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Some Studies on Pseudomonas Infection in Experimentally Infected *Oreochromis niloticus*

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ABSTRACT

Bacterial fish diseases are the major problems in aquaculture as it found naturally in the fish environment and under certain strees condition causes severe economic losses to fish farms. This study was to achieve the aim of this study, the following points were done : 1) Evaluation of the virulence of isolated *Pseudomonas flourescence*. 2) And effect of isolated strain of *Pseudomonas fluorescence* on different blood parameters and serum enzyms. Examination the potency of locally prepared bacterin against isolated strain of *Pseudomonas flourescence*. the clinical signs were manifested as, loss of scales from some areas of the body with excessive mucus allover the body surface and petechial haemorrhages over the body . The lymphocyte showed a tendancy to decreasing from the 1st week to the 4th.While, the monocyte level decreased from the 1st week to the 3rd week . The Phagocytic activity and index increased progressively from the 1st week to the 4th week of the experiment. The results also showed that, the total protein, albumin, globulin and albumin/globulin ratio differ significantly among infected and control group and among different weeks. The antibody titer and RLP the antibody titer in the groups treated with *P. Flourescence* only of lower RLP than that of the control groups**.**

INTRODUCTION

Fish diseases are a substantial source of great losses to aquaculturists. Production costs are increased by fish diseases outbreaks because of the investment lost in dead fish, cost of treatment and decreased growth during convalescence. (Floyd, 2003).

The most prevalent diseases affecting fish farms in Egypt were Motile *Aeromonads, Pseudomonas* species, *Streptococcus* Spp. *and Staphylococcus* Spp.*; Vibrio* Spp. and *Flexibacter* Spp. (Khalil et al., 2001) .

MATERIALS AND METHODS

A- Materials :

Fish for experimental infection

A total number of ninty apparently healthy ***O. niloticus***, were obtained from private fish farm. Fish were transported a live to the laboratory of Departement of poultry and fish disease, Faculty of Veterinary Medicine, Alexandria Univeristy in plastic bags contained water enriched by air (2/3).Average body weight of fish about (50 ± 5 gm).

Fish pathogens :

a. Bacteria used for preparation of bacterin and challenge . (***Pseudomonas floursence***) isolated strains kindly obtained from Poultry and Fish Diseases Dept.

b. ***Candida albicans*** used for phagocytes was kindly provided by Poultry and Fish Diseases Dep. Fac. of Vet. Med. Alex. Univ.

B- Methods :

1. Clinical and Postmortem examinations

The collected fish were examined clinically according to the method described by ***McVicar (1982)*** .

A. Experiment I

2. Chronic experiment

Another same 60 apparently healthy ***O. niloticus*** were equally divided into 2 groups; each group contains 2 replicates (10 fish / each replicate). First group was inoculated I/M with 0.2 ml of 1/10 LD_{50} of tested bacteria. The second group was inoculated I/M with 0.2 ml / fish of normal saline and served as a control group. All infected and control fish were observed daily to record their general health condition, clinical signs and mortalities. Experimental period was 28 days. Postmortem examination was performed on dead fish. The survivors at the end of the observation were sacrificed and examined for postmortem changes and specimens for histopathological studies were collected.

3. Haematological examination

Differential leucocytic count

Blood film was done according the method described by ***Lucky (1977)***. The percentage and absolute value for each type of cells were calculated according to ***Schalm (1986)*** .

Determination of phagocytic activity and phagocytic index

Phagocytic activity was determined according to ***Kawahara et al. (1991)*** .

$$\text{Phagocytic index (PI)} = \frac{\text{No.of yeastcells phagocytized}}{\textit{No.of phagocytic cells}}$$

WBCs, RBCs counts and Packed cell volume (PCV %)

Were determined according to ***(Stoskopf, 1993)*** .

Blood hemoglobin

Blood hemoglobin (Hb gm %) was assessed by cyanomtahemoglobin method ***(Drubkin, 1964)***.

Haematocrite value

According to ***(Blaxhall and Daisley, 1973).***

Clinico-biochemical analysis

1) Determination of serum total protein

Serum total protein was determination according to ***Doumas et al. (1981)*** using commercial kits produced by Pasteur Lab.

2) Determination of serum albumin

Serum albumin was determined according to ***Reinhold (1953)*** using commercially available kits of Chemroy.

3) Determination of serum globulin

Serum globulin was determined by subtract the total serum albumin from total serum protein according to ***(Coles, 1974).***

Protein fractions determination

Blood serum was used for the determination of the relative concentrations (%) of major protein fractions of fish serum ***Bossuyt and Sporrow (1998)*** and ***Boyanton, and Blick, (2002)***.

B. Experiment II

Thirty *O. niloticus* were equally divided into three groups.

Antibody titration against Pseudomonas flourecence bacterin :

For determination of antibody titration the design of the experiment as in the following Table :

Treatmemt	No. Of fish
Infected fish+ bacterin	10
Control +ve with bacterin	10
Control –ve	10

Evaluation of potency of prepared vaccine against Pseudomonas flourecence

Bacterin preparation

Pseudomonas flourecence isolate was used in the bacterin preparation according to the method described by *Sakai et al. (1984) and Badran (1990).* The preparation of bacterin for injection was carried out according to the method of *Badran (1990).* The formalin inactivated bacterin cells were mixed with an equal volume of 0.85% sterile saline . Bacterial number was adjusted to Macferland's No. 2 (approximately 6 X 10^8 cells / ml).

Antibody titration against Pseudomonas flourecence bacterin

Detection of immune response to *Pseudomonas flourecence* was evaluated by microagglutination (MA) test according to the method described by Badran (1990).

Challenge test

$$RLP = 1 - \frac{\%\ mortality\ of\ vaccinated\ fish}{\%\ mortality\ of\ control}\ x\ 100$$

According to *Newman and Majnarich (1982).*

4) Statistical analysis

The data of hematological and biochemical examinations of exposed fish were statistically analyzed using t-test, Duncan-test after ANOVA and simple correlation according to (SAS, 1987).

RESULTS

1. Results of Clinical and postmortem lesions in experimentally infected fish

The experimentally infected fish species (*O. niloticus*) with the strains of *Pseudomonas fluorescens* showed the following clinical signs : paleness coloration of the body , loss of eye , ascitis, scale loss and exophthalmia, fin and tail rot, darkness coloration of the body and tail rot.

2. Post mortem findings for experimentally infected fish

Internally, organs are friable and have a generalized hyperemic appearance ; the kidney and spleen are swollen ;and the liver is often mottled with hemorrhage increased with light areas. The enlarged abdomen with ascitis . The body cavity contain a clear fluid but more often the fluid is bloody and cloudy. congestion of all internal organs especially gills ,liver and kidneys with necrosis of liver and kidneys (Fig. 1, 2 and 3).

3. Effect of Pseudomonas fluorescens infection on differential leucocytic counts, Phagocytic activity and Phagocytic index, T.WBCs and T.RBCs.

Table (1) indicated the significant effect of ***Pseudomonas fluorescens*** infectoion ($P < 0.01$) on differential leucocytic counts in ***O. niloticus*** fish at different weeks of infection.

The lymphocyte showed a tendancy to decreasing from the 1st week to the 4th of the experiment than that of the control group . While, the monocyte level decreased from the 1st week to the 3rd week of the experiment but at the 4th week of the experiment it increased and returned to its normal level.

Fig. (1) : O. niloticus *infected with* ***P. Flourescence*** *at 2nd week showing unilateral exophthalmia .*

Fig. (2) : O. niloticus infected with P. Flourescence 3rd week showing skin errosion and eye loss .

Fig. (3) : O. niloticus infected with P. Flourescence at 1st week showing congestion of all internal organs .

The results in Table (1) indicated the significant effect of ***Pseudomonas fluorescens*** infectoion ($P < 0.01$) on Phagocytic activity and Phagocytic index in ***O. niloticus*** fish at different weeks of infection.

The Phagocytic activity increased progressively from the 1st week to the 4th week of the experiment in infected fish and the maximum level of Phagocytic activity observed at the 4th week of the experiment but in the infected group lower than that of the control group.

The Phagocytic index, also increased progressively from the 1st week to the 4th week of the experiment but in control group higher than that of infected group.

The results in Table (2) indicated the significant effect of ***Pseudomonas fluorescens*** infectoion ($P < 0.01$) on T.WBCs and T.RBCs in ***O. niloticus*** fish at different weeks of infection.

Table (1) : ***Effect of Pseudomonas infection on differential leucocytic counts, Phagocytic activity, phagocytic index, T. WBCs and T. RBCs counts at different weeks of experiment .***

Week	Group	Lymph	Monocyte	PA	PI	T. WBCs	T. RBCs
1st week	Infected	BC 46±3.40	C 12±2.20	C 16±3.55	B 1.4±0.04	C 25±5.22	C 1.3±0.55
	Control	D 44±4.40	D 10±1.20	B 19±4.55	A 1.6±0.05	B 27±3.77	C 1.4±0.33
2nd week	Infected	CD 45±4.40	D 10±1.20	B 18±3.77	AB 1.5±0.06	BC 26±5.44	BC 1.5±0.33
	Control	AB 47±4.30	A 15±3.50	B 19±3.41	A 1.7±0.07	AB 28±5.22	C 1.4±0.44
3rd week	Infected	C 45±4.50	D 10±1.3	B 19±3.56	A 1.6±0.06	BC 26±6.22	AB 1.6±0.55
	Control	A 48±4.60	BC 13±1.20	A 21±4.53	A 1.7±0.07	BC 26±5.33	A 1.7±0.52
4th week	Infected	D 44±4.33	C 12±1.20	AB 20±5.22	AB 1.5±0.05	A 28±4.88	AB 1.6±0.52
	Control	A 48±4.22	AB 14±1.22	AB 20±5.22	B 1.4±0.04	A 29±4.55	A 1.8±0.43

Means within the same column of different letters are significantly different at (P < 0.01).

The T.WBCs increased progressively from the 1st week to the 4th week of the experiment in infected fish and the maximum level of T. WBCs observed in the infected fish at 4th week of the experiment, but its level lower than that of the control groups .

While, the T.RBCs also increased progressively from the 1st week to the 4th week of the experiment, but its level lower than that of the control groups.

4. Total protein, albumin, globulin and albumin / globulin ratio level in different fish groups at different periods :

The results in Table (2) showed that, the total protein, albumin, globulin and albumin/globulin ratio differ significantly among infected and control group and among different weeks.

The total protein level increased progressively from the first week to the last week and the level of total protein in infected group lower than that of control group. While the albumin level showed increasing then decreasing level of albumin and in the 4th week it increased and the albumin level in infected groups lower than that of the control groups. While, the globulin level not show any significant difference among the control and infected groups but its level in the 1st weeks lower than that of the last weeks.

The albumin/globulin ratio showed that, its level decreased gradually from the first week to the last week but in the last week it return to its normal level. But, the albumin globulin ratio level in infected group lower than that of the control group all over the period of the experiment.

5. Effect of P. Flourescence infection on serum T- globulin level (α - globulin, β -globulin and γ -globulin) at different weeks of experiment

Fig. (4, 5, 6 and 7) indicated the significant effect on total serum globulin fractions (α - globulin, β -globulin and γ - globulin) levels among different treatment groups at different weeks of the experiment.

Table (2) : ***Total protein, Albumin, globulin and albumin/globulin ratio level in different fish groups at different periods.***

Time	Group	Number	Total protein	Albumin	Globulin	Albumin/globulin ratio
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st week	Infected	3.00	G 4.30±1.30	E 2.40±0.45	D 1.90±0.74	D 1.27±0.20
	Control	3.00	D 4.60±1.60	B 2.70±0.70	D 1.90±0.74	B 1.42±0.40
2nd Week	Infected	3.00	F 4.40±1.30	C 2.60±0.65	E 1.80±0.45	AB 1.45±0.42
	Control	3.00	E 4.50±1.44	B 2.70±0.74	E 1.80±0.43	A 1.5±0.500
3rd Week	Infected	3.00	C 4.70±1.45	2.40±0.74	A 2.30±0.44	F 1.04±0.30
	Control	3.00	B 4.90±1.49	C 2.60±0.61	A 2.30±0.43	E 1.14±0.25
4th Week	Infected	3.00	D 4.60±1.47	D 2.50±0.51	C 2.10±0.31	1.20±0.22
	Control	3.00	A 5.10±1.52	A 2.90±0.71	B 2.20±0.20	C 1.31±0.23

Means within the same column of different litters are significantly different at (P < 0.05)

The serum globulin level increased in the 3rd and 4th of the experiment and the level of serum globulin increased in control group than infected one all over the period of the experiment.

The α - globulin level increased in infected group than that of the control group.While, the β -globulin level decreased in infected group than that of the control group. The level of γ -globulin level decreased in infected group than that of the control group. In general our results cleared that, infected groups of lower serum globulins level than control group.

6. Antibody titer (logarithmic value) of Pseudomonas fluorescens in different fish groups at different periods :

Table (3) showed that, the antibody titer level differ significantly ($P < 0.01$) among infected and control groups and among different weeks of the experiment.

The antibody titer level in control groups higher than the infected groups and its level in in the 1st and 4th week at the same level while at 2nd and 3rd weeks it of higher value.

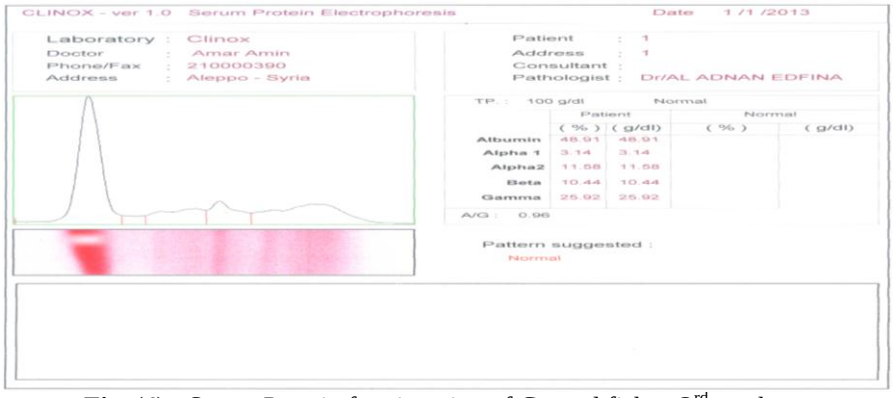

Fig. (4) : Serum Protein fractionation of Control fish at 3rd week

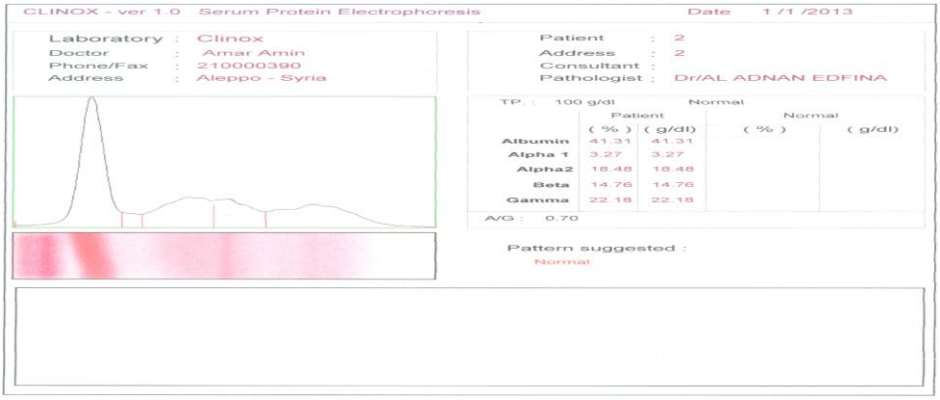

Fig. (5) : Serum Protein fractionation of ***P. Flourescence*** infected fish at 3rd week

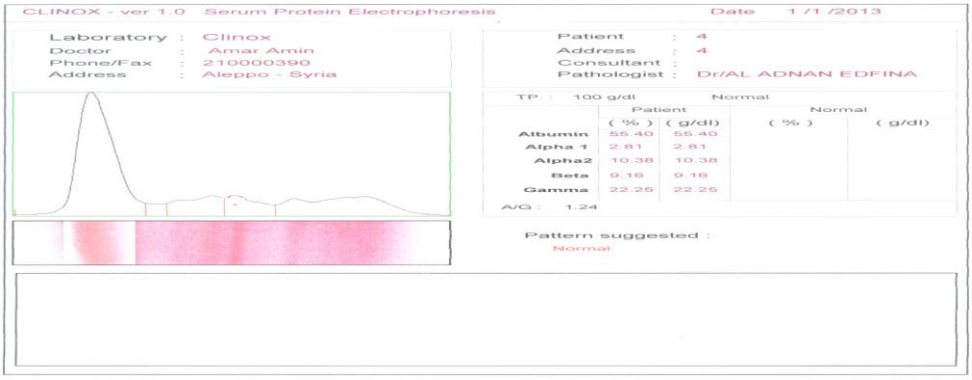

Fig. (7) : Serum Protein fractionation of ***P. Flourescence*** infected fish at 4th week

Table (3) : *Antibody titer (logarithmic value) of* ***Pseudomonas fluorescens*** *in different groups at different weeks.*

Time	Group	Number	Antibody titer
			Mean Std. Error
1st week	Infected	3.00	A 0.477±0.07
	Control	3.00	B 0.60±0.04
2nd Week	Infected	3.00	B 0.60±0.05
	Control	3.00	B 0.60±0.01
3rd Week	Infected	3.00	A 0.477±0.07
	Control	3.00	C 0.69±0.06
4th Week	Infected	3.00	A 0.477±0.07
	Control	3.00	B 0.60±0.03

Means within the same column of different litters are significantly different at (P < 0.05)

Table (4) : ***Mortality % and RLP in different fish groups at different periods.***

Groups	Mortality	RLP
Infected + Vaccinated	6	1 – 6/8 =2/8 = 25 % C
Control +ve	5	1 – 5/8 = 3/8 = 37.5 % B
Control –ve	8	0 A

Means within the same column of different litters are significantly different at (P < 0.05)

7. Relative level of protection against Pseudomonas fluorescens in different fish groups at different periods :

Table (4) showed that, the relative level of protection differe significantly (P < 0.01), the higher level of protection observed in infected + vaccinated group, followed by control +ve group and finally the control –ve group.

DISCUSSION

Fish diseases due to bacterial infections are the major problems in aquaculture as it found naturally in the fish environment and under certain strees condition causes severe economic losses to fish farms *(Olsson et al., 1998)* .

In experementally infection with *P. Flourescence* the clinical signs were manifested as, loss of scales from some areas of the body with excessive mucus allover the body surface and petechial haemorrhages over the body .

The clinical signs & P.M. lesions mainly due to septicaemic effect of *P. Flourescence* infection and its endotoxin which affecting body of fish , these observation were partially similar to those reported by *(Hicks, 2008)* .

El-saka (2006) reported that there was a high mortality percentage among the experimentally infected fish under different stress conditions. These conditions were overcrowdness, external parasitism and transportation with mortality rates of 83%, 70% and 60% in *O. niloticus*, respectively, and all of these stress conditions facilitated the infection with *P. Flourescence* .

The present results cleared that the Effect of Pseudomonas fluorescens infection on differential leucocytic counts:-

The lymphocyte showed a tendancy to decreasing from the 1st week to the 4th of the experiment than that of the control group.While, the monocyte level decreased from the 1st week to the 3rd week of the experiment but at the 4th week of the experiment it increased and returned to its normal level.

Dovale et al. (2002) reported that neutrophils and macrophages are important phagocytic cells depending on the opportunity to encounter the invading agent, macrophages from peritoneal exudates showed a greater capacity for engulfing bacteria *Esteban et al. (1977)* .

In the present work injection of *P.Flourescence* increased the neutrophils count at the first week and significant decrease in its count in the second week.

Also, the current investigation agreed with the result of *Siwicki and Dunier (1993)* . In the present study there was increase in both lymphocytes and monocytes especially with increase of total leucocytic count at the first weeks of infection and this mainly due to the direct effect of stress on the immune response and activation of first line of defense to resist the infection through cellular immune response.

Lymphocyte may performs T-cell equivalent or as natural killer cells *(Ellis, 1981)*

The T.WBCs increased progressively from the 1st week to the 4th week of the experiment in infected fish and the maximum level of T. WBCs observed in the infected fish at 4th week of the experiment, but its level lower than that of the control groups.

These results nearly agreed with those of *(Fumihiko et al., 2008)* where they reported that fish fed bacteria of causative agent of disease show neutrophilia and in general there was a leucocytopenia.

Also these results agreed with those of *(Fernandez et al., 2003)* indicated that the Pseudomonas produces products which causes lysis and destruction of RBCs and reduces its number and its Hb content.

The Phagocytic activity and index increased progressively from the 1st week to the 4th week of the experiment in infected fish and the maximum level of Phagocytic activity observed at the 4th week of the experiment but in the infected group lower than that of the control group.

The significant (P< 0.01) decrease of phagocytic activity and phagocytic index of *Pseudomonas florescence* infected fish than the control fish may be attributed to the destructive action of this pathogen on liver, kidney, spleen and other haemopiotic organs *(Thampuran et al., 2008),* so it causes leucocytopenia and decrease the phagocytic activity and phagocytic index.

This suppression may be mediated directly via the corticosteriod receptors on macrophages or indirectly through the enhanced production of certain factors by the macrophages themselves, which suppress the secretion of other macrophage products (e.g. α-2 macroglobulin) *(Pickering et al., 1981 and Thampuran et al., 2008*).

The total serum proteins were useful in diagnosis of fish diseases *(Mulcahy, 1967).* In the present work, significant decrease in albumin, globulin, total protein and albumin/globulin (A/G) were recorded .

The results also showed that, the total protein, albumin, globulin and albumin/globulin ratio differ significantly among infected and control group and among different weeks.

Infection which causes liver damage that causes decreases of serum protein concentration *Lee and Marks (2009).*

(Dennis et al., 2008) stated that chronic liver disorder is usually accompanied by hypoalbuminaemia. Both hypoglobulinaemia and hypoalbuminaemia confirmed the recorded hypoproteinaemia, which was associated with liver damage .

Naglaa (2004) mentioned that, the mechanisms causing reduction of total serum protein due to bacterial infection was not clear but may be due to the following processes :

1. Loss of protein through vascular leaking caused by increased permeability due to histamine release *Ellis (1981)* .
2. Impaired synthesis of serum proteins due to liver damage and anorexia in diseased fish. *Evenberg et al. (1986)* .
3. Non specific proteolysis of serum proteins. *Ellis (1981).*

The antibody titer and RLP the antibody titer in the groups treated with *P. Flourescence* only of lower RLP than that of the control groups .

This results attributed to the action of bacterial toxin or whole bacteria and extra-cellular products on liver, kidney, spleen and other haemopiotic organs as considered as a stress factor on this organs *(Fucks et al., 1986 and Gekle et al., 1998),* which causes decreases of the relative level of protection of the fish against any stress conditions as well as fish infected with *P. Flourescence (El-Gamel, 2005)* .

REFERENCES

Badran, W.B. (1990) : Studies on Pseuodmonas species isolated from farmed animal. Zagazig. Vet. J., 21 (1): 37.

Blaxhall and Daisley, (1973) : Vibrio cholerae Non-01 isolated from Ayu fish (Plecoglossus altivelis) in Japan. Applied and environmental microbiology .Vol. 58, N9: 3078-3082 .

Bossuyt, T.J. and Sparrow, R.A.H. (1998) : The bacterial flora in the alimentary tract of fresh water salmonid fishes Can.J.Microbiol, 20: 1219-1228.

Boyanton, B. and Blick, K.E. (2002) : Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. L. I. N. Biochem. 6, 24.

Coles, E. H. (1974): Veterinary clinical pathology. PP. 211 – 213. W. B. Saunders Company, Philadelphia, London, toronto.

Dennis M; T, Yoshida; S, Atsuta and M, Kobayashi. (2008) : Enhancement of resistance to Vibriosis in rainbow trout, Oncorhynchus mykiss (walaum), by oral administration of Clostridium butyricum bacterin. Journal of Fish Diseases, 18, 187 – 190.

Doumas, D. T. N.; Simon, Yassibanda.; Phang, L. N. T.; Marcel, R. K.; Malika, Gouali. (1982): Enteropathogenic Escherichia coli O157 in Bangui and N' Goila, CVenteral African Republic : a brief report. Am. J. Trop. Med. Hyg., 75(3), 2006, pp. 513-515.

Dovale, M. K; T, Kitao, L. S, Raungpan and T, Yoshida (2002) : First record of association of A. hydrophila with mortalities. Fisheries Sci. J. 1 (2), 90 - 95.

El-Gamel, J. T. (2005) : Abnormalities of plasma enzymes. In: Biochemistry in clinical Practice, D. L. Williams and V. Marks, (eds.). William Heinemann, London: PP. 221-250.

Ellis , A. E. (1981): Serodiagnosis and vaccines, Lee town, W. Va, U.S.A. Develop. Biol. Standard. 49: 337 – 352. Karger, Based in International Symposium on Fish Biologics.

El-Saka, A. G. (2006) : Bacterial management strategies for stock enhancement of warm water marine fish: a case study with common snook (centropomus undecimalis). Bulletin of Marine Science. 62, 573 - 588.

Esteban, A. M.; Kilen, J. W.; Hejbol, E. K.; Zinck, T.; Varming, K. and Byatt, A. L. (1977) : Growth performance and respiratory burst activity in rainbow trout treated with growth hormone and vaccine. Fish & Shellfish Immunology. 7, 297 – 304.

Evenberg, H. and Egusa, S. (1986) : Characteristic of Pseudomonas species from an epizootic of pond cultured cells (Anguilla Japanica). Bulletin of the Japanese Society of Specific Fisheries, 38: 577-587.

Fernandez, G.; Nussel, E.; Arrbetsrred, S. and Praventive, M. E. D. (2003) *:* Determination of cholesterol in blood. Ann. Clin. Biochem. 1. 10: 25.

Floyd, R. F (2003): Introduction to fish health management. Department of fisheries and aquatic sciences ,Florida Cooperative extension service , Institute of food and agricultural sciences, University of Florida , circular 921.

Fucks, W.; Kulow, H. and Schreckenbach, K. (1986) *:* Fish diseases . Vol. 1, 5th Ed., A. A. Balkema / Rotterdam.

Fumihiko K.; Midori H.; Tetsuya H.; Katsuhiko O.; Kanji S.; Takashi M. and Norio O.; (2008) : Molecular typing of Japanese Escherichia coli O157 : H7 isolates from clinical specimens by multilocus variable-number tandem repeat analysis and PFGE . J Med Microbiol 57 (2008), 58-63; DOI: 10.1099/jmm.0.47213-0

Gekle, M.; B. Gassner.; R. Freudinger.; S. Mildberger.; S. Silbernagl.; W. Pfaller and and H. Schramek. (1998): Characterization of an ochratoxin -A- dedifferentiated and cloned renal epithelial cells line. Toxicol. Appl. Pharmacol. Sept.; 152 (1): 282-91.

Hicks, K. M. (2008) *:* Fish Medicine. W. B. Saunders Compony, Harcourt Brace Jovanovich, Inc.

Kawahara, E. ; T. Ueda and S. Nomura. (1991): In vitro phagocytic activity of white-spotted shark cells after injection with Aermonas salmonicida extracellular products. Gyobyo Kenkyu, Japan, 26 (4): 213-214.

Khalil, R. H ; S. T. Atallah, M. K. Soliman; S. G. M. Ismail and N. Mahfouze (2001): Economic losses due to fish diseases at the farm level. Aquaculture Europe 2001- [New Species, New Technologies] Trondheim, Norway, August 3-7, 2001.

Lee, R.J., and Marks, L.G. (2009) : Mycotic aspects of epizootic ulcerative syndrome (EUS) of Asian fishes. Journal of fish Diseases 16(3):169-184**.**

Lucky, (1977) : Handbook of histopathologic and histochemical staining. 3rd Ed., Buterworth London . ***Lysenko, O (1961):*** Pseudomonas on attempt at general classification. J. of Microbiol. 25, 379.

McVicar P. T. (1982) : Fish Diseases and disorders. Vol. 3 Viral, Bacterial and Fungal infections edited by P. T. K. Woo, New York, USA.

Mulcahy, E. D. (1967) : Notes on a bacterial disease of rainbow trout in a Colorado hatchery. Progressive Fish Culturist, 13: 139.

Naglaa, M. F. (2004) : Occurrence of Antibiotic-Resistant and Plasmid DNA Harbouring Bacterial Pathogens in Stressed Polluted Water Environment of Lake Manzala, Egypt Research Journal of Microbiology Year: 2009 | Volume: 4 | Issue: 2 | Page No.: 59-66.

Newman, S. R. and Majnarich K. M. (1982) : Molecular diversity and growth features of Flavobacterium columnare strains isolated in Finland. Diseases of Aquatic Organisms, 70:55-61

Olsson, J. C. ;Joborn ,A.; Westerdahl , A. ; Blomberg , L.; Kjelleberg ,S .; and Conway , P.L. (1998): Survival ,persistence and proliferation of V. anguillarum in juvenile turbot ,Scophthalmus maximus (L.) intestine and faeces . Journal of fish diseases ,21 ,1-9

Pickering, G.; Nussel, and Praventive, M. E. D. (1981): Determination of cholesterol in blood. Ann. Clin. Biochem. 1. 10: 25.

Reinhold, R. R. (1953): Determination of serum albumin. Clin. Chem., 21: 1370-1372.

Sakai, N.; Surendraraj, A. and Surendran, P. K. (1984) : Prevalence and characterization of typical and atypical *Escherichia coli* from fish sold at retail in Cochin, India. Central Institute of Fisheries Technology, Matsyapuri P.O., Cochin - 682 029, India. Journal of Food Protection.

SAS. (1987): Statistical analysis system. User's Guide Statistics. SAS Institute Cary, North Carlina.

Schalm, O. W. (1986): Veterinary hematology. 4th Ed., Lea and Febiger, Philadelphia.

Siwicki, A. D. and Dunier, A. M. (1993) : Transmission to eels, portals of entry, and putative reservoirs of Vibrio vulnificus serovar E. (biotype 2). Appl. Environ. Microbiol. 67, 4717 – 4725.

Stoskopf, K. M. (1993): Fish Medicine. W. B. Saunders Compony, Harcourt Brace Jovanovich, Inc.

Thampuran, N.; Surendraraj, A. and Surendran, P. K. (2008): Prevalence and characterization of typical and atypical Escherichia *coli* from fish sold at retail in Cochin, India. Central Institute of Fisheries Technology, Matsyapuri P.O., Cochin - 682 029, India. Journal of Food Protection.

بعض الدراسات على عدوى السودوموناس فى أسماك البلطى النيلى المستزرعة

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أمراض الأسماك البكتيرية تعتبر من أهم أنواع الأمراض المنتشرة فى البيئة المائية التى تصيب الأسماك وتؤثر على الناحية الاقتصادية فتم إجراء هذه الدراسة لتقييم عترة السودوموناس فلوريسنس ودراسة تأثيرها على مختلف مقاييس الدم والأنزيمات .

تم فحص وتصنيف وعزل بكتيريا السودوموناس فلوريسنس وتسجيل الأعراض المصاحبة لها خارجيا وداخليا والتى تتلخص فى فقدان القشور واحتقانات داخلية وخارجية وتآكل الزعانف واحمرار فى مختلف أجزاء الجسم .

أما بالنسبة لقياسات الدم فوجد بها نقصان على طول التجربة المزمنة من الأسبوع الأول إلى الأسبوع الرابع . أما بالنسبة لأنزيمات الدم فوجد بها تغيرات تدريجية سواء بالنقصان أو الزيادة على طول التجربة أما بالنسبة لاختبار الأجسام المضادة واختبار التحدى فكان أعلى فى المجموعة الضابطة عنها فى المجموعة المصابة ببكتيريا السودوموناس فلوريسنس .