Advanced Studies for Diagnosis of Aeromonas Septicemia in *Oreochromis Niloticus*

Eissa I. A. M., Maather El-lamei, Takwa Isamil*, Fatma Youssef*,Shimaa Mansour*

*Dept. of Fish Diseases and Management, Fac. of Vet. Medicine, Suez Canal Univ. *: Animal Health Research Institute, Ismailia branch.*

Abstract

A total number of two hundreds (200) naturally infected and freshly caught *Oreochromis niloticus* were collected randomly and seasonally from western lagoon (abranch from Lake Temsah in Ismailia governorate). The clinical picture of septicemia in the affected *O. niloticus* were observed and examined to isolate and identify the bacteria causing motile aeromonas septicemia. The most isolated bacteria was *Aeromonas sobria* which identified by using traditional methods and Vitek 2 system. Also confirmed by detection of 16s rRNA gene using PCR. The virulence of *A. sobria* was determined by detection of hemolytic activity on blood agar and hemolysin gene using PCR. The total prevalence of *A. sobria* in *O. niloticus* was 63%, with the highest prevalence in summer (92%) followed by spring (80%) then autumn (60%) while the lowest prevalence was in winter (20%). *A. sobria* was isolated with high prevalence from kidneys, liver, spleen and intestines at rates of 25.39, 23.02, 19.84 and 15.08 % respectively. The lowest prevalence was recorded from gills and skin lesions as 10.32 and 6.35 % respectively. The experimentally infected *O. niloticus* by (I/P) showed the same picture recorded in the naturally infected fish. The mortality rate and reisolation of the injected bacteria were tried and recorded.

Key words: *Oreochromis niloticus*, PCR, *Aeromonas sobria*, Prevalence.

Introduction

Without doubt, Nile tilapia, *Oreochromis niloticus* (L.) is considered one of the most important species due to its growth performance and disease resistance so it considered one of the most important and largely cultivated species in Egypt ***(Abdel Rhman et al., 2009).*** The infectious diseases are the most prominent causes that put the lives of fish at risk with consequent negative impact on growth and productivity ***(Eissa et al., 2009).*** Bacterial pathogens are among the most critical etiological agents which lead to mass

mortalities in Egyptian aquaculture ***(Eissa et al., 2010).***

Aeromonas septicemia is a fatal infectious disease of warm and cold-blooded animals often caused by *Aeromonas hydrphila, A. sobria* and *A. caviae* ***(Das et al., 2013).*** They cause a hemorrhagic septicaemia in cultured and wild freshwater fish such as tilapias ***(Yu et al., 2010).*** Motile mesophilic aeromonads were commonly found in freshwater reservoirs and soil. Also, they have been isolated from the gastrointestinal contents of fish, reptiles, amphibia and higher vertebrates causing various diseases under environmental stress conditions as overcrowding, poor water quality, organic pollution, and hypoxia ***(Janda and Abbott, 2010).***

Aeromonas species grow at a wide range of temperatures but were isolated with increase frequency during warmer months ***(Horneman and Morris, 2007) .*** Also, they produce many extracellular enzymes which enhance its virulence and pathogenicity, including nucleases, cytolytic toxins, lipases, lecithinase and amylase ***(Percival et al., 2014).***

VITEK 2 compact system is an advanced method used for rapid identification of Aeromonas species with avoiding false or weak positive and negative reaction which often occurs in routine laboratories and reducing the human faults as probable contamination during handling or incubation and misreading, so it improved the results for identification of Aeromonas species ***(Barry et al.,2003).*** Recently, PCR is an advanced method used for identification of Aeromonas species and specific for the detection of virulence factors. It avoids the time consuming in biochemical and other DNA-based methods ***(Eissa et al., 2015a).*** The present study was planned to investigate the clinical signs and the postmortem lesions of Motile Aeromonas Septicemia in *Oreochromis niloticus*, isolation and identification of the causative agent with traditional and advanced methods, experimental infection with the isolated bacteria and recording the total and seasonal prevalence of MAS disease.

Materials and Methods

Naturally collected fish

Two hundreds (200) of naturally collected freshly caught *Oreochromis niloticus* weighting 200 ±30g. Most showing signs of septicemia (congestion and hemorrhages distributed all over the body surface and fins with skin ulcer and scale detachment).They were collected randomly and seasonally from western lagoon (a branch from Lake Temsah in Ismailia governorate). They were transported in ice box to the wet laboratory of Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University.

Clinical picture

Clinical signs and postmortem examination were adopted according to the methods described by ***Austin and Austin (2007).***

Bacterial isolation and identification

Under complete aseptic condition, fish specimens (lesions of skin, gills and internal organs as liver, kidneys, intestines and spleen) were inoculated over nutrient agar and incubated at 37˚C for 24 hrs. Re-inoculation of cultured bacteria occurred until separated colonies appeared. The suspected purified colonies were picked up for further identification by inoculation of these bacteria on different media as MacConkey agar, RS agar and Aeromonas agar according to ***Austin and Austin (2007).*** Identification of Aeromonas species was done by using biochemical tests and Vitek 2 system (bioMérieux, France). Confirmatory identification was done by detection of 16s rRNA gene using PCR. Virulence of the isolated bacteria was identified by detection of hemolytic activity on blood agar and detection of hemolysin gene using PCR according to ***Eissa et al. (2015a).***

Polymerase Chain Reaction

PCR was used to detect the presence of the haemolysin (Asa1) and 16S rRNA genes in the five isolates of *Aeromonas sobria.* The primers used (Asa1 F: 5`-TAAAGGGAAATAATGACGGC G-3` and Asa1 R: 5`-GGCTGTAGGTATCGGTTTTCG-3` for haemolysin gene at 249bp and A16S F: 5`-GGGAGTGCCTTCGGGAATCAG A-3` and A16S R: 5`-TCACCGCAACATTCTGATTTG-3` for 16S rRNA gene at 356bp ***(Wang et al., 2003).*** Extraction of DNA was performed by heating of bacterial suspension at 100 C° for 4 min. for complete lysis of cell membrane forming DNA suspension. The DNA suspension was centrifuged at 4000 rpm for 4 min at 4C°. Then the supernatant was removed leaving the pellet (contain lysozyme, lysed cell membrane and protein). The DNA purity was measured by spectrophotometer (Jenway apparatus) at 260 / 280 nm optical density (O.D) which should be (1.7 – 2). PCR amplification was performed using master cycler (Eppendorf AG,Germany). The reaction mixture for multiplex PCR according to Biotek corporation company as 10µl of master mix was mixed with 1 µl of each primer (F & R) and 4 µl distilled water to all the tubes and then add 2 µl DNA template of each bacteria to one tube and to the control negative 2 µl distilled water added instead of the DNA reaching a total volume 20µl. Fifty PCR cycles was run under the following conditions ***(Wang et al., 2003)*** as initial denaturation at 95°C for 5 min, followed by 50 cycles of denaturation at 95°C for 0.5 min, annealing of the primers at 59°C for 0.5 min, and primer extension at 72°C for 0.5 min. with final

extension at 72°C for 7 min. Agarose gel electrophoresis was performed in 1.5% agarose at 100 volt for 30 minutes and the gel was placed inside BioSpectrum Imaging System (UVP, AG, Germany) and the photo of gel was saved on specific software of the computer.

Experimental infection

A total number of 40 apparently healthy *Oreochromis niloticus* were collected alive with an average body weight 100±10g and divided into two replicate equal groups (1,2 and 3,4). Each group contained 10 fish and kept in four fully prepared glass aquaria which supplied with dechlorinated tap water and conducted with electric air pump in a water temperature of 25±1°C . They were fed on commercial pelleted feed containing 30% crude protein for one week before injection for acclimation. The first and second groups were served as control and the third and fourth groups were experimentally challenged. They were infected by intra-peritoneal route (I/P) with 0.1 ml saline and 0.1 ml saline containing 1 x 10^7 cells/ml (LD50) of 24 hrs bacterial cultures of *Aeromonas sobria* respectively according to ***Wahli et al. (2005).***They were monitored and observed daily for any clinical signs and mortalities for one week post infection. Postmortem examination was done for dead fish to detect the internal lesions and bacterial reisolation of *Aeromonas sobria* was done from such fish.

Results

Clinical picture

The examined naturally infected *Oreochromis niloticus* showed clinically one or more signs from the following ; hemorrhages all over the body surface, base of dorsal, pectoral and caudal fins and redness around eyes. Characteristic ulcers were observed on skin varied from shallow to deep necrotizing ulcers with detachment of the scales. In most cases, gills were congested and eroded. Abdominal distension, inflamed vent as well as bilateral exophthalmia were seen in some cases. Postmortem findings, were represented as congestion and enlargement of liver, spleen, kidneys , gall bladder and intestines (**Plate,1**).

Bacterial examination

The most isolated bacteria were identified as Aeromonas *sobria* in examined naturally infected *Oreochromis niloticus* by using traditional method and Vitek 2 system. *Aeromonas sobria* was manifested as Gram-negative, rod shaped and pleomorphic. *Aeromonas sobria* colonies were smooth, circumscribed, circular, flat, pale like shaped and translucent on MacConkey agar while it showed yellow colonies on RS agar. On Aeromonas agar, it was green with black center. Biochemically, The isolated bacteria showed motility and gave positive reaction for oxidase, catalase, indole , citrate, starch

hydrolysis, methyl red, vogaus proskauer and gelatin liquefaction.

Results of identification of hemolysin and 16S rRNA genes of pathogenic *Aeromonas sobria* using PCR

Fig. (1) revealed presence of hemolysin and 16S rRNA genes in the selected five isolates of *A. sobria* (these isolates were previously checked for hemolysis on blood agar and chosen according to the highest degree of hemolysis). The five isolates of *Aeromonas sobria* showed bands with hemolysin gene at 249 bp and showed bands with 16SrRNA gene at 356bp.

Prevalence of *Aeromonas sobria* in naturally infected *O. niloticus*

The total prevalence was 63%. The highest prevalence was in summer 92% followed by spring 80 % then autumn 60% while the lowest prevalence was in winter 20% (**Fig,2**).

Prevalence of *Aeromonas sobria* isolates from different organs and tissues of naturally infected *O. niloticus*

Fig (3) showed that *Aeromonas sobria* isolates were isolated from kidneys, liver, spleen and intestines with high prevalence 25.39, 23.02, 19.84 and 15.08 % respectively. The lowest prevalence of *Aeromonas sobria* isolates was recorded from gills and skin lesions at rate of 10.32 and 6.35 % respectively.

Experimental infection

The total mortality rates for the first and second groups which served as control and injected with saline were 0 and 10% respectively. While the total mortality rates for the third and fourth groups post infection with *A. sobria* were 90 and 70% respectively (**Table,1**).

The clinical picture in the experimentally infected *Oreochromis niloticus* was represented as hemorrhages distributed all over the body surface, lower jaw and base of fins, scale detachment and erosion of fins. The internal organs appeared congested and enlarged (liver, spleen, kidneys, gall bladder) (**Plate,1**). The reisolation of isolated bacteria was identified as *A. sobria*.

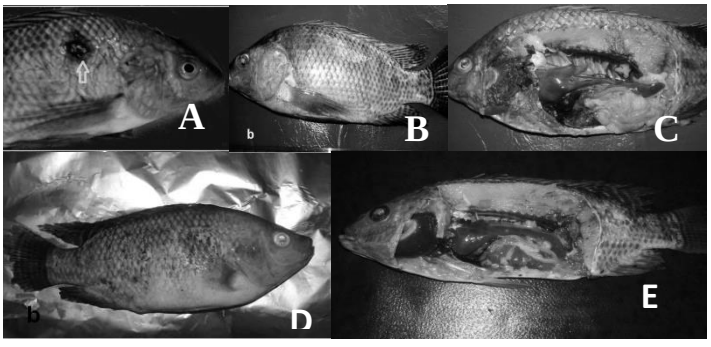

Plate (1): *Showing naturally infected Oreochromis niloticus a) suffered from hemorrhagic ulcer (arrow), b) suffered from hemorrhages all over the external body surface and fins, c) suffered from septicemic lesions in internal organs . Showing experimentally infected O. niloticus d) suffered from hemorrhages distributed all over the body surface and fins, scales detachment and erosion of the fins, e) suffered from septicemic lesions in internal organs.*

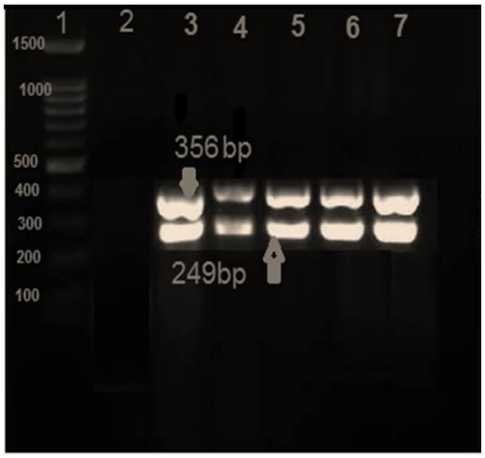

Fig. (1): *Detection and identification of Aeromonas sobria hemolysin (249bp) and 16S rRNA (356bp) genes by amplification of fragments in the multiplex PCR assay. Lanes 1: 100bp ladder (Genedirex); lane 2: negative control. lane 3-7: A. sobria showing the hemolysin and 16S rRNA genes (249 and 356 bp fragments, respectively).*

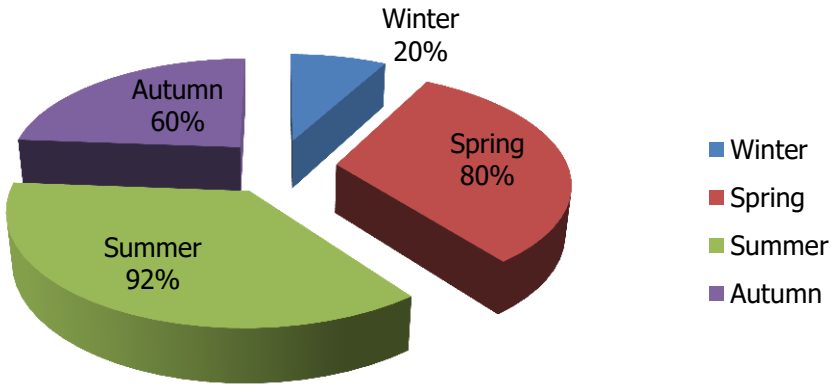

Fig (2) *Seasonal prevalence of Aeromonas sobria infection in examined O. niloticus in different seasons.*

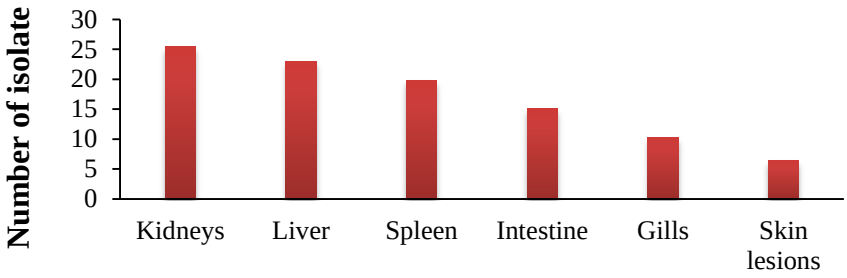

Fig (3): *The prevalence of A. sobria isolates from different organs and tissues of naturally infected O. niloticus*

Table (1) *Mortality rates in experimentally infected O. niloticus with Aeromonas sobria*

Group No.	**Fish No.**	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Total	%
1 (control)	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	0	**0**
2 (control)	**10**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	0	**10**
3 (challenged)	**10**	**0**	**4**	**2**	**1**	**1**	**1**	**0**	**9**	**90**
4 (challenged)	**10**	**0**	**3**	**2**	**1**	**1**	**0**	**0**	**7**	**70**

Discussion

Fish diseases play a role as a limiting factor in fish production and causing mass mortalities and severe economic losses. The most observed clinical signs of naturally infected *Oreochromis niloticus* were congestion and haemorrahges distributed all over the body surface and fins with skin ulcers and scales detachment. These results were nearly similar to that recorded by ***Austin and Austin (2012); Majtán et al. (2012); Roberts (2012); Fard et al. (2014) andEissa et al. (2015a).***Such signs may be due to multiplication of motile Aeromonads inside intestines (motile aeromonads compose a part of the normal intestinal microflora of healthy fish). During stress and changes in environmental conditions and production of extracellular toxins as haemolysin and aerolysin, enterotoxins and enzymes as protease, lipase and gelatinase causing damage of intestinal wall and absorbed from the intestine then traveled through the blood stream to the other internal organs as liver, kidneys and spleen ***(Cipriano, 2001).***

In this study, postmortem examination showed enlargement and congestion in internal organs as liver, anterior and posterior kidneys, spleen and intestines as recorded by

Eissa et al.(2011); Austin and Austin(2012); Roberts(2012); Shayo et al. (2012); Fard et al. (2014) and Eissa et al. (2015a).The liver and kidneys were degenerated by toxins of *Aeromonas sobria* causing destruction in hepatic cells and renal tubules ***(Cipriano, 2001).***

This study revealed that all the isolates of *Aeromonas sobria* produced pale like shaped and translucent colonies on MacConkey agar

as they were non lactose fermenter. Also, they produced on Rimler Shotts agar yellow colonies since they have the ability to ferment maltose that exists in the medium and gave green with black center on Aeromonas agar. The colonies of *A. sobria* on TCBS (thiosulfate citrate bile salt sucrose agar) appeared yellow in colour because its potentiality to ferment sucrose that exists in the medium and on blood agar giving round and B-haemolytic colonies ***(Jayavignesh et al., 2011; Alsaphar and Al-Faragi, 2012 and Eissa et al., 2015a).***

The biochemical properties as citrate utilization, production of H**2**S

on TSI and methyl red were used to differentiate between the four species of Aeromonas (*A. sobria, A. veronii, A. jandaei* and *A. hydrophila). Aeromonas sobri*a showed positive reactions in these tests while the other species were negative as described by ***Eissa et al.(2011); Austin and Austin (2012); Roberts (2012) and Eissa et al.(2015a).***

The results of Vitek 2 compact system, in this study, revealed *A. sobria* was positive for D-cellobiose, L-proline Arylamidase, lipase, β –galactosidase , β-N-acetylglucosaminidase and citrate utilization with no production of H_2S while it was negative for L-Pyrrolydonyl Arylamidase, Glutamyl ArylamidasepNA, Beta-Alanine ArylamidasepNA, Glycine Arylamidase, Glu-Gly-Arg-Arylamidase, Ala-Phe-Pro-Arylamidase, Tyrosine Arylamidase, α–galactosidase, β-N-acetyl galactosaminidase, urease, lysine decarboxylase, ornithine decarboxylase, phosphatase, α-glucosidase, β -glucosidase ,β–glucuronidase, β –xylosidase. These results were in agreement with ***Barrey et al. (2003).***

Hemolysin gene was detected by PCR, which was the virulent factor of Aeromonase species. The hamolysin gene was detected in five isolates. The hemolysin gene (asa1) PCR amplified product size was 249 bp. No bands appeared in any position on the gel except for the predicted bands. Similarly ***Eissa et al. (2015b)*** detected hemolysin gene in the the four chosen isolates of isolated *Aeromonas veronii biovar sobria*. ***Das et al. (2013)*** found that 76.78% of *A. sobria* isolates were positive for asa1 gene of 249bp fragment. Also, a majority of the *Aeromonas biovar sobria* isolates

(67%) carried hemolysin (asa1) genes ***(Wang et al., 2003).***

In the present study, the total prevalence of *A. sobria* in naturally infected *O. niloticus* was 63%. Higher observation was recorded by ***Eissa et al. (2015a)*** who found *Aeromonas biovar sobria* was 86.25%. Also, ***Fard et al.(2014)*** found*A. sobria* in about 85 % of Goldfish *Carassius auratus* during fish mass mortalities and ***Das et al.(2013)*** who identified 77.78% in catfish *Clarias batrachus* showing the signs of Aeromonas septicemia as *A. sobria* and ***Eissa et al. (2011)*** who found 98% of the examined carp showing clinical alteration and infected with *A. sobria*. Similar observations were recorded by ***Sugumar et al., (2002)*** who found *A. sobria* in 60.6% of fingerlings of *Labeo rohita* during the mass mortalities. Lower prevalence was recorded by ***John and Hatha (2013)*** who found *A. sobria* in 40.57% of the examined ornamental fishes. This difference in results may be attributed to fish species, environment, age and total number of the examined fish and time of the study.

The results of this study revealed that the highest prevalence was observed in summer 92% followed by spring 80 % then autumn 60% while the lowest prevalence was in winter 20%. This may be due to increase in temperature which often occur in the summer and spring ***(Tam et al., 2011). I***ncreased temperature act as stress factor on fish so decrease its resistance and increase virulence factors produced by bacteria leading to increase spread of disease during summer and spring ***(Marcogliese, 2008).***

In this study, the *A. sobria* isolates were manifested with the highest prevalence from kidneys by 25.39% and from liver by 23.02%. These results were in agreement with that met by ***Fard et al. (2014)*** who found *A. sobria* was the highest prevalence in kidneys and liver of Goldfish *Carassius auratus* during fish mass mortalities while ***Sugumar et al.(2002)*** found *A. sobria* with the highest prevalence in kidneys and liver of fingerlings of *Labeo rohita* during mass mortalities . Also, ***Wahli et al. (2005) and Majtán et al. (2012)*** who reported that the pathogenic *A. sobria* had identified as a causative agent in diseased *Perca fluviatilis* and *Garra rufa mass* mortalities in Slovakia. Moreover, ***Austin and Austin (2012)*** reported that *A. sobria* was isolated from spawning gizzard shad (*Dorosomace pedianum)* in Maryland. Also, ***Kozińska (2007); Goldschmidt-Clermont et al. (2008); Orozova et al. (2009); Loch and Faisal (2010) and Eissa et al. (2011)*** who isolated *A. sobri*a from common carp, farmed European perch, rainbow trout, whitefish, black and common carp respectively.

The clinical signs observed in the experimentally infected *O. niloticus* with *A. sobria*, in present study, were haemorrahges distributed all over the body surface, lower jaw and base of fins , scales detachment, unilateral and bilateral exophthalmia and erosion of fins. Regarding the postmortem examination, observed in this study in experimental fish it was revealed congested to enlarged internal organs as liver, spleen, kidneys and gall bladder.These results were in agreement with the results of ***Wahli et al. (2005), Eissa et al. (2011), Fard et al. (2014), Anyanwu et al. (2015), Eissa et al.(2015a) and Monir et al. (2015).***

In the present study, the mortality rates in experimentally infected *Oreochromis niloticus* with *A. sobria* by intra-peritoneal route (I/P) were representing 70 -90 % of the total fish. These results were higher than that obtained by ***Anyanwu et al. (2015)*** who reported that the mortality rate of experimentally infected *Clarias gariepinus* with *A. sobria* was 50%.Also, nearly similar to ***Monir et al. (2015)*** who recorded that the mortality rate of experimentally infected Shing (*Heteropneustes fossilis*) with *A. sobria* was 70% and ***Eissa et al. (2011)*** who found that mortality rate of experimentally infected common carp with *A. sobria* by I/P route was 70%**.** Also, ***Wahli et al. (2005)*** recorded that the mortality rate of experimentally infected perch, *Perca fluviatilis L.* with *A. sobria* was 70% within 6 days post infection. These variations may be attributed to the difference in fish species, number, site, and bacterial virulence.

References

Abd El- Rhman, A.M.; Khattab, Y.A.E. and Shalaby, A.M.E. (2009): "Micrococcus luteus and pseudomonas species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus "* Fish Shellfish Immunol., 27: 175-180.

Alsaphar , S. A.A. and Al-Faragi , J.K.H. (2012): "Detection and study of the experimental infection of Aeromonas strain in the common carp (*Cyprinus carpio* L.)" Iraqi Journal of Veterinary Medicine, 36 (2): 222–230.

Anyanwu, M.U.; Chah, K.F. and Shoyinka, V.S.(2015): " Evaluation of pathogenicity of motile Aeromonas species in African catfish" International Journal of Fisheries and Aquatic Studies, 2 (3): 93-98.

Austin, B. and Austin, D.A. (2007): "Bacterial fish pathogens, Diseases of farmed and wild fish" Fourth Edition, Praxis publishing Ltd, Chichester, UK.

Austin, B. and Austin, D.A.(2012):"Bacterial fish pathogens, Diseases of farmed and wild fish" Fifth Edition, Springer Praxis in Aquaculture in Fisheries,

Praxis Publication Ltd., Chichester, UK. 457.

Barry, J.; Brown, A.; Ensor, V.; Lakhani, U.; Petts, D.; Warren, C. and Winstanley, T.(2003): "Comparative evaluation of the VITEK 2 Advanced Expert System (AES) in five UK hospitals" J. of Antimicrobial Chemotherapy, 51: 1191–1202.

Cipriano, R.C. (2001): "*Aeromonas hydrophila* and motile Aeromonad septicemias of fish" Fish Dis., 68: 1- 24.

Das, A.; Rathore, A.; Janani, C.; Hemanth, S. and ArvindBalakrishnan, R. (2013): "Diagnosis of motile *Aeromonas sobria* from catfish with septicemia by PCR" IOSR J. Agri. Vet. Sc., 2 (6): 87- 91.

Eissa, A.E.; Moustafa, M.; El-Husseiny, I.N.; Saeid, S.; Saleh, O. and Borhan, T. (2009): "Identification of some skeletal deformities in some
freshwater teleost raised Egyptian aquaculture" Chemosphere, 77: 419- 425.

Eissa, A. E.; Zaki, M.M. and Abdel Aziz, A. (2010): "Flavobacterium
columnare / Myxobolus tilapiae Concurrent Infection in the Earthen Pond Reared Nile Tilapia (*Oreochromis niloticus*) during the Early Summer" Interdisciplinary Bio Central., 2 (5):1-9.

Eissa, I.A.M.; Badran, A.H.; Azza Abdel-Rhman, M.M. and SomayahAwad, M.M. (2011): "Studies on cultured Black Carp (*Mylopharyngodon picens*) regarding *Aeromonas sobria* septicemia for the first time in Egypt" SCVMJ,XVI(1).

Eissa, I. A.M.; Maather El-Lamie, M.M.; Mona Abdel El-Wahab, M.; Desuky, E.; Mona Zaki, S. and Bakry, M. (2015a): "*Aeromonas veronii biovar sobria* a Causative Agent of Mass Mortalities in Cultured Nile Tilapia in El-Sharkia governorate, Egypt" Life Science Journal, 12 (5).

Eissa, I. A.M.; Maather El-Lamie, M.M.; Mona Abdel El-Wahab, M.; FatmaYousseff, M.; Mona Zaki, S. and Bakry, M.(2015b): "Detection of Hemolysin gene and Antibiogramme of *Aeromonas veronii biovar sobria* Isolated From Mass Mortalities in Cultured Nile Tilapia in El-Sharkia governorate,Egypt" Life Science Journal, 12 (5).

Fard, A. N.; Azadikhah, D. and Abdi, K. (2014) :"Occurrence reported the *Aeromonas sobria* infection in Goldfish (*Carassius auratus*)" Iranian journal congress. at Tehran. 18.

Goldschmidt-Clermont, E.; Wahli, T.; Frey, J. and Burr, S.E. (2008):
"Identification of bacteria from the normal flora of perch, *Perca fluviatilis* L.,and evaluation of their inhibitory potential towards Aeromonas species" Journal of Fish Diseases, 31 (5): 353–359.

Horeman, A. and Morris, J.G.(2007): "Aeromonas infect.

Drug" Microbial infect. Dis., 58-231.

Janda, J.M. and Abbott, S.L.(2010): "The genus Aeromonas: taxonomy, pathogenicity and infection" Clin. Microbiol. Rev., 23: 35–73.

Jayavignesh, V. ;Kannan, K.S. and Bhat, A.D.(2011): "Biochemical characterization and cytotoxicity of the *Aeromonas Hydrophila* isolated from Catfish " Archives of Applied Science Research, 3 (3): 85-93.

John, N. and Hatha, A.A.M. (2013): " Distribution, Extracellular Virulence Factors and Drug Resistance of Motile Aeromonads in Fresh Water Ornamental Fishes and Associated Carriage Water" International Journal of Aquaculture, 3 (17): 92-100.

Kozinska,A. (2007): "Dominant pathogenic species of mesophilic aeromonads isolated from diseased and healthy fish cultured in Poland" Journal-of-Fish Diseases, 30 (5): 293-301.

Loch, T.P. and Faisal, M. (2010): "Infection of Lake Whitefish (*Coregonus clupeaformis*) with Motile Aeromonas spp. in the Laurentian Great Lakes" Journal of Great Lakes Research, 36 (1): 6-12.

Majtán, J.; JaroslavČerny, AlenaOfúkaná, Peter Takáč and Milan Kozánek (2012): "Mortality of therapeutic fish *Garra rufa* caused by *Aeromonas sobria*" Asian Pacific Journal of Tropical Biomedicine, 85-87.

Marcogliese, D.J. (2008): "The impact of climate change on the parasites and infectious diseases of aquatic animals" Revue Scientifique et Technique-Office International desEpizooties, 27: 467-484.

Monir, M. S.; Ahammed, T.; Borty,S.C.; Bagum, N.; Islam, M.A. and Mahmud, Y. (2015): "Pathogenesis of Aeromonas species in Stinging Catfish Shing (*HETEROPNEUSTES FOSSILIS*) of Bangladesh" An International peer-Reviewed Journal**,** 4 (1): 2319-4758.

Orozova, P.; Barker, M.; Austin, D.A. and Austin, B. (2009): "Identification and pathogenicity to rainbow trout, *Oncorhynchus mykiss* (Walbaum), of some aeromonads" Journal of Fish Diseases, 32: 865–871.

Percival, S. L.; Yates, M. V.; Williams, D., Chalmers, R. and Gray, N.(2014): "Microbiology of Waterborne Diseases (Second Edition)" Microbiological Aspects and Risks, 49–64.

Roberts, R.J.(2012): "Fish Pathology Fourth Edition. by Blackwell Publishing Ltd" 367-369.

Shayo, S.D.; Mwita, C.J. and Hosea, K. (2012): "Ulcerative Aeromonas Infections in Tilapia (*Cichlidae: Tilapiini*) from Mtera Hydropower Dam, Tanzania" 1: 115.

Sugumar, G.; Karunasagar, I. and Karunasagar, I.(2002): "Mass

mortality of fingerlings of *Labeo rohita* (Hamilton) in a nursery pond" Indian J. Fish., 49 (3) : 305-309.

Tam, B.; Gough, W.A. and Tsuji, L. (2011): "The impact of warming on the appearance of furunculosis in fish of the James Bay region, Quebec, Canada" Regional Environmental Change, 11: 123–132.

Wahli, **T.; Burr, S.E.; Pugovkin, D.; Mueller, O. and Frey, J. (2005):** "*Aeromonas sobria, a* causative agent of disease in farmed perch, *Perca fluviatilis* L" Journal of Fish Diseases, 28 (3): 141-150.

Wang, G.; Clark, C. G.; Liu, C.;, Pucknell, C.; Munro, C. K.; Kruk, T. M. A. C.; Caldeira, R.; Woodward, D. L. and Rodgers, F. G.(2003): "Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria by* multiplex PCR" Journal of Clinical Microbiology, 41 (3): 1048-1054.

Yu, J.H.; Han, J.J.; Kim, H.J.; Kang, S.G. and Park, S.W. (2010): " First report of *Aeromonas veronii* infection in farmed Israeli carp *Cyprinus carpio* in Korea" J.Fish Pathol., 23 (2) : 165-176.

الملخص العربي

تم تجميع عدد 200 سمكة بلطي نيلي موسميا وبصورة عشوائية بمنطقة بحيره التمساح بمحافظة الإسماعيلية. تم تسجيل الأعراض الظاهرية والصفة التشريحية للمرض في اسماك البلطي النيلي وقد تم فحص الاسماك من اجل عزل وتصنيف البكتريا المسببه لمرض التسمم الدموي الايروموناسي المتحرك. وجد أن معظم البكتريا المعزولة تنتمي لميكروب الايروموناس سوبريا الذي تم التعرف علية بالطرق التقليدية و بجهاز الفيتك2 كما تم التأكد منه عن طريق تحديد جين 16س. تم الكشف عن ضراوة الميكروب وذلك عن طريق الزرع على أجار الدم وكذلك تحديد جين الهيموليسين باستخدام تفاعل البوليميريز المتسلسل. انتشار المرض كان بنسبة 63% وكان أكثر الانتشار في موسم الصيف ثم الربيع ثم الخريف ثم الشتاء بنسبة92 % و 80 % و 60 % و20% على التوالي. الايروموناس سوبريا المعزوله من الاعضاء الداخلية للأسماك بحيث كانت اكثر نسبه انتشار في الكلي و الكبد و الطحال و الامعاء بنسبه 25.39 % و23.03% و 19.84 % و15.08 % ع التوالي و اقل نسبه انتشار في الخياشيم والعلامات الجلدية بنسبه 10.32 % و 6.35 %ع التوالي .لقد تم اجراء العدوي لأسماك البلطي النيلي معمليا بالبكتريا الايروموناس سوبريا المسببه لمرض التسمم الدموي الاليروموناسي المتحرك والمعزولة من الأسماك المصابة طبيعيا و كانت نسبه النفوق حوالي 80% من الاسماك التي تم حقنها .