, liver, kidney, spleen, intestine and brain. Proliferative interlamellar hyperplasia with fusion in gills, vacuolar degeneration of the hepatic cells, renal tubular and hemopoietic tissue necrosis and splenic lymphocytic necrosis and depletion were recorded in infected fish.

Key words: Nile tilapia · *Pseudomonas fluorescens* · Histopathological changes

INTRODUCTION

Bacterial pathogens are the causative agents of most serious disease problems in both wild and cultured fish causing mortalities and severe economic losses [1] Pseudomonas infections has been incriminated as an important bacterial infection among fish and appear to be stress related disease of freshwater fish especially under culture conditions [2].

Pseudomonades are opportunistic gram negative pathogens, naturally occur in aquatic environment and as a part of normal gut flora of healthy fish, it causes outbreak when the optimum environmental conditions change [3], which characterized by fin rot, petechial hemorrhage, darkness of the skin, detached scales, abdominal ascitis and exophthalmia [4]. The infection with pseudomonas in fish causes decrease in the lymphocytes, monocytes and neutrophils, there is a increase in the level of WBCs, decrease in RBCs, Hb %, there is also decreasing of the level of serum proteins (Albumin and total protein) [5]. The pseudomonas infection caused increasing of alkaline phosphatase, GPT and GOT level [6, 7].

This study was carried out to determination of the clinical signs, postmortem, hematological, biochemical changes and histopathological changes of *Pseudomonas fluorescens*.

MATERIALS AND METHODS

Experimental Fish: Thirty apparently healthy Nile tilapia (55 ± 10 g. BW), were obtained from a private fish farm. Fish were transported alive in metal tank containing water enriched by oxygen sources to the Laboratory of National Institute of Oceanography and Fisheries (NIOF). All fish were acclimatized for two weeks before beginning of the experiment. Fish were kept in prepared glass aquaria (90 ×50 ×35 cm). Fish were fed on a commercial fish diet

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containing 25 % crude protein. The diet was daily provided at 3% of body weight as described by Eurell *et al*. [8].

Bacteria: Local isolates of *Pseudomonas fluorescens* (*P. fluorescens*) strain were obtained from Animal Reproduction Researches Institute, Alexandria, Egypt (Dep. of Microbiology).

Experimental Design: The fish were divided into two groups; each group comprised of fifteen fish kept in glass aquaria. The first group were injected with 0.2 mL of physiological saline and designated as the control. The fish in second group were IP inoculated with 0.2 ml of *P. fluorescens* at concentration $2.5x10^5$ CFU/ml. All fish were observed daily for any clinical signs, abnormal behavior or mortalities for a period of thirty days post-infection.

Hematological Studies: Blood samples were taken three times during 1st, 2nd and 4th week post infected. Blood film was prepared according to the method described by Lucky [9]. Red blood cell (RBCs) and White blood cell (WBCs) counts were counted by haemocytometer according to Stoskopf [10]. Blood hemoglobin (Hb) was assessed by cyanomtahemoglobin method [11]. Differential leucocyte counts were calculated according to Schalm [12].

Biochemical Studies: The activity of the liver enzymes, Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) were determined according to Reitman and Frankel [13] beside Alkaline phosphatase (ALP) was measured according to Aoki, [14] by using commercial kits produced by Pasteur Lab. Moreover, serum total protein was determination according to Domuas *et al*. [15] was measured by colorimetric methods and Albumin according to Reinhold [16]. Furthermore, the activity of the kidney creatinine was determined according to Henry [17] and urea was determined according to Patton and Crouch [18].

Histopathological Studies: Following necropsy, tissue specimens were collected from gills, hepatopancreas, kidney, spleen, brain and intestine. These specimens were collected during 1st, 2nd and 4th week post infected from every group. Tissue specimens were rapidly fixed in 10% neutral buffered formalin solution for at least 24 hrs. The fixed specimens were processed through the conventional paraffin embedding technique. Paraffin blocks were prepared from which 5 microns thick sections were obtained. These sections were stained with Haemtoxyline and Eosin (H and E) according to the method described by Culling [19].

Statistical Analysis: Data collected were analyzed using T-independent test to assess the significance of different parameters between control and pseudomonas fluorescence groups using Statistical Analysis System [20] software.

RESULTS

Clinical Signs, Mortalities and postmortem examination: The clinical signs were observed at the second day post injection. The recorded mortalities of the experimental infected fish were 53.3% (8 fish). Fish showed loss of balance, excessive mucus secretions on skin and gills, detachment of scales and skin ulceration (Fig. 1a), ascites with slightly protruded reddish vent (Fig. 1b) and hemorrhages all over the body surface, frayed and torn tail and fins (Fig. 1c). Internally these fishes showed abdominal dropsy with reddish ascetic exudates, liver paleness and enlargement in some fishes and congested with necrotic patches in other fishes, kidney and spleen was congested (Fig. 2d) and enlarged and hemorrhagic enteritis in some fish.

Hematological Studies: As shown in Table (1). Red blood cells counts and Hemoglobin estimation showed a significant decrease in the infected group when compare with control group along period of the experiment. As well, the infected Nile tilapia showed a significant increase in WBCs counts, neutrophils, lymphocytes and monocytes along period of the experiment when compare with control group.

Biochemical Studies: As illustrated in Table (2). The serum AST and ALT activities showed a significant increase in the infected group compared with control group in a time dependent manner. The serum ALP activity was increased significantly in the infected compared with control group at 2nd and 4th week. The serum total protein and albumin level were decreased in the infected group compared with control group along period of the experiment. The serum concentration of urea was not significantly increased in the infected group if compared with the control group. However, the serum concentration of creatinine was increased significantly in comparison with the control group.

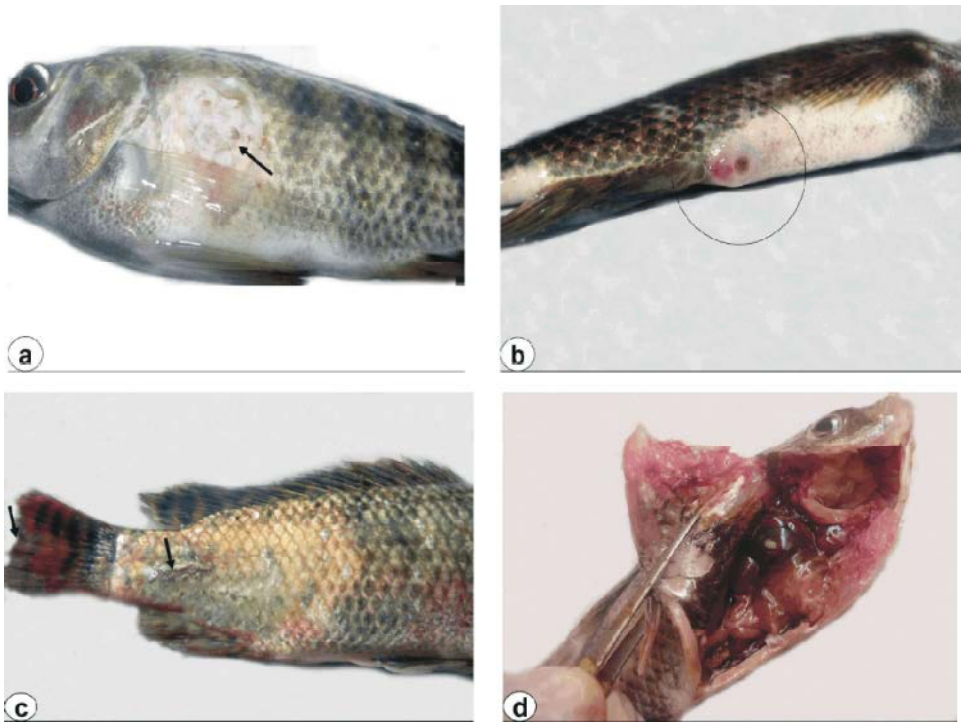

Fig. 1: Nile tilapia during the infection with *P. fluorescens* showing (a) Detachment of scales and skin ulceration (arrow). (b) Slightly protruded reddish vent. (c) Frayed and torn tail and fins (arrows). (d) Congestion of internal organ.

Table 1: Hematological parameters of Nile tilapia during the experimental infection

Period (week)	Groups	RBCs10^6 / mm^3	Hb (g/dl)	WBCs 10^3/ mm^3	Neutrophils 10^3/ mm^3	Lymphocytes 10^3/ mm^3	Monocytes 10^3/ mm^3
1st	Control	3.30±0.00^a	8.75±0.09^a	13.00±0.58^b	45.50±2.60^b	11.50±0.87^c	3.50±0.29^b
	P. fluorescens	2.00±0.12^b	5.60±0.12^b	19.90±0.06^a	80.00±1.15^a	16.50±0.87^c	3.00±0.58^b
2nd	Control	2.93±0.07^a	8.83±0.17^a	13.73±0.24^b	56.00±1.00^b	16.67±0.33^b	4.00±0.58^b
	P. fluorescens	1.90±0.00^b	6.10±0.12b	17.75±0.26^a	63.50±2.60^a	26.00±4.04^b	9.50±0.87^a
4th	Control	3.47±0.03^a	8.80±0.12^a	12.03±0.26^b	54.33±1.20^b	12.33±0.33^d	2.33±0.67^c
	P. fluorescens	2.80±0.06^b	6.80±0.06^b	19.11±0.06^a	79.63±0.78^a	16.50±0.29^d	14.27±1.59^a

Values are means ± standard errors.

WBCs, white blood cells count; RBCs, red blood corpuscles count; Hb, hemoglobin

Means bearing different superscripts within the same column within the same period are significant at ($P<0.05$)

Table 2: Biochemical parameters of Nile tilapia during the experimental infection

Period (week)	Group	ALT (U/l)	ASTU/l)	ALP(U/l)	Total protein (g/dl)	Alb.(g/dl)	Urea (mg %)	Creatinine (mg %)
1st	control	58.20±1.27^b	83.50±0.87^b	13.50±0.29^b	11.05±0.55^a	2.70±0.06^a	16.25±0.32^a	0.55±0.09^b
	P. fluorescens	65.00±1.73^a	100.00±8.66^b	11.50±2.02^b	8.85±0.32^b	1.95±0.09^b	17.10±1.33^a	1.20±0.12^a
2nd	control	63.00±1.00^b	88.67±2.40^b	13.33±0.57^b	11.70±0.25^a	2.83±0.09^a	17.53±0.62^a	0.73±0.13^b
	P. fluorescens	70.00±1.73^a	116.50±2.60^a	15.70±0.17^a	9.70±0.35^b	1.90±0.15^b	18.45±0.26^a	0.95±0.09^b
4th	control	66.00±1.00^b	80.67±0.67^b	11.00±0.58^b	8.63±0.13^a	2.73±0.07^a	20.47±0.18^a	0.63±0.07^b
	P. fluorescens	70.27±0.15^a	122.90±0.06^a	14.30±0.17^a	8.30±0.06^a	1.55±0.03^b	21.30±0.06^a	0.90±0.00^a

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

Means bearing different superscripts within the same column within the same period are significant at ($P<0.05$).

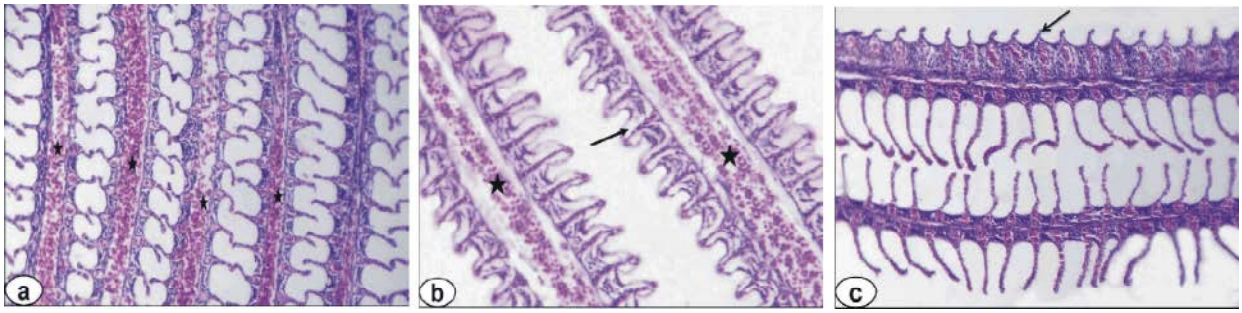

Fig. 2: Photomicrograph of gills of Nile tilapia infected with *P. fluorescens* and stained with HE showing (a) congestion of branchial blood vessels (stars X160) (b) congestion of branchial blood vessels and lamellar lifting (arrow X250) (c) unilateral stunting of secondary lamellae (arrow X250)

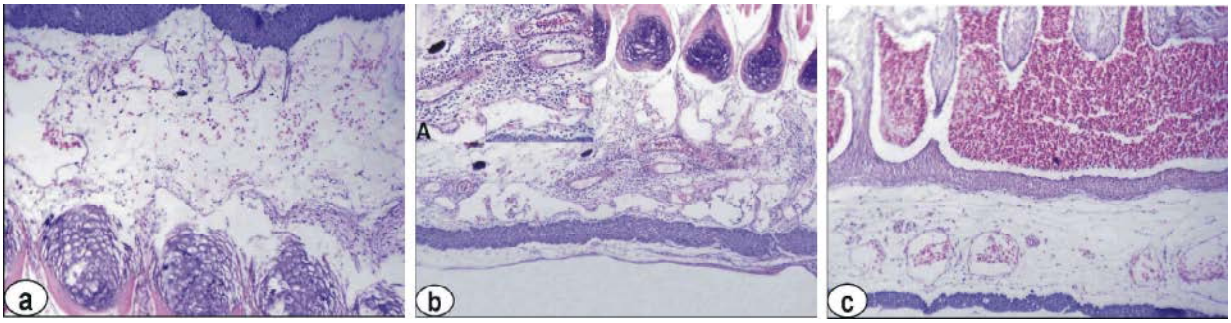

Fig. 3: Photomicrograph of gill arch of Nile tilapia infected with *P. fluorescens* and stained with HE(X160) showing (a) edema (b) intense mononuclear cells and EGCs infiltration (A inset X400) (c) severe hemorrhages

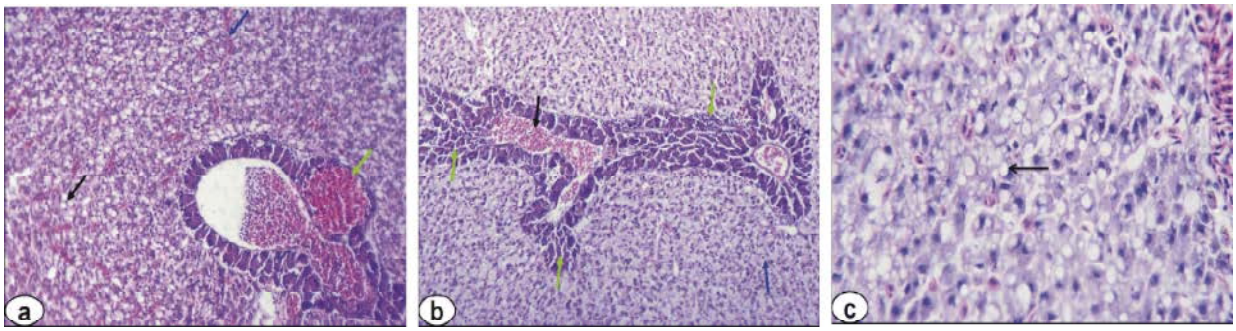

Fig. 4: Photomicrograph of hepatopancreas of Nile tilapia infected with *P. fluorescens* and stained with HE showing (a) fatty change (black arrow) beside congestion of pancreatic acini (green arrow) and hepatic sinusoid (blue arrow X 160) (b) hydropic degeneration of the hepatocytes (blue arrow) beside congestion of pancreatic acini (black arrow) and mild necrotic pancreatic acini (green arrow X 160) (c) fatty changes (black arrow X250)

Histopathological Findings

Gills: The microscopically findings in gills were congestion of branchial blood vessels (Fig. 2a) during 1st week post infection besides lamellar lifting where separation between lining epithelium of the secondary lamellae from the capillary beds with edema (Fig. 2b) during 2nd week. Moreover, unilateral stunting of secondary gill lamellae due to hyperplasia of the basal epithelium of primary gill lamellae (Fig. 2c) at the end of experiment was noticed.

Gill Arch: The gill arch showed edema which represented by faint eosinophilic fluid (Fig. 3a) during 1st week of the experiment. Moreover, intense mononuclear cells and EGCs infiltration (Fig. 3b) beside sever hemorrhage

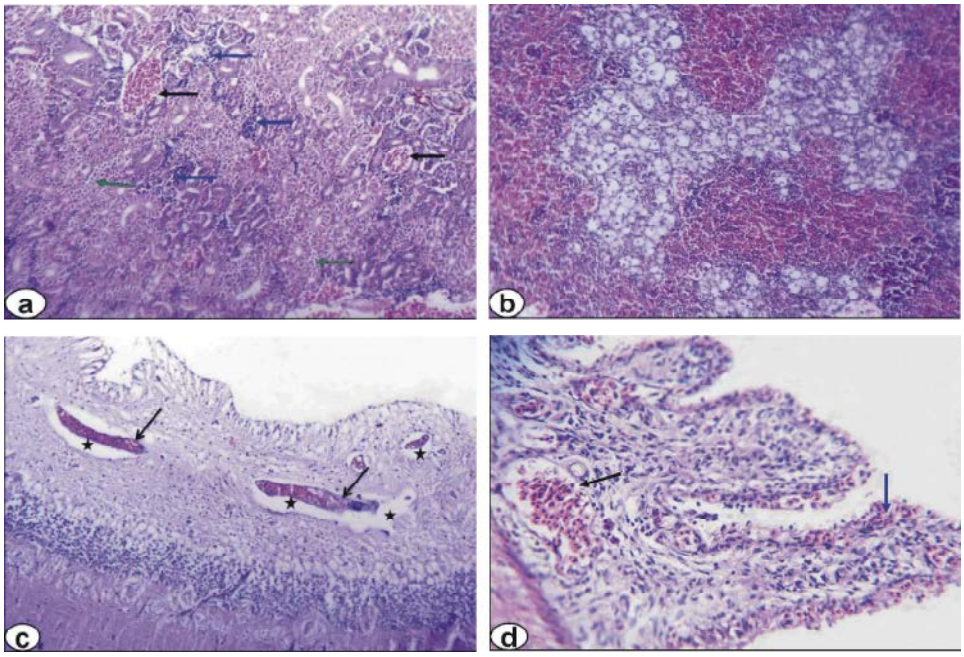

Fig. 5: Photomicrograph of kidney, spleen, brain and intestine of Nile tilapia infected with *P. fluorescens* and stained with HE showing (a) kidney: congestion of blood vessel (black arrow), depletion of hemopiotic elements (blue arrow) and tubular necrosis (green arrow X160) (b) spleen: Lymphoid depletion and apoptosis with infiltration of tingible-bodies macrophages (Starry sky appearance) X160 (c) brain: congestion of blood vessels (arrows) with moderate distension in Virchow-Robin space by edema (stars X160) (d) intestine: congestion of blood vessel (black arrow) and hemorrhagic enteritis where RBCs replaced necrotic enterocytes (blue arrow X250)

(Fig. 3c) were noticed during 2nd and 4th week of the experiment, respectively.

Hepatopancreas: The detectable lesions during 1st week were moderate fatty change which characterized by sharp edged vacuoles, flattened nucleus and signet ring appearance beside congestion of pancreatic acini and hepatic sinusoid (Fig. 4a). Moreover, during 2nd week there was hydropic degeneration of the hepatocytes beside mild necrotic pancreatic acini (Fig. 4b). Furthermore, at the end of experiment the severe fatty change of the hepatocytes (Fig. 4c) was noticed.

Kidney: The kidney exhibited congestion of blood vessel, depletion of hemopiotic elements and mild tubular necrosis (Fig. 5a) along period of the experiment.

Spleen: The encountered lesions were activation of melanomacrophage centers (MMCs) and mild to moderate depletion of white pulp during 1st and 2nd week of experiment. Moreover, there was Lymphoid depletion and apoptosis with infiltration of tingible-bodies macrophages (Starry sky appearance) (Fig. 5b) at the end of experiment.

Intestine: The intestine showed congestion of blood vessel and hemorrhagic enteritis where RBCs replaced necrotic enterocytes (Fig. 5c) along period of the experiment.

Brain: The only detectable lesion was congestion of blood vessels with moderate distension in Virchow-Robin space by edema (Fig. 5d) during 2nd and 4th week.

DISCUSSION

Experimental infected fish showed external signs and symptoms such as loss of balance, excessive mucus secretions on skin and gills, detachment of scales and skin ulceration, ascites with slightly protruded reddish vent and hemorrhages all over the body surface, frayed and rotten tail and fins. Internally, organs have a generalized hyperemic appearance; the kidney and spleen are swollen; and the liver is often mottled with hemorrhage increased with light areas. The body cavity contains bloody and cloudy fluid. The intestine is flaccid, hyperemic and is a void of food. These signs were nearly similar to the result recorded by Stoskopf [10] and

Khairnar *et al.* [21]. The clinical signs and P.M. lesions may be attributed to the role of plasmids in virulence which differs in number according to pathogenicity [22]. Further work is necessary, however, to resolve the precise role of this large plasmid in the pathogenicity of *P. fluorescence*.

RBCs count and Hb concentration were a significant decreased in *P. fluorescence* infected group. These results attributed to the Pseudomonas infections which cause destruction of RBC that causes the decreasing numbers of RBCs in fish blood and consequently with decreasing of Hb concentration. These agreed with those of Fernandez *et al.* [23] indicated that the pseudomonas produces a product which causes lysis and destruction of RBCs and reduce its number and its Hb content.

WBCs, Neutrophilis, lymphocytes and Monocytes were significant increase in *P. fluorescence* infected group. The neutrophilis and monocytes, phagocytic cells as tissue macrophages they work in role of defense mechanism of the host by adhering and engulfing it [24, 25].

In the present work serum aspartate aminotransferase and serum alanine aminotransferase were a significant increased in *P. fluorescence* infected group. These results attributed with [26-29]. Moreover, serum ALT and AST activities are considered a sensitive indicator to evaluate hepatocellular damage [30, 31]. Significant increase in ALT and AST may reflect the hepatic damage leading to extensive liberation of the enzymes into blood circulation [29, 32]. Serum alkaline phosphatase was a significant increased in *P. fluorescens* infected group.

Serum albumin and Serum total protein showed a significant decrease in *P. fluorescens* infected group. This may attributed to liver damage [33, 34].

The urea and creatinine were non-significant and significant increase respectively, may be as a result of renal dysfunction either by bacterial invasion and/or by bacterial toxins which was closely similar to that achieved by Rehulka and Minaoik [35].

In aquatic organisms, the gills represent a vital organ, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Toxic substances may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms [36]. The gills showed congestion of branchial blood vessels, lamellar lifting and unilateral stunting of secondary gill lamellae due to hyperplasia of the basal epithelium of primary gill lamellae. The bacteria produce an extracellular hyperplasia inducing factor which can produce typical lesions as epithelial hyperplasia associated with lamellar fusion.

The gills arch showed edema, hemorrhage and dilatation of blood vessels with leukocytic infiltration and increase number of EGC there lesions may be due to process of acute inflammation was initiated by the action of the released vasoactive amines on the microcirculation of the area and the release of cell breakdown products [37].

In the present study, the hepatopancrease exhibited congestion of pancreatic acini and hepatic sinusoid beside varying sizes of cytoplasmic vacuoles of the affected hepatocytes and mild necrotic pancreatic acini. These findings nearly similar to those reported by Aly [38] and Zaki [39] in common carp.

The renal lesions were in the form of congestion of blood vessel, depletion of hemopiotic elements and mild tubular necrosis [39].

Upon the microscopical examination of the spleen, there was activation of melanomacophage center in the spleen is assumed to be a result of stimulation of this organ by mild action of bacterial toxin. Besides there were lymphoid depletion and apoptosis of lymphocytes with infiltration of tingible-bodies macrophages (Starry-sky appearance).

Regarding the microscopical examination of the intestine, there were congestion and hemorrhagic enteritis.

The detectable brain lesions were congestion of blood vessels with moderate distension in Virchow-Robin space by edema.

CONCLUSION

Pseudomonas fluorescens is mainly a primary pathogen to fish with hematological, biochemical changes and marked pathological alterations. The information will help in controlling and treating the incidents of bacterial infections in aquaculture ventures as well as in capture fisheries. This knowledge will be of significant to fish farmers in maintenance of fish health for improvement of fish productions and ultimately reflects the economy of the farmers and nation as a whole.

REFERENCES

1. Roberts, R.J., 2001. Fish pathology, 3rd Edition W. B. Saunders, Philadelphia, PA.

2. Kitao, T., T. Aoki, M. Fukudome, K. Kawano, Y. Wada and Y. Mizuno, 1993. Serotyping of *Vibrio anguillarum* isolated from fresh water fish in Japan. Journal of Fish Diseases, 6: 175-181.
3. Angelini, N.M. and G.N. Seigneur, 1988. Disease of the fins of *Rhamdia sapo*. Isolation of the etiological agents and experimental infection Research Microbiology, 20: 37-48.
4. Khalil, S.A., R.H. Khalil, T.T. Saad and M.H. Safaa, 2010. Studies on *pseudomonas septicemia* among cultured *Oreochromus niloticus*. Journal of the Arabian Aquaculture Society, 5(1): 55-64.
5. Austin, B. and D. Austin, 1987. Bacterial Fish Pathogens: Disease in Farmed and Wild fish. Ellis Horwood. Chichester, pp: 97-107.
6. Austin, B. and D. Austin, 1993. Bacterial fish pathogens. Pseudomonadaceae representatives, pp: 253.
7. Badran, A.F., 1993. Studies on pseudomonas septicemia outbreak among cultured freshwater fishes with special reference to its control, Zagzig Veterinary Journal, 21(4): 737-746.
8. Eurell, T.E., S.D. Lewis and L.C. Grumbles, 1978. Comparison of selected diagnostic tests for detection of motile *Areomonas septicemia* in fish. American Journal Volume Research, 39(8): 1384-1386.
9. Lucky, Z., 1977. Methods for Diagnosis of Fish Disease. American Publishing Co. New York.
10. Stoskopf, M.K., 1993. Fish Medicine. Saunders Comp. Philadelphia.
11. Drubkin, D., 1964. Spectrophotometric methods XIV. The crystographic and optical properties of the hemoglobin of man in comparison with those of other species. Journal of Biology Chemistry, 164: 703-723.
12. Schalm, O.W., 1986. Veterinary hematology 4thedition, Lea and Febiger, Philadelphia.
13. Reitman, S. and S. Frankel, 1957. A colorimetric method for determination of serum glutamic oxalo acetic and glutamic pyruvic transaminase. American Journal Pathology, 26: 1-13.
14. Aoki, T., T. Arai and S. Egusa, 1977. Detection of R plasmids in naturally occurring fish-pathogenic bacteria, Enterobactereacea. Microbiology Immunology, 21(2): 77-83.
15. Domuas, B.T., D.D. Bayso, R.J. Carter, T. Peters and R. Schaffer, 1981. Determination of total serum protein. Clinical Chemistry, 27: 1642-1643.
16. Reinhold, R.R., 1953. Determination of serum albumin. Clinical Chemistry, 21: 1370-1372.
17. Henry, R.J., 1974. Clinical Chemistry principles and technical. 2nd Edition Harper and Row, pp: 525.
18. Patton, C.J. and S.R. Crouch, 1977. Annual Chemistry, 49: 464-469.
19. Culling, C.F., 1983. Handbook of Histopathological and Histochemical Staining Techniques. 3rd Edition; Butter Worth-London.
20. Statistical Analysis System (SAS), 2004. Statistical user's Guide. International Cary, New York City. USA.
21. Khairnar, K., M.P. Raut, R.H. Chandekar, S.G. Sanmukh and W.N. Paunikar, 2013. Novel bacteriophage therapy for controlling metallo-beta-lactamase producing *Pseudomonas aeruginosa* infection in Catfish. BMC Veterinary Research, 26(9): 264.
22. Austin, B. and D.A. Austin, 2007. Bacterial fish pathogens, Diseases of Farmed and Wild Fish, 4th Edition. ISBN 978-1-4020-6068-7 Springer Dordrecht Berlin Heidelberg New York.
23. Fernandez, A.I., R. Knuesel, H. Segner and T. Wahli, 2003. A survey of viral diseases in farmed and feral salmoids in Switzerland. Center for Fish and wild life Health, Institute of Animal Pathology, University of Bern, Switzerland, Journal of Fish Diseases, 26: 167-182.
24. Bruno, D.W. and A.L. Munro, 1989. Hematological assessment of Rainbow trout, *Salmo gairdneri* Richardson and Atlantic Salmon *Salmo Salar* L., infected with *Renibacterium Salmoninarium*. Journal of Fish Diseases, 9: 195-204.
25. Alicja, T. Kodama, M. Moustafa, T. Mikani and H. Izawa, 2000. Development of a selective-differential medium for the isolation of Enterobactereacea and its application in epidermiological studies. Journal of Fish Diseases, 15: 243-254.
26. Kachmor, H.U., 1970. Methods of enzymatic analysis. 2nd Edition Verlag Chemistry Weinheim and Academic Press Inc. New York and London.
27. Vermu, S.R., S.P. Gupta and Tyagi, 1981. Studies on the toxicity of Lindon on *Cloisa fossiatus*. Part 1: TLM measurement and histological changes in certin tissues. Gegenbaurs Morphology Jashrsb. Leibzig, 121: 38-54.
28. Richardi, J.C. and W.E. Huff, 1983. Effects of acute Ochratoxicosis on blood pressure and heart rate of broiler chickens. Poultry Science, 62: 2164-2168.

29. Fucks, P.E., G. Colletb, E. Ingerslvec, S. Secombesb, N. Lorenzend and A.E. Ellisa, 2003. DNA vaccination against Koi Herps Virus (KHV) in rainbow trout: size, dose, route of injection and duration of protection-early protection correlates with Mx expression. Fish and Shellfish Immunology, 15: 39-50.
30. Raa, J.T., 1984. Abnormalities of plasma enzymes. In: Biochemistry in clinical Practice, D.L. Williams and V. Marks. William Heinemann, London, pp: 221-250.
31. Abohegab, D.M., W. Ahne, K.L. Denham, A.M. Sheppard, G.R. Taylor and K. Way, 1992. Nucleotide sequence analysis of the glycoprotein gene of putative Spring viraemia of carp virus and pike fry Rhabdovirus isolates reveals four genogroups. Disease Aquaculture Organization, 53(3): 203-10.
32. Seyfakth, W., 1982. Uber das Studium an Chondriosomen de Soorhefe (Manilla albi- cans) und ihr Nachweis mit Triphenyltetra-zoliumchloride. Naturvvissenschaften.
33. Lee, M. and R. Marks, 2009. Suscepility of Zebrafish *(Danio rerio*) to a model pathogen, Spring viremia of Carp virus. Comparative Medicine, 53(5): 514-21.
34. Dennis, K., S.J. Bark R.M. Le Deuff, P.D. Martin, D.M. Stone and G.R. Taylor, 2008. Isolation of Rhabdo virus during outbreaks or disease in Cyprinid fish species at fishery sites in England. Disease Aquatic. Organization, 57(1-2): 43-50.
35. Rehulka, J. and B. Minaoik, 2007. Blood parameters in brook trout *Salvelinus fontinalis* (Mitchill, 1815), affected by columnaris diseases. Aquaculture Research, 38: 1182-1197.
36. Au, D.W.T., 2004. The application of histocytopathological biomarkers in marine pollution monitoring: A review Marine Pollution, 48: 817-834.
37. Robert, R.J., 1978. "Fish Pathology" Baillier Tindal, London, pp: 194-195.
38. Aly, S., 2001. Light and electron microscopic studies on Pseudomoniasis among Common carp (*Cyprinus carpio* L.). Suez Canal Veterinary Medicine Journal, IV(1): 95-103.
39. Zaki, M.M., 2009. Occurrence of Antibiotic- Resistant and Plasmid DNA Harbouring Bacterial pathogens in Stressed Polluted Water Environment of Lake Manzala, Egypt Research Journal of Microbiology, 4(2): 59-66.