STUDY ON VIBRIOSIS IN *MUGIL CAPITO* IN EL-DAKAHLIA AND DAMITTA GOVERNORATES, EGYPT

El-Bouhy Z.[1]; El-Nobi, G.[1]; Abd elhakeem El-Murr[1] and Samar Abd El-Hakim[2]

[1]*Department of Fish Diseases and Management, Faculty of Vet. Medicine, Zagazig University, Egypt.*

[2]*Animal Health Research Institute, Mansoura, Egypt.*

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Abstract

Vibriosis is considered the most important threatening disease problem facing aquaculture specially *Mugil capito (Liza ramada*). This study aimed to conduct bacteriological study on Vibriosis in different fish farms in El-Dakahlia and Damitta governorates. The isolated bacteria on tryptic soya agar (TSA) with 2% sodium chloride gave circular creamy colored colonies. On thiosulfate citrate bile salt sucrose agar (TCBS) gave yellow colonies for *Vibrio alginolyticus* and gave green colonies for *Vibrio parahemolyticus*. PCR yielded a single specific and clear amplificated band of expected size 737Bp and 387Bp for *V. alginolyticus* and *V. parahemolyticus* respectively. The total prevalence of Vibriosis infection in *Mugilcapito* in study was 56.11%.The highest infection rate was recorded during summer season with prevalence rate 88.88% followed by spring 63.63%, winter 45.90% and the lowest was during autumn with prevalence rate 37.50%.Total prevalence of *Vibrio alginolyticus* among naturally infected *Mugil capito* was 69.76%. While *Vibrio para hemolyticus* in *Mugil capito* was 30.24%.

INTRODUCTION

Mullets are an important component of Egyptian aquaculture and are considered as one of the most important cash crops from artisanal fisheries in the numerous lagoons throughout the country. Mullet has a very wide geographic distribution. The gray or stripped mullet is found in the coastal waters, estuaries and lagoon of tropical and subtropical zones.

Vibrio bacteria occur widely in aquatic environment and are part of the normal flora of coastal seawater and are opportunistic pathogens in many

marine animals (Austin and Austin, 2007). The infectious reservoir in cultured fish were numerous including, apparently healthy fish harboring Vibrios, shellfish, rotifers Invertebrates and scavenger fish feeding around cages (Mizuki *el al.*, 2006).

The genus Vibrio comprises more than 45 species, most of which are widely distributed in marine environment. The Vibrio species affected all type of fish of either marine or freshwater fish all over the world in different areas of Asia, America, Australia, Africa and Europe (Reham, 2009).

Vibrio alginolyticus and *Vibrio parahemolyticus* are responsible for mass mortalities among fish stocks in many marine fish farms throughout the Mediterranean area and severe economic losses in aquaculture worldwide (Snoussi *et al.,* 2008). *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are important halophilic Gram negative pathogens, which cause serious episode to marine fish and shellfish (Marhual *et al*., 2010).

This study was planned to carry out a bacteriological survey on Vibriosis in different fish farms in (El-Dakahlia and Damitta governorate). Also to isolate and identify *Vibrio* isolates encountered in *Mugil capito* fish with molecular identification of isolated strains of *Vibrio*.

MATERIALS AND METHODS

Fish used for field studies:

A total number of 319 *Mugil capito (Liza ramada)* of different body weight ranging 70 - 450g were collected randomly from El-Serw brackish water fish farms (2‰ salinity) (EL-Dakahlia governorate) and El-Ratama fish farms (40‰ salinity) (Damitta governorate) in the period from January 2014 to end of the year during four seasons. Fish were transferred alive to Animal Health Research Institute (AHRI) - Al Mansoura branch and subjected to clinical, post mortem and bacteriological examination for isolation of *Vibrio* spp.

Clinical examination:

External clinical examination was performed using the method described by Schaperclaus *et al*. (1992) and Internal (Post mortem) examination according to Austin and Austin (2007).

Bacteriological examination:

Sampling and primary isolation of bacteria was carried out according to (Noga, 1996), Purification of bacterial isolates (Austin and Austin, 2012), Identification of bacterial isolates through Gram stain (Shape and arrangement of bacteria according to method described by Lucky (1977), Motility test and Oxidase test (Buller, 2004)

Identification of the isolates by API® 20 E: Analytical profile index system according manufacture guide (Buller, 2004), Growth of bacteria in different concentration of sodium chloride, other conventional test: Catalase test, V.P. reaction and Hydrogen sulphide on TSI media

Molecular identification of *Vibrio* by polymerase Chain reaction (PCR):

Procedures for total genomic of *Vibrio sp.* Samples were done according to protocol of Omega Co. (USA.LMt.). The reaction consists of 40 cycles; each cycle consisted of denaturation at 94 ^{o}C for 30 sec followed by annealing at 30^{o}C for 30 sec and extension at 72^{o}C for 30 sec (Di pino *et al.*, 2005). There was an initial delay for 15 min at 95 ^{o}C at the beginning of the first cycle and 10 min delay at 72^{o}C at the end of the last cycle as a post extension step the product was stored at –20 ^{o}C or 4^{o}C. Gel documentation system was applied for data analysis using Total lab analysis software (Geldoc-it, UVP, England).

Table 1. Target gene, oligonucleotide primer sequence and PCR amplicon (Bp) for *Vibrio alginolyticus* and *Vibrio parahemolyticus*.

Gene	Sequence	Molecular weight
Collagenase ***(Di pino et al.,2005).***	F: (5-CGAGTACAGTCACTTGAAAGCC-3)	737 Bp
	R: (5- CACAACAGAACTCGCGTTACC-3)	
PR72H ***(Lee et al.,1995)***	Vp32:(5-CGAATCCTTGAACATACGCAGC-3)	387 Bp
	VP33: (5-TGCGAATTCGATAGGGTGTTAACC-3)	

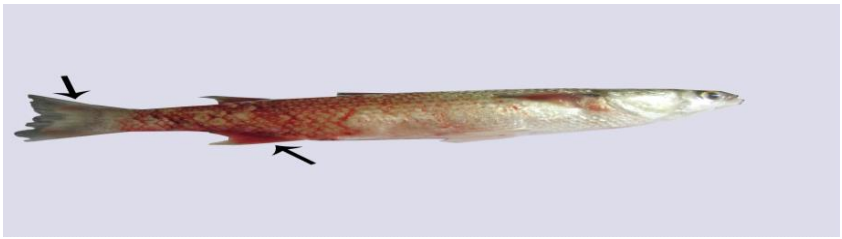
RESULTS AND DISCUSSION

Clinical signs and post mortem changes of infected *Mugilcapito* withVibriosis:

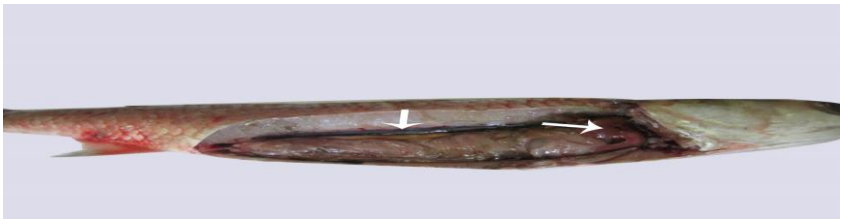
The common clinical signs in examined naturally infected *Mugilcapito* with *Vibrio spp.* were dark coloration of skin, detached scales, hemorrhage on several parts of the body surface, mouth, base of fins, abdomen, opercula and around the anal opening, distended abdomen, eroded fins (Figure 1A).

Post mortem finding showed pale enlarged liver with hemorrhagic on it edge, congested kidney, congested enlarged spleen, enlarged gall bladder, ascetic fluid in the abdominal cavity, liquefaction of internal viscera (Figure 1B).sings and post mortem finding agreed well with Khalil and Abd El-Latif (2013) for Mugil capito. Also These results are in agreement with Jale and Gulsen (2008) for Dicentrarchus labrax; Marzouk *et al*. (2009) for Red Grouper, Sea bass and Sea bream; Abd El-Galil and Mohamed (2012) for Gomphosus caeruleus.

Sidrophore-mediated iron-sequestering system followed harmful effect of extracellular products as cytotoxins, enterotoxins, lytic enzymes, protease, collagenase, chondroitin sulfatase, amylase, and different hemolysins. These substances responsible for invasive and proliferative processes in fish. Furthermore, destruction of critical components of both circulatory and immune systems by these extracellular products (Anzaldi and Skaa 2010; Labella *et al*., 2011and Naka *et al*., 2013).



1. A.



1. B.

Figure 1. A. Naturally infected *Mugil capito* with *Vibrio* spp. showing haemorrhages on several parts of the body surface, base of anal fin and erosion of caudal fin.

1. B. naturally infected *Mugil capito* with *Vibrio* spp. showing congested enlarged liver and congested kidney.

Bacteriological examination (Etiological agents):

Vibrio alginolyticus appeared as large convex yellow colored colonies on TCBS agar Subculture on TSA agar with 2% salt gives swarmed pale large colonies. Gram staining of isolated bacteria revealed that they were Gram-negative straight to slightly curved rods under oil immersion lens (Figure 2A & B).

Vibrio parahemolyticus appeared as green colored colonies on TCBS agar. The colonial appearance of *V. parahemolyticus* displayed, green convex colonies on TCBS agar and no swarming appeared when sub-cultured on TSA agar. The Gram staining displayed Gram negative rods and relatively pleomorphic.

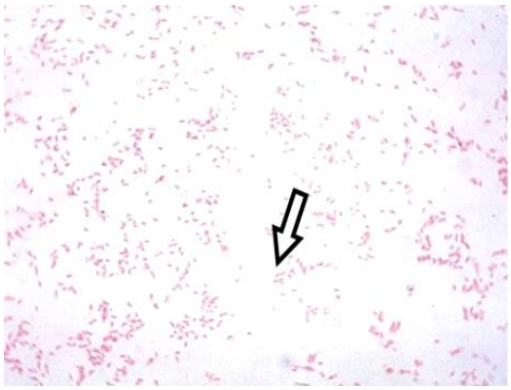
Eleonor *et al.* (1997); Buller (2004) and Liu *et al.* (2004) isolated *Vibrio alginolyticus and Vibrio parahemolyticus* by these same characters

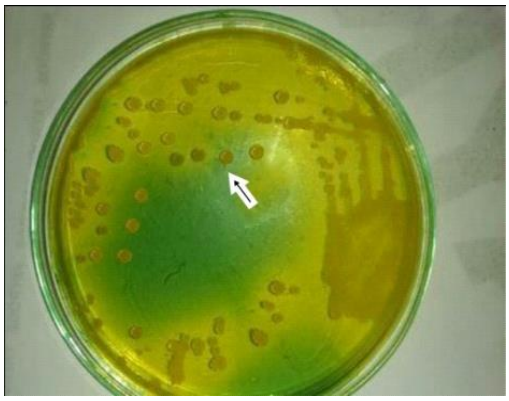
Vibrio alginolyticus strains were oxidase and catalase positive, sensitive to Vibriostatic agent O/129, growth at wide range of temperature (20-35^{o}C) and no growth appeared at 0% NaCl but was able to grow at different concentration of salt 2%, 3%, 4%, 6%, 8% and10%. These results completely agree with Zorrilla *et al*. (2003) in Spain in different marine fishes.

Biochemical identification:

Vibrio spp. are sensitive to Vibriostatic disc O/129 (150 μg). Positive reaction for lysine decarboxylase, indole production, tryptophane desaminase, gelatinase, glucose and mannitol fermentation. Negative for Ortho-nitro-phenyl galactosidase, arginine dehydrolase, citrate utilization, H2S production, urea hydrolysis, inositol, sorbitol, rhamnose, mellibiose, and arabinose fermentation.

Vibrio parahemolyticus strains were Gram-negative motile rods, oxidase and catalase positive, sensitive to Vibriostatic agent O/129. No growth appeared at 0% and 10% NaCl but was able to grow at different concentration of salt 2%, 4%, 5%, 6%, 8%. Yiagnisis and Athanassopoulou (2011) found that *V. parahemolyticus* grew in up to 8% NaCl and no growth at 0% and 10% NaCl and recorded that *V. parahemolyticus* positive for lysine decarboxylase, indole production, tryptophan deaminase, gelatinase, glucose and mannitol. On the other hand these strains were negative for ortho-nitro-phenylgalactosidase, arginine dehydrolase, ornithine decarboxylase citrate utilization, H2S production, urea hydrolysis, VP, inositol, sorbitol, sucrose, rhamnose, mellibiose and amygdalin fermentation.





2. A. **2. B.**

Figure 2. A. Gram- negative straight to slightly curved rods of *Vibrio alginolyticus* and *Vibrio parahemolyticus* (1000X).

2. B. Large yellow colored colonies of *Vibrio alginolyticus* on TCBS agar.

Molecular identification:

Phenotypic methods of identification is problematic, does not seem firm enough to confirm tested *Vibrio* strains. Molecular identification is a must tool in which molecular biology tools provide rapid and accurate method for diagnosis (Picard and Bergeron, 2002 and Pusterla *et al*., 2006).

Three isolates were positively reacted to the collagenase gene primers to produce unique, specific and clear band of the suspected size (737Bp), corresponding to the (737Bp) internal fragment of the collagenase gene primers (Figure 3) These results agree with the findings of Di Pinto *et al.* (2005); Marhual *et al.* (2010) and Lajnef *et al.* (2012)

Four isolates were positively reacted to the pR72H gene primers to produce unique, specific and clear band of the suspected size (387Bp), corresponding to the (387 Bp) internal fragment of the pR72H gene primers (Figure 4). Also, Lee *et al.* (1995); Robert-Pillot *et al.* (2002) and Bermúdez-Almada *et al.* (2014) demonstrated that amplification of the pR72H fragment, for amplicons of 387 Bp, is a powerful tool for reliable identification of *V. parahaemolyticus*.

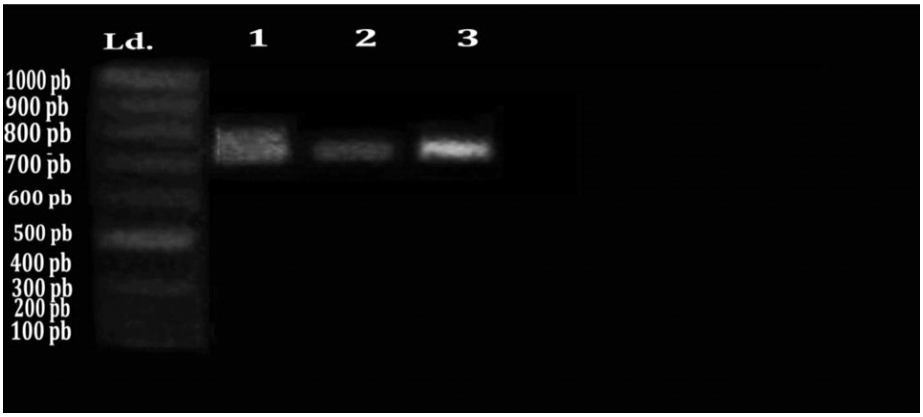


Figure 3. Agarose gel electrophoresis of products obtained after PCR amplification of collagenase gene of three *V. alginolyticus* strains extracted from *Mugilcapito* yielded (737 Bp) using forward and reverse primers.

Ld. Indicate 100bp size ladder.
1indicate*V. alginolyticus*strains isolated from spleen.
2 indicate *V. alginolyticus*strains isolated from liver.
3 indicate *V. alginolyticus*strains isolated from kidney.

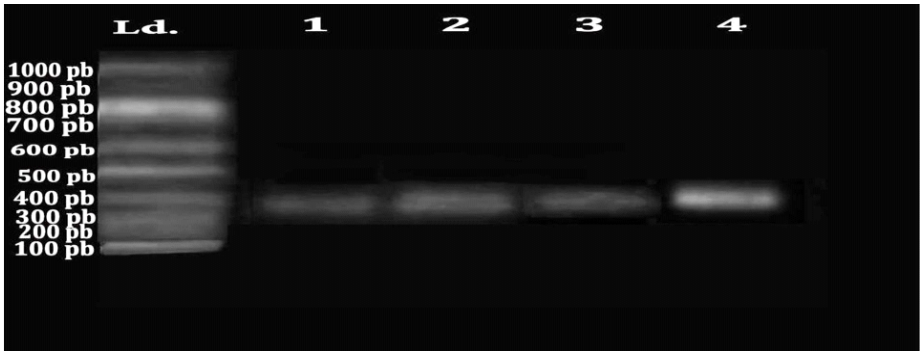


Figure 4. Agarose gel electrophoresis of products obtained after PCR amplification of the pR72H gene of four *V. parahemolyticus* strains extracted (387Bp) using forward and reverse primers.

Ld. Indicate 100bp size ladder
1indicate*V. parahemolyticusstrains* isolated from gill.
2 indicate *V. parahemolyticus*strains isolated from spleen.
3 indicate *V. parahemolyticus*strains isolated from liver.
4 indicate *V. parahemolyticus*strains isolated from kideny.

Epizootiology of Vibriosis:

Prevalence of *Vibrio spp.* infection:

At the present study the result of total prevalence of *Vibrio spp.*is 56.11%.This result nearly agree with El-Gendy (2013) who recorded 44.1% prevalence of isolated microorganism from some salt water fish. In contrast this result was slightly lower than 69.9% previously recovered from Sea bream in southwestern Spain (Zorrilla *et al.*, 2003). Also Akayli *et al.*, (2008) reported that the percentage of Vibriosis due to Vibrio spp. was 70%. While our results were higher than 34.28% of mortalities in some marine fish recorded by Moustafa *et al*. (2010).This difference in prevalence might be attributed to different locality and species variation.

Percentages of infection of Vibriosis from El-Serw and El-Ratama fish farms were 52.15% and 61.65% respectively (Figure 5). Higher prevalence in El-Ratama fish farm than El-serw fish farm may be due to water salinity in El Ratama fish farm ranged from (33.50- 45.50 ppt) while water salinity in El Serw fish farm ranged from (0.80-3.40 ppt). Tantillo *et al.* (2004); Austin and Austin (2012); Chen *et al*. (1999) and Sabir *et al.* (2013.) They demonstrated that, *Vibrio* species mostly of marine origin, halophilic, and higher values of salinity enhanced more *Vibrio* infections.

The seasonal prevalence of isolated bacteria was highest during summer season with prevalence rate 88.88% followed by spring (63.63%), winter (45.90%) and the lowest was autumn with prevalence rate 37.5%.Similarly, Eissa *et al.* (2013) found that the highest seasonal prevalence was recorded in summer (56 %) followed by spring (48%) then autumn (26.67%) and winter (13.33%). Also, Sabir *et al.* (2012) recorded that highest outbreaks of V. alginolyticus were during the warmer seasons.Also, Nagib (2011) observed that high prevalence of bacterial infections was correlated with high temperature recorded in summer season. This result was in agreement with previously reported by Moustafa *et al*. (2010) who reported that the total

prevalence of bacterial infection was recorded in summer season (40.81%) while the lowest was recorded in winter (15.91%).

This can explained by higher temperatures reduced immune capability and decreased resistance to infection so fish become susceptible to septicemic diseases (Lawson, 1995 and Cheng *et al*. 2005).

The total prevalence of *Vibrio alginolyticus* among naturally infected *Mugil capito* was 69.76%. Our results were in accordance with previously reported by Sabir *et al*. (2012) who recorded 70. 2% prevalence rate. This result was higher than 37.5% previously reported by El- Adawy (2010). Also, this result higher than those previously reported by Hussain (2002) who mentioned that the total prevalence of Vibrio alginolyticus was 14.61%. Similarly, this result was higher than those reported by Zorrilla *et al.* (2003) who recorded that Vibrio alginolyticus represent 21.35%. Furthermore, Wafeek *et al*. (2007) isolated V. alginolyticus from Grey mullet fish (Mugilcephalus) collected from Sharm El-Sheikh with percent of 39%. In contrast, this result was lower than prevalence recorded by Abdel-Aziz *et al*. (2013) who found that total prevalence of V. alginolyticus for sea bream and sea bass were 82.19% and 87.28% respectively.

Total prevalence of V. parahemolyticus among naturally infected Mugil capito was 30.24%. This result was slightly higher than Hassanein (2007) who mentioned that V. parahemolyticus in shrimp was 23.7%.The same result were slightly higher than 21.5% previously recovered from crayfish and periwinkle (Adebayo-Tayo *et al*., 2011).Similarly, this result higher than prevalence recorded by Abdel-Aziz *et al*. (2013) who reported total prevalence of *V. parahemolyticus*for sea bream and sea bass were 10.27% and 6.79% respectively.

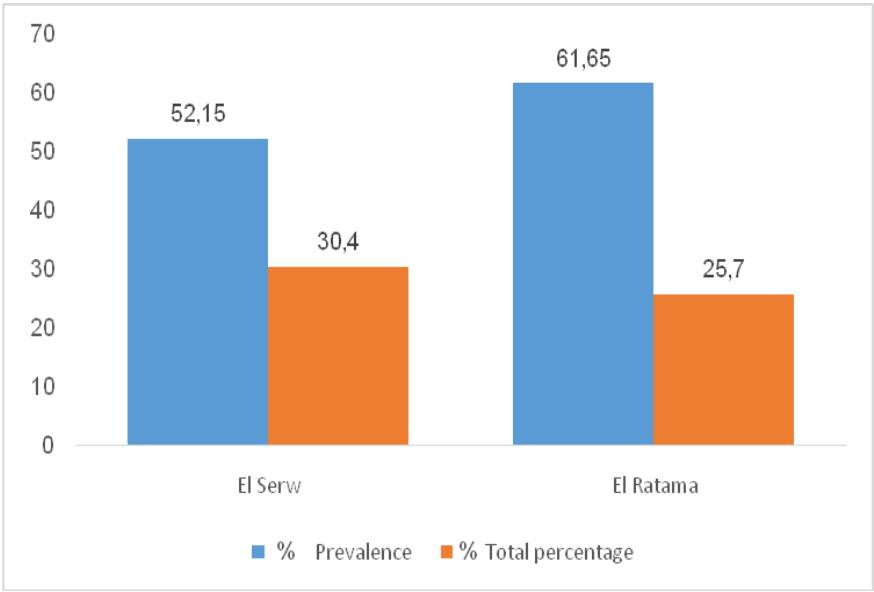


Figure 5. Showing prevalence of Vibriosis among *Mugil capito* in relation to different localities.

REFERNCES

Abdel-Aziz, M.; A. Eissa; M. Hanna and Abou M. Okada, 2013. Identifying some pathogenic *Vibrio /Photobacterium* species during mass mortalities of cultured Gilthead seabream *(Sparus aurata)* and European sea bass *(Dicentrarchus labrax)* from some Egyptian coastal provinces. International Journal of Veterinary Science and Medicine, 1(2):87-95.

Abd El-Galil, M.A. and M. H. Mohamed, 2012. First isolation of *Vibrio alginolyticus* from ornamental Bird Wrasse fish (*Gomphosus caeruleus*) of the Red Sea in Egypt. Journal of Fisheries and Aquatic Science, 7 (6): 461-467.

Adebayo-Tayo, B.C.; I.O. Okonko; M.O. John; N.N. Odu; J.C. Nwanze and M.N. Ezediokpu, 2011. Occurrence of Potentially Pathogenic *Vibrio Species* in Sea Foods Obtained from Oron Creek. Advances in Biological Res., 5 (6): 356-365.

Akayli, T.; G. Timur; B. Aydemir; A.R. Kiziler; O. Coskun; G. Albayrak and E. Arican, 2008. Characterization of *Vibrio alginolyticus* Isolates from

Diseased Cultured Gilthead Sea Bream, *Sparusaurata*. The Israeli Journal of Aquaculture – Bamidgeh, 60: 89-94

Anzaldi, L.L. and E.P. Skaa, 2010. Overcoming the heme paradox: heme toxicity and tolerance in bacterial pathogens. Infect Immun., 78: 4977–4989.

Austin, B. and D.A. Austin, 1987. Bacterial Fish Pathogen; Disease in farmed and wild fish. John and Sons Chichester.

Austin, B. and D.A. Austin, 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. Fourth edition ed. Chichester, UK: Praxis Publishing Ltd; p. 594 p. Lee K.K. (1995) Pathogenesis Studies on *Vibrio alginolyticus*in the Grouper, *Epinephelus-Malabaricus*, Bloch-Et-Schneider. Microbial Pathogenesis, 19: 39-48.

Austin, B. and D.A. Austin, 2012. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. Springer Science + Business Media Dordrecht 2012.

Balebona, M.C.; M.A. Morifiigo; A.D. Faris; K.I. Krovacek; M.A. Bordas and J.J. Borrego, 1995. Influence of salinity and pH on the adhesion of pathogenic *Vibrio* strains to *Sparusaurata* skin mucous. Aquaculture.132: 113-120.

Bermúdez-Almada, M.C.; A. Espinosa-Plascencia; M.L. Santiago-Hernández; C.J. Barajas-Borgo and E. Acedo-Félix, 2014. Behavior of Oxytetracycline in Shrimp Cultured *Litopenaeus vannamei* and sensitivity to three antibiotic of bacteria of Vibrio isolated from the organisms. Biotecnia., 3: 29-37.

Buller, N.B., 2004. Bacteria from fish and other aquatic animals: A practical identification Manual, CABI Publishing, Wallingford, UK. 361 pp.

Chen, F.R.; P.C. Liu and K.K. Lee, 1999. Purification and partial characterization of a toxic serine protease produced by pathogenic *Vibrio alginolyticus*. Microbios. 98 (390): 95-111.

Cheng, W.; L. Wangb and J. Chen, 2005. Effect of water temperature on the immune response of white shrimp *Litopenaeus vannamei* to *Vibrio alginolyticus*. Aquaculture, 250: 592– 601.

Di pino, A.; G. Ciccarese; G. Tantillo; D. Catalano and V.T. Forte, 2005. A Collagenase-Targeted Multiplex PCR Assay for Identification of *Vibrio alginolyticus, Vibrio cholerae,* and *Vibrio parahaemolyticus*. Journal of Food Protection, 68(1): 150–153.

Eissa, I. A. M.; H.I. Derwa; M. El-Lamei; A. Desuki; M.S. Zaki and H. El-Sheshtawy, 2013. Iron in water and some marine fishes in relation to vibriosis at Lake Temsah. Life Science Journal 10 (3): 2520-2528.

El- Adawy, M.M., 2010. Studies on bacterial pathogens affecting *Mugil capito* fish. Thesis, M. V. Sc. (bacteriology) Fac. Vet. Med., Suez Canal University.

Eleonor, V.A.; L.A. Tendencia and Dureza, 1997. Isolation of *Vibrio* species from Penaeusmonodon (*fabricious*) with red disease syndrome. Aquaculture, 154: 107-114.

El-Gendy, M.Y., 2013. Epizootiological and molecular studies on the common septicemic bacterial infections of some salt water fishes. PhD thesis, fish diseases and management, Faculty of Veterinary Medicine, Cairo University, Egypt, 212 pp.

Hassanein, 2007. Studies on some problems facing shrimp culture in Egypt. PhD Thesis, Fish diseases and management, Faculty of veterinary Medicine, Zagazig University.

Jale, K. and T. Gulsen, 2008. Marine *Vibrios* associated with diseased sea bass (*Dicentrarchus labrax*) in Turkey. Journal of fisheries Sciences, 21 (1): 66-76.

Hussain, R.A., 2002. Studies on some bacterial infections affecting certain marine fishes in the Arabian Gulf of Kingdom of Saudi Arabia. Thesis, M.V.Sc., Fac. Vet. And anim. Res. King Faisal Univ.S.

Khalil, R.H. and H.M. Abd El-Latif, 2013. Effect of *Vibrio alginolyticus* on *Mugilcapito*. Journal of the Arabian Aquaculture Society, 8 (1): 193-204.

Labella, A.; C. Berbel; M. Manchado; D. Castro and J.J. Borrego, 2011. *Photobacterium damselae subsp. damselae* an Emerging pathogen affecting New Cultured Marine Fish Species in Southern Spain. Recent Advances in Fish Farms, 9: 135-152.

Lajnef, R.; M. Snoussi; S. Balboa; A. Bastardo; H. Laabidi; A. Chatti; H. Abdennaceur and J. Romalde, 2012. Molecular typing of *Vibrio alginolyticus* strains isolated from Tunisian marine biotopes by two PCR-based methods (ERIC and REP). African Jour. of Microbio. Research, 6(22): 4647- 4654.

Lawson, T.B. 1995. Fundamentals of Aquaculture Engineering. Chapman and Hall, New York. 355pp.

Lee, C.Y.; S.F. Pan and C.H. Chen, 1995. Sequence of a cloned pR72H fragments and its use for detection of *Vibrio parahaemolyticus* in shell fish with PCR. Applied and Environmental Microbiology.61: 1311–1317.

Liu, P .C.; J.Y. Lin; W.H. Chuang and K.K. Lee, 2004. Isolation and characterization of pathogenic *Vibrio harveyi* (*V-carchariae*) from the farmed marine cobia fish *Rachycentroncanadum* L. with gastroenteritis syndrome. World Journal of Microbiology and Biotechnology, 20: 495 – 499.

Lucky, Z., 1977. Methods for diagnosis of fish diseases. AmerindPubl.Co. PV. Ltd., New York, 1st Ed.

Marhual, N.P.; B.K. Das; M. Sadique; A.K. Swain; B.K. Mishra; N.K. Maiti and A.E. Eknath, 2010. Molecular identification and typing of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* strains isolated from black tiger shrimp *Penaeus monodon.* J. Aquaculture in Trop., 25: 25-33.

Marzouk, M.S.; M.I. Hanna and M.K. Kenawy, 2009. Monitoring the Cause of Mortality in some Marine Fishes in Matrouh Governorate, Egypt during the summer 2008. American- Eurasian J. Agric. and Environ. Sci., 5 (2): 148-158.

Mizuki, H.; S. Whasio; T. Moita; S. Itoi and H. Sugita, 2006. Distribution of fish pathogen *Listonella anguillarum* in the Japanese flounder hatchery. Aquaculture, 261: 26-32.

Moustafa, M.; A.M. Laila; M.A. Mahmoud; W.S. Soliman and M.Y. El-gendy, 2010. Bacterial infections affecting marine fishes in Egypt. J. of American Science, 6 (11): 603-612.

Nagib, T.M., 2011. Polymerase chain reaction for diagnosis of some fish bacterial pathogen. Thesis, M. V. Sc. (bacteriology). Faculty of veterinary Medicine, Zagazig University.

Naka, H.; M. 55Liu; L.A. 55Actis and J.H. Crosa, 2013. Plasmid and chromosome-encoded siderophore anguibactin systems found in marine *Vibrios*: biosynthesis, transport and evolution. Biometals., 26 (4): 537-47.

Noga, E.J. 1996. Fish disease Diagnosis and Treatment. Mosby-Yearbook, Inc. St. Louis, MO. 367 pp

Reham, A.M. 2009. Some studies on Vibriosis on cultured fish. M. V. Sc., Fac. of Vet. Med., Alexandria University, Dept. Poultry and Fish Diseases.

Robert-Pillot, A.; A. Guénolé and J.M. Fournier, 2002. Usefulness of R72H PCR assay for differentiation between *Vibrio parahaemolyticus* and *Vibrio alginolyticus* species: validation by DNA-DNA hybridization", FEMS Microbiol. Lett., 215: 1-6.

Picard, F.J. and M.G. Bergeron, 2002. Rapid molecular theranostics in infectious disease. Durg Discov. Today, 21: 1092-1101.

Pusterla, N. Madigan, J.E. and Leutenegger, C.M. 2006. Real time polymerase chain reaction: a novel molecular diagnostic tool for equine infectious diseases. J. Vet. Intern.med. 20 (1) :3-12.

Sabir, M.; M.M. Ennaji; B. Bouchrif and N. Cohen, 2012. Characterization of *Vibrio alginolyticus* Trh Positive from Mediterranean Environment of Tamouda Bay (Morocco). World Environment, 2 (4): 76-80.

Schaperclaus, W.; H. Kulow and K. Schreckenback, 1992. Fish Disease.Vol. 1, 5th Ed., A. A. Balkema / Rotterdam.

Snoussi, M.; H. Hajlaoui; E. Noumi; S. Zanetti and A. Bakhrouf, 2008. Phenotypic molecular characterization of *Vibrio alginolyticus* strains recovered from juveniles and older *Sparus aurata* reared in a Tunisian marine farm. Ann Microbial, 58: 141-146.

Tantillo, G.M.; M. Fontanarosa; A. Di Pino and M. Musti, 2004. Updated perspective on emerging *Vibrio* associated with human infections. Lett. Appl. Microbiol., 39 (2): 117-126.

Wafeek, M.; M. Abou El- Atta and G. Ebrahem, 2007. Heavy metals and bacterial distribution in different organs of Grey mullet (*Mugil cephalus*) cultured in different environmental conditions. Egypt J. Aquat. Biolo &Fish, 11 (3): 1125-1144.

Yiagnisis, M. and F. Athanassopoulou, 2011. Bacteria isolated from diseased wild and farmed marine fish in Greece. Recent advances in fish farms, Dr. FarukAral 1Ed ., 153-168.

Zorrilla, I.; M. Morinigo; D. Castro; M. Balebona and J. Borrego, 2003a. Intraspecific characterization of *Vibrio alginolyticus* isolates recovered from cultured fish in Spain. J. Appl. Microbiol., 95: 1106-1116.

دراسة علي عدوي الفبريو في الطوباره في محافظتي الدقهلية ودمياط

زينب مصطفي عبدالسلام[١]، جمال النوبي[١]،
عبدالحكيم المر[١]، سمر عبدالحكيم[٢]

[١] *قسم امراض ورعاية الاسماك –كلية الطب البيطري– جامعة الزقازيق –مصر.*
[٢] *معهد بحوث صحة الحيوان–المنصورة–مصر.*

الملخص العربي

يُعتَبرُ مرض الفبريو من اهم وأكثر المشكلاتَ المرضِية التي تُواجهُ الاستزراع السمكي وبخاصة اسماك العائلة البورية. تم تجميع عدد ٣١٩ سمكه طوباره من محافظتي الدقهليه و دمياط وخضعت للفحص الميكروبيولوجي لعزل وتصنيف عترات الفبريو وعمل اختبار الضراوة للعترات المعزولة من الاسماك واستخدام البيولوجيا الجزيئيه في التشخيص.

اظهرت طرق التشخيص التقليديه ان البكتريا المعزوله على المنابت البكتيريه (TSA) باضافه ٢% كلوريد الصوديوم اظهرت مستعمرات بكتيريه مستديره و لونها كريمي بينما البكتريا المعزوله على المنابت البكتيريه (TCBS) اظهرت مستعمرات بكتيريه صفراء اللون بالنسبه للفبريو الجينوليتيكس و مستعمرات بكتيريه خضراء اللون بالنسبه للفبريو باراهيموليتكس . البكتريا المعزوله هي عصيات سالبه الجرام منحنيه ومتحركه تحت العدسه الزيتيه . باستخدام التفاعلات البيوكميائيه فهي ايجابيه لتفاعل الاوكسيداز والكتاليز وتظهر حساسيه لاقراص الفبريو البيوكيميائية (١٢٩/O) ١٥٠ µg . تنمو عند مدي واسع من الملوحه تتراوح بين ١–٨%. تظهر العترات تفاعلات متباينه وتم تأكيد دلك بأستخدام نظام API®٢٠ E.

انتج تفاعل البلمره المتسلسل (باندات) وحيده ومتخصصه وواضحه للحجم المتوقع Bp٧٣٧ و Bp ٣٨٧ لكلا من الفبريو الجينوليتيكس و الفبريو باراهيموليتكس علي التوالي.

وقد اكدت النتائج ان اجمالى انتشار الاصابه بميكروب الفبريو فى اسماك الطوباره فى هذه الدراسه في المتوسط هو ٥٦.١١% وكانت اعلي نسبة اصابة في فصل الصيف (٨٨.٨%).يليها الربيع ثم الشتاء بينما كانت اقل نسبة اصابة في فصل الخريف (٣٧.٥%).