Mansoura Veterinary Medical Journal

STUDIES ON SOME BACTERIAL DISEASES IN *OREOCHROMIS NILOTICUS* WITH SPECIAL REFERENCES TO TREATMENT

Viola*, H. Zaki.[1], Ahmed*, M.EL-gamal[2]and Yasmin, A.Reyad

[1]**Department of internal medicine, infectious and fish diseases, faculty of veterinary medicine, Mansoura University*
[2]**Animal Research Institute (Mansoura branch).*

ABSTRACT

The present research carried out to study the common bacterial infections in Oreochromis niloticus (Nile tilapia) in Manzala area at Dakahlia governorate and possible antimicrobial agents used for treatment. A total number of 400 fish were randomly collected from Manzala private farms at Dakahlia governorate and subjected to the clinical, bacteriological and histopathological examination.

The highest prevalence of bacterial isolates during the whole period of examination of naturally infected O.niloticus was recorded for A.hydrophila (22.66%), followed by V.alginolyticus (19.01%), V.parahemolyticus (13.80%), Streptococcus spp. (12.24%), A.caviae (11.72%), V.cholera (10.16%), A.salmonicida (7.55%), while the lowest prevalence was recorded for Klebsiella oxytoca (2.86%).

The seasonal highest total prevalence of bacterial isolates from examined naturally infected O. niloticus was recorded in spring (30.21%), followed by autumn (28.39%), then summer (22.40%) and the lowest prevalence was recorded in winter (19.01%).

Histopathological findings of the tissue samples which collected from different organs of naturally infected O.niloticus revealed that spleen show marked hemosiderosis and sever hemorrhage, gills showsever congestion of lamellar capillaries with marked aneurysm, necrosis and hemorrhage of lamellar epithelium and liver show sever hydropic degeneration and necrosis of hepatocytes, Ciprofloxacin was the most effective antibiotic against all isolated bacterial strains.

Keywords: Oreochromis niloticus- bacterial diseases- antibiogram

INTRODUCTION

Tilapia represents the second major group of fresh fish Produced from aquaculture after *Cyprinidae* in the world ***(Mjoun et al., 2010)***. Among tilapia species, the Nile tilapia *(Oreochromis niloticus)* is the most widely cultured fish due to rapid growth, acceptance of natural foods and dietary supplements is easy , propagation is simple, its large size, tolerance to environmental conditions, and resistance to diseases are features that make tilapia a desirable food fish (***Pech et al., 2017)***.

The bacteria that had been associated with lethal consequences in *O.niloticus* were *Aeromonas spp., Vibrio spp.* and *Streptococcus spp. (**Daskalov, 2006; Najiah et al., 2012** and **Marcel et al., 2013).***

Vibriosis is considered one of the most prevalent fish diseases caused by bacteria belong to the genus *Vibrio*; *Vibrio parahaemolyticus, V. alginolyticus, V. cholera (non-O1)* which represent the major fish pathogens ***(Kim and Bang, 2008).***

The most bacterial diseases in freshwater aquaculture, including furunculosis (*Aeromonas salmonicida*) and some types of streptococcosis that responsible of main economic losses in cultured fish worldwide. **(*Toranzo et al., 2005)***.

MATERIALS AND METHODS

A total number of 400 fish (*Oreochromis niloticus)* were randomly collected from private Manzala farms at Dakahlia governorate during the period of October 2015 to November 2016. Fish samples transferred as soon as possible to the laboratory of fish Disease and Management, Faculty of Veterinary Medicine, Mansoura University.

For clinical, post-mortem (P. M) and bacteriological examination, fish were subjected to both clinical and post-mortem examination according to methods described by (***Schaperclaus, 1992***).

Bacteriological examination:

Samples from liver, kidney, spleen and gills cultured on tryptone soya broth **(Oxoid, CM 0129)**.

Identification of bacterial isolates: according to the methods described by ***(Austin and Austin, 2007).***

Biochemical characters using conventional tests:

The biochemical tests used in our investigation were: Catalase test, Oxidase test, Indole test, Voges - Proskauer test (V. P) , Methyl Red test (M.R), Citrate utilization test, Urease test , Sugar fermentation test, H2S production test, Nitrate reduction test, O/129 susceptibility test , and Gelatin liquefaction according to ***(Austin and Austin, 2007).***

API 20NE system for identification of bacterial strains:

Final confirmation of each strain achieved using the identification by using the analytical profile index of API 20NE system ***(Buller, 2004).***

Histopathological Studies:

Tissue specimens were taken from (Liver, kidney, spleen and gills) of naturally infected *Oreochromis niloticus* and gently removed, then fixed in 10% neutral buffered formalin for 24 hours, processed by a conventional method, sectioned and stained with Haematoxyline and Eosin (H&E) in accordance with ***(Roberts, 2012).***

Antibiogram sensitivity test:

The antimicrobial sensitivity of some isolated bacteria species against the following antimicrobial agents (Ciprofloxacin CIP (5mg), Nalidixic acid NA(30mg) , Colistin sulphate CT(25mg), Lincomycin MY(10 ug), Oxytetracycline T(30 mcg), Nitrofuration F(300 mcg), Erythromycin E(15 mcg) , Flumequine UB (30 ug)) were carried out by the disc diffusion method on Muller Hinton agar **(Oxoid CM0337).** Antibiotic discs obtained from **(Oxoid).** Each species of bacteria categorized as Sensitive(S), Resistant (R) or Intermediate (IM) according to the interpretive limits for Clinical and Laboratory Standards Institute ***(NCCLS, 2003).***

Table (1): Total prevalence of bacterial isolates from examined naturally infected *O. niloticus*.

Type of Bacteria	Bacterial isolates	
	No.	%
A. Hydrophila	87	22.66
A. Caviae	45	11.72
A. salmonicida	29	7.55
V. alginolyiticus	73	19.01
V. parahemolyticus	53	13.80
V. cholera	39	10.16
Streptococcus spp.	47	12.24
Klebsiella oxytoca	11	2.86
Total	**384**	**100.00**

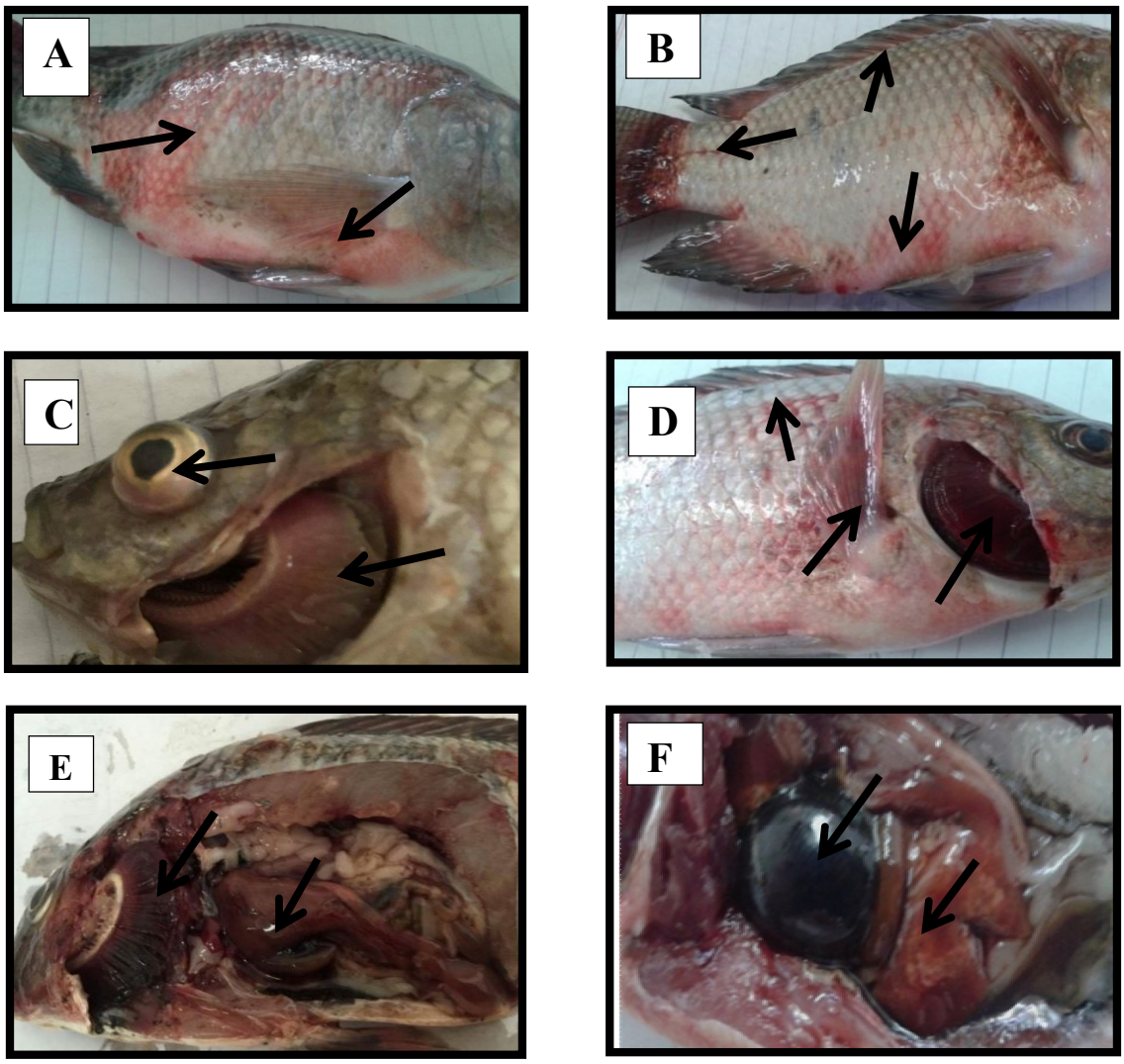
Table (2): Seasonal prevalence of bacterial isolates in naturally infected *O.niloticus*.

Season	Bacterial infection	
	No.	%
Autumn	109	28.39
Winter	73	19.01
Spring	116	30.21
Summer	86	22.40
Total	384	100.00

Table (3): Antibiogram of most important bacterial isolates.

Types of M.O / Type of Antibiotic	*Klebsiella oxytoca*	*Aeromonas salmonicida*	*Vibrio parahemolyticus*	*Streptococcus species*
Ciprofloxacin CIP (5mg)	S	S	S	S
Nalidixic acid NA(30mg)	R	S	S	R
Colistin sulphate CT(25mg)	S	R	R	S
Lincomycin MY(10ug)	R	S	R	R
Oxytetracycline T(30mcg)	R	S	R	S
Nitrofuration F (300mcg)	S	S	R	S
Erythromycin E(15mcg)	S	S	IM	IM
Flumequine UB(30ug)	30mm	39mm	35mm	25mm

R:Resistant S:Sensitive IM:Intermediate



Photo(A) Naturally, infected *Oreochromis niloticus* show hemorrhagic patches on ventral and lateral aspect of fish.

Photo(B) Naturally, infected *Oreochromis niloticus* show hemorrhagic patches on ventral and lateral aspect of fish, dorsal fin, and caudal fin.

Photo(C) Naturally, infected *Oreochromis niloticus* show exophthalmia and pale gills.

Photo (D) Naturally, infected *Oreochromis niloticus* show general congestion of the body including pectoral fin, dorsal fin and the gills.

Photo(E) Naturally, infected *Oreochromis niloticus* show congested dark hemorrhagic gills and congested liver.

Photo(F) Naturally, infected *Oreochromis niloticus* show distended gall bladder and congested liver with hemorrhagic patches.

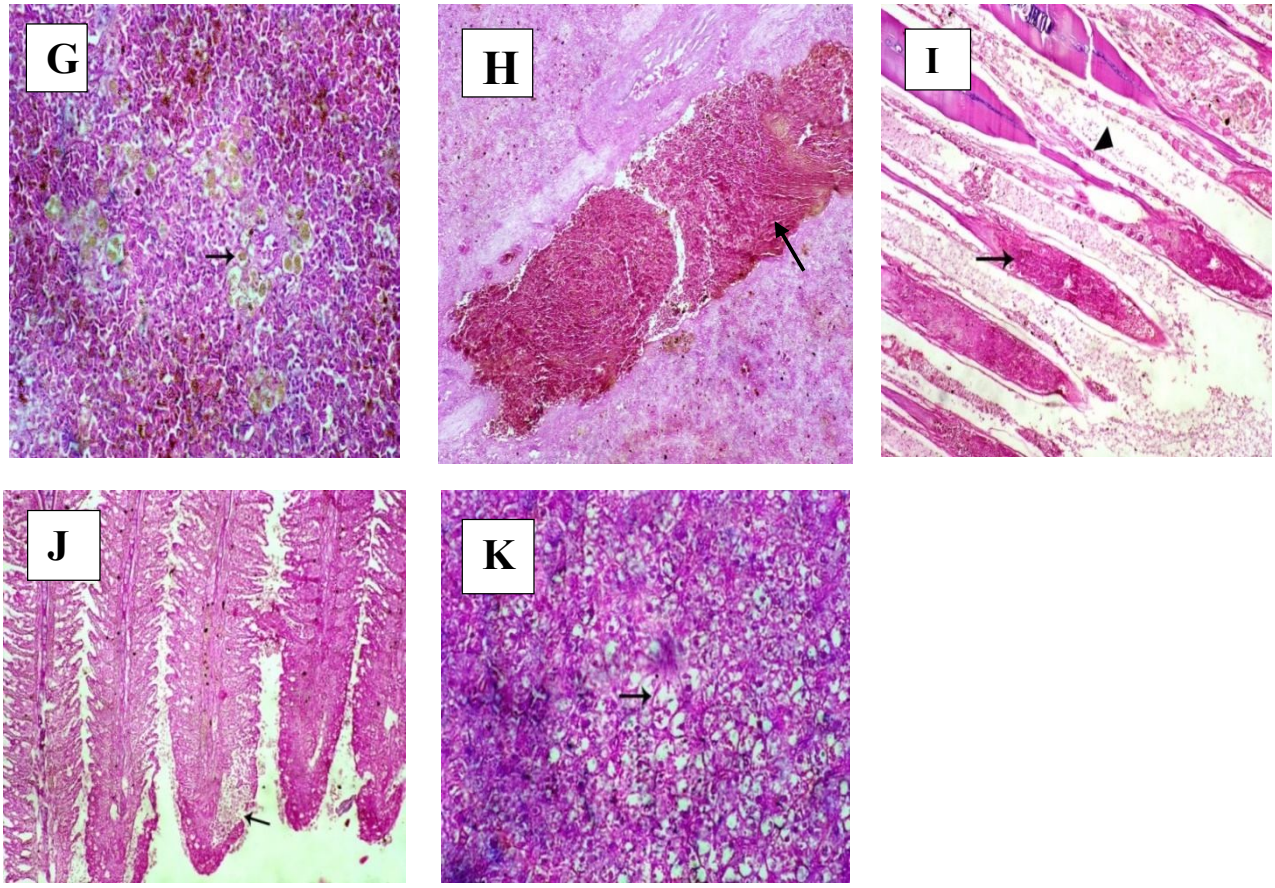


Photo (G) Spleen show marked hemosiderosis (arrow) (H&E 400x).

Photo(H) Spleen showing severe hemorrhages (H&E 100x).

Photo(I) Gills showing sever congestion of lamellar capillaries (arrow) with marked aneurysm (arrow head) (H&E, 100x).

Photo(J) Gills showing necrosis and hemorrhage of lamellar epithelium**.** (H&E, 100 x).

Photo(K) Liver showing severe hydropic degeneration and necrosis of hepatocytes. (H&E, 100 x).

RESULTS & DISCUSSION

In regards to the clinical signs and post-mortem lesions of the examined naturally infected *Oreochromis niloticus* were hemorrhagic patches on ventral and lateral aspect of fish, dorsal fin, caudal peduncle, caudal fin , pectoral fin , dark congested gills, exophthalmia and pale gills, general congestion of the body include gills, the eye and under the lower jaw. **(Photo A, B, C, D)** .Such clinical signs and post-mortem findings were described by (***Toranzo et al., 2005*****;** ***Austin and Austin, 2007;*** and ***Salati, 2011***).

Post-mortem examination results show distended gall bladder and congested liver with hemorrhagic patches as shown in (**Photo E, F)**. Similar post-mortem findings were described by ***(Salvador et al., 2005*** and ***Younes et al., 2016)***.

Interpretation the prevalence of the isolated bacterial isolates the highest prevalence of bacterial isolates during the whole period of examination of naturally infected *O.niloticus* was recorded for *A. hydrophila* (22.66%), followed by *V. alginolyticus* (19.01%), *V. parahemolyticus* (13.80%), *Streptococcus spp*. (12.24%), *A. caviae* (11.72%), *V. cholera* (10.16%), *A. salmonicida* (7.55%), while the lowest prevalence was recorded for *Klebsiella oxytoca* (2.86%) recorded in **(Table 1)**. These results agree with ***Younes et al., (2016)*** who isolated *Vibrio spp*. by prevalence of (87.5%); followed by *Aeromonas hydrophila* by prevalence (25%), also isolate *Streptococcus spp.* and *Klebsiella oxytoca* with few numbers from apparently healthy *O.niloticus* arroud Qarun lake.

Regarding the seasonal highest total prevalence of bacterial isolates from examined naturally infected *O. niloticus* was recorded in spring (30.21%), followed by autumn (28.39%), then summer (22.40%) and the lowest prevalence was recorded in winter (19.01%) recorded in **(Table 2)** These results agree with similar results recorded by ***(Pech et al., 2017)*** who recorded that the highest incidence of bacterial genera occurs in spring. These results disagreed with ***(Salem, 2015; Abd El-Maksoud, 2016)*** and ***(El-Son, 2016)*** who recorded the total prevalence of bacterial infections during different seasons, actually the highest rate of infections (36.94%) was detected in winter season in comparison to (33.49%) in summer season, (17.24%) was detected in autumn and (12.31%) was detected in spring.

Regarding the histopathological findings of the tissue samples collected from different organs of naturally infected *O. niloticus*, our results revealed that spleen show marked hemosiderosis **(Photo G)** and show severe hemorrhage **(Photo H).** Moreover, gills showing sever congestion of lamellar capillaries (arrow) with marked aneurism **(Photo I).** Gills showing necrosis and hemorrhage of lamellar epithelium **(Photo J)** and liver showing severe hydropic degeneration and necrosis of hepatocytes **(Photo K).** Our results were in agreement with (***Hussein et al., 2003*** and ***Rey et al., 2009***).

On overview of the sensitivity of different isolated bacterial species to a number of antimicrobial agents during this study **(Table 3),** our results showed that Ciprofloxacin (CIP) is the most effective antibiotic against different isolated bacterial species. These results agree with (***Abdel- Latif and Sedeek, 2017),*** on the other hand, *Aeromonas salmonicida* and *Vibrio parahaemolyticus* show resistance against to Colistin sulphate (CT). On the other hand, all isolated bacterial species show sensitivity against Nitrofuration (F) except *Vibrio*

parahemolyticus. These results were supported by ***(Abbass et al., 2000*** and ***AL-Othrubi et al., 2014)*** who recorded that ciprofloxacin used as ulternative treatment in severe cases of infection by *V. parahemolyticus.*

REFRENCES

Abbass, A. A.; Shaheen, A. A.; Abd El- Aziz, A. M. and ***Mona, M.S. (2000):*** Clinico-pathological and laboratory investigations on vibriosis in some fishes**. Zag. Vet. J. 28, 115-124.**

***Abd El- Maksoud, S. M.F. A. (2016)*:** Some studies on gill diseases of freshwater fishes with special reference to control methods. **M. V. Sc. Thesis, Fac. Vet. Med. Mans. Univ.**

Abdel- Latif, H.M.R and Sedeek, E.K. (2017): Diversity of enterobacteriaceae retrieved from diseased cultured *Oreochromis niloticus*. **International Journal of Fisheries and Aquatic Studies; 5 (1):29-34.**

Al-Othrubi, S. M. Y.; Kqueen, C. Y.; Mirhosseini, H.; Abdul Hadi, Y. and ***Son, R. (2014):*** Antibiotic Resistance of *Vibrio parahaemolyticus* Isolated from Cockles and Shrimp Sea Food Marketed in Selangor, Malaysia. **Clinical Microbiology 3: 148– 154.**

Austin, B. and Austin, D. (2007): Bacterial fish pathogens: disease of farmed and wild fish. **Springer Science & Business Media.**

Buller, N.B. (2004): Bacteria from fish and other aquatic animals: A practical identification manual. **CAB International publishing Cambridge, Oxoford shire, UK, PP: 361.**

Daskalov, H. (2006): The importance of *Aeromonas hydrophila* in food safety. **Food Control**, **17(6), pp.474-83.**

El- Son, M. A.M. (2016): Effect of Some Environmental Parameters on Spreading of Bacterial Fish Diseases**. M. V. Sc. Thesis. Fac. Vet. Med. Mans. Univ.**

Hussein, M.M.; Ibrahim, E.M. and ***Younis, A.A. (2003):*** Comparative pathological studies on motile aeromonads infection in Oreochromis niloticus with referring to their treatment. **Egypt. J. Comp. Path. & Clinic. Path., 16 (2): 178-192.**

Kim, M. N. and Bang, H. J. (2008): Detection of marine pathogenic bacterial Vibrio species by multiplex polymerase chain reaction (PCR). **J. Environ. Boil. 29:543-546.**

Marcel, G.; Sabri, M.Y.; Siti-Zahrah, A. and ***Emikpe, B.O. (2013):*** Water condition and identification of potential pathogenic bacteria from red tilapia reared in cagecultured system in two different water bodies in Malaysia. **African Journal of Microbiology Research, 7(47), pp.5330-37.**

Mjoun, K.; Rosentrater, K. and ***Brown, M. (2010): Tilapia:*** Environmental biology and nutritional requirements**. South Dakota Cooperative Extension Service. 1-6 pp.**

Najiah, M.; Aqilah, N.; KL, L.; Khairulbariyyah, Z.; Mithun, S.; Jalal, K.; Shaharom-Harrison, F. and ***Nadiah, M. (2012):*** Massive Mortality Associated with *Streptococcus agalactiae* Infection in Cage-cultured Red hybrid Tilapia *Oreochromis niloticus* in Como River, Kenyir Lake. **Malaysia. J. Biol. Sci., 12(8), pp.438-42.**

NCCLS, (2003): Performance standards for

antimicrobial disc susceptibility tests: approved standard, National Committee for Clinical Laboratory Standard of Antimicrobial Susceptibility, Document M2-AS, Pennsylvania, USA.

Pech, Z.G.H.; Chavez, C.M.R. and ***Reynoso, F.L. (2017):*** Pathogenic bacteria in *Oreochromis niloticus* Var. stirling tilapia culture**. Fish Aqua J 8:197. Doi: 10.4172/2150-3508.1000197.**

Rey, A.; Verjan, N.; Ferguson, H. W. and ***Iregui, C.(2009):*** Pathogenesis of Aeromonas hydrophila strain KJ99 infection and its extracellular products in two species of fish. **Veterinary Record, 164, 493-499.**

Roberts, R. J. (2012): Fish Pathology**, 3rd edn.W. B. Saunders, Philadelphia, PA.**

Salati, F. (2011): *Enterococcus seriolicida* and *Streptococcus spp.* (*S. iniae, S. agalactiae* and *S. dysgalactiae*). In Fish Diseases and Disorders. Viral, Bacterial and fungal infections, **Vol 3. CAB International: 375-396.**

Salem, M. A. A. (2015): Studies on some predominant bacterial diseases in some freshwater fish in Dakahlian farms**. M. V. Sc. Thesis, Fac. Vet. Med. Mans. Univ.**

Salvador, R.; Muller, E.E.; Freitas, J.C.; Leonhadt, J.H.; Giordano, L.G.P. and ***Dias, J.A. (2005):*** Isolation and characterization of *Streptococcus* spp. group B in Nile Tilapia (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northen region of Parana State, Brazil. **Ciencia Rural. *Santa Maria*, *35*, 1374-1378.**

Schaperculus, W. (1992): Fish diseases. A. A. **Balkema Rotterdam, (1): 498-503.**

Toranzo, A.E.; Magarinos, B. and ***Romalde, J. L.(2005)*** **:** A review of the main bacterial fish diseases in mariculture systems**. Aquaculture, 246(1):37-61.**

Younes, A.M.; Fares, M.O.; Gaafar, A.Y. and ***Laila, A. Mohamed (2016):*** Isolation of *Vibrio alginolyticus* and *Vibrio vulnificus* Stains from cultured *Oreochromis niloticus* Around Qarun Lake, Egypt**. Global Veterinaria 16 (1): 01-05, ISSN 1992-6197© IDOSI Publications, 2016 DOI: 10.5829/idosi.gv.2016.16.01.10214**.

الملخص العربي

دراسات على بعض الامراض البكتيرية في اسماك البلطي النيلي مع اشاره خاصة للعلاج

*[1]فيولا حسن زكى، *[2]احمد محمد الجمل، ياسمين أحمد رياض

[1]قسم الامراض الباطنة والمعدية والاسماك، كليه الطب البيطري-جامعه المنصورة
[2]معهد بحوث صحة الحيوان فرع المنصورة، مركز البحوث الزراعية

أجريت هذه الدراسة للتعرف على اكثر الامراض البكتيرية انتشارا في اسماك البلطي النيلي في منطقة المنزلة التابعة لمحافظة الدقهلية وحساسيتها تجاه بعض المضادات الحيوية المستخدمة في العلاج وكذلك التغيرات الباثولوجية المصاحبة لهذه الامراض البكتيرية حيث تم تجميع عدد ٤٠٠ سمكه من مزرعة خاصة المنزله في محافظه الدقهلية وبعد الفحص الخارجي والصفة التشريحية والعزل البكتيرى وكذلك الفحص النسيجى للاسماك المصابة بالعدوى البكتيرية تبين من هذه الدراسة ان اعلى نسبه أصابه كانت للايروموناس هايدروفيلا بنسبه (٢٢,٦٦%) ثم الفيبريو الجينولتكس (١٩,٠١%) يليها فيبريو باراهيموليتيكس (١٣,٨٠%)،يليها الاستربتوكوكس (١٢,٢٤%)،ايروموناس كافى (١١,٧٢%)،فيبريوكوليرا (١٠,١٦%)، ايروموناس سالمونيسيدا (٧,٥٥%)، بينما سجلت اقل نسبه أصابه للكليبسيلا اوكسيتوكا بنسبه (٢,٨٦%).بالاضافه الى ذلك تم تسجيل اعلى نسبه أصابه موسميه كليه للبكتيريا المعزولة في اسماك البلطي النيلي كانت في فصل الربيع بنسبه (٣٠,٢١%)، يليها الخريف (٢٨,٣٩%) ، يليها فصل الصيف (٢٢,٤٠%)، بينما سجلت اقل نسبه أصابه بكتيرية في فصل الشتاء بنسبه (١٩,٠١%).

بدراسه التاثير الباثولوجى للبكتيريا المسببه للمرض التي عزلت من الانسجه المختلفه من اسماك البلطى النيلى المصابه طبيعيا كشفت النتائج ان الطحال اظهر نزيف شديد والخياشيم حدث موت للخلايا والتلف الشديد للخيوط الفرعيه وتجمع واحتقان الدم في الاوعيه الدمويه وتنخر وموت الخلايا والانسجه والكبد عن وجود فجوات في الخلايا الكبديه ،احتقان شديد في الوريد المركزى في الكبد وانحلال المياه ونخر في خلايا الكبد ، واظهرت المضادات الحيوية سيبروفلوكساسين لها تأثير فعال في منع نمو جميع الميكروبات المعزولة.