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SOME STUDIES ON VIBRIOSIS IN FARMED MUGIL CEPHALUS IN DAKAHLIA GOVERNORATE

(With 5 Tables)

By

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**بعض الدراسات على الإصابة بمرض الفيبريوزس في أسماك البورى
المستزرعة في محافظة الدقهلية**

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أجريت هذه الدراسة على عدد ٨٠ سمكة من أسماك البورى المستزرعة في محافظة الدقهلية وبالفحص الظاهري تبين وجود أنزفة واحتقان على الجلد وتآكل الزعانف وبالفحص البكتريولوجى للعينات كانت نسبة تواجد ميكروبات الفيبريو المعزولة ٣٣,٧٥% التى صنفت مورفولوجيا وبيوكيميائيا إلى ١٦ (٥٩,٢٥%) فيبريو أنجويلارم نوع سي، ٨ (٢٩,٦٢%) فيبريو أوردلى، ٥ (١٨,٥١%) فيبريو دامسلا و ٣ (١١,١١%) فيبريو فالنفيكس وبدراسة مدى ضراوة ميكروبات الفيبريو أنجويلارم نوع سي فى أسماك البورى الطبيعى كانت نسبة النفوق عالية (٨٠%) بين الأسماك التى تم حقنها وباجراء اختبار الحساسية للمعزولات البكتيرية وجد أنها حساسة لكل من الأنتروفلوكساسين ، سلفات الكواليستين، نيتروفوران، أوكسى تتراسيكلين، تتراسيكلين وسيفوتاكسيم.

SUMMARY

80 naturally infected farmed *Mugil Cephalus* were collected from a private fish farm at Dakahlia Governorate, revealed clinically congestion and haemorrhages on skin and fins rot. The fish were examined bacteriologically for detection of *Vibrio* species. The obtained results revealed that 27 (33.75%) were positive for *Vibrio* species which identified morphologically and biochemically to *V. anguillarum* biotype C 16 (59.25%), *V. ordalii* 8(29.62%), *V. damsela* 5 (18.51%) and *V. vulnificus* 3(11.11%). The pathogenicity of isolated *V. anguillarum* biotype C strains in *Mugil* fish revealed high mortality (80%) in experimentally healthy fish. Sensitivity test of the isolated strains showed that *Vibrio* spp. were sensitive for Enrofloxacin, Colistin sulphate, Nitrofurantoin, Oxytetracycline, Tetracycline and Cefotaxim.

Key words: *Mugil cephalus*, vibriosis, fish diseases

INTRODUCTION

Fish are regarded as being most popular and more perishable than other high protein foods. The flesh of healthy fish is considered bacteriologically sterile. However, they are sometimes contaminated with bacterial pathogens and thus can inflict heavy losses in fish and causing diseases in man. Vibriosis is an enzootic disease of fish all over the world. It occurs among various fish species predominately in marine water, brackish water and freshwater fish. (Hacking and Budd, 1971; Kitao *et al.*, 1983).

Losses from vibriosis have attained considerable importance and become a serious threat to fish production especially with the increasing utilization of sea, brackish and inland waters near the coast to cultivated and fatten fish of various species (Schaperclaus *et al.* 1992). Epizootics of vibriosis take place in fish in presence of overcrowding, poor hygiene and organical polluted water (Kitao *et al.*, 1983; Noga, 1995). *Vibrio* is Gram-negative, non spore forming, facultative anaerobe and rod shaped bacterium, either curved or straight, it is motile by a single polar flagellum. *Vibrio* species grow in a wide temperature 5 – 35°C and rarely at 37°C. It grows well on most common laboratory media in the presence of 3 – 10% NaCl. (Cowan *et al.*, 1975).

Family Vibrionaceae including *V. anguillarum*, *V. damsela*, *V. ordalii*, *V. vulnificus*, *V. alginolyticus*, *V. fischeri* and *V. fluvialis* (Oliver and kaper, 1997 and Hurley *et al.* 2006). *Vibrio anguillarum* is the most common fish pathogen that affecting fresh water as well as marine fishes (Hacking and Budd, 1971); Rock and Nelson, 2006).

Some *Vibrios* produce hemolysin which may cause anemia and proteases which may cause muscle damage, reducing the keeping quality, marketability of fish and so economically losses (Hjeltnes and Roberts, 1993).

Vibriosis among various fishes cause acute, subacute and chronic infection as well as external signs as erythema at the base of fins, in the mouth and along the grooves of the lower jaw (Bullock, 1987).

This investigation was planned to study the prevalence of *vibrio* species in *Mugil cephalus* fish, isolation and identification of the recovered *Vibrios*, pathogenicity to such fish and in vitro sensitivity to antibiograms.

MATERIALS and METHODS

Fish:

A total number of 80 diseased fish (*Mugil cephalus*) (170g. \pm body weight) were collected from private fish farms in Dakahlia Governorate. Diseased fish showed wide spread skin and fin haemorrhages, sloughed skin, fin rot and anal congestion.

Fish specimens were transferred to laboratory and bacteriologically examined.

Bacteriological examination:

Specimens of fish gills, skin, liver, spleen and kidneys were taken under complete aseptic precautions for bacteriological examination of vibrio species according to Schaperclaus *et al.* (1992) and Austin and Austin, (1993). The samples were inoculated into Brain Heart Infusion (BHI) broth and peptone water containing 3% sodium chloride tubes and adjusted at pH 8.5 and incubated aerobically at 25°C over night. Loopfuls from the inoculated tubes were streaked on (BHI) agar with 3% sodium chloride. Moreover, Thiosulphate Citrate Bile Salt Sucrose agar (TCBS) was also used as a selective media and incubated at 25°C for 24 hours according to Inglis *et al.* (1993) and Quinn *et al.* (1994). The typical colonies were picked up on Trypticase Soya Agar (TSA) slant with 3% NaCl and incubated at 25°C for 24 hours. The isolates were morphologically and biochemically identified by Gram-stain, oxidase and catalase tests, motility, carbohydrate fermentation, TSI slant and other biochemical tests according to Overman *et al.* (1985) and Elliot *et al.* (1995).

Experimental infection:

The isolates were grown separately on BHI broth for 24 hours, then 0.2 ml dose (5×10^5 CFU/ml) was intraperitoneally injected to *Mugil cephalus* fish (5 fish for each isolate).

Furthermore 5 fish were used as a control group. The inoculated fish were observed during 3-weeks for the development of pathological changes.

Reisolation of the inoculated organism from internal organs of freshly dead fish was carried out.

Antibiogram activity:

Bacterial isolates were tested for their susceptibility towards ten antibacterial agents according to Koneman *et al.* (1992) using the following drugs; Amoxicillin, Ampicillin, Enrofloxacin Colistin sulphate, Nitrofurantoin, Cefotaxime, Oxytetracycline, Erythromycin,

Lincomycin and Tetracycline. The interpretation of results was carried according to Bio-Merieux Manual (1986).

RESULTS

Table 1: Biochemical properties of the isolated vibrio used for identification

Vibrio Strain	Biochemical properties											
	Oxidase	Catalase	V. Proskauer	Gelatin liquefaction	H ₂ S production	Arginin decomposition	Lysine decarboxylase	Acid from sucrose	Acid from arabinose	Acid from glucose	Acid from manitol	Acid from lactose
<i>V. anguillarum</i>	+	+	+	+	-	+	-	+	±	+	+	-
<i>V. ordalii</i>	+	+	-	+	-	+	-	+	-	+	+	-
<i>V. damsela</i>	+	+	-	±	-	+	±	-	-	-	-	-
<i>V. vulnificus</i>	+	+	-	-	±	-	+	±	-	-	-	±

+ = positive - = Negative ± = positive or negative

Table 2: Prevalence of Vibrio species in *Mugil cephalus*.

No. of examined samples	Positive samples		Vibrio species			
	No.	%	<i>V. anguillarum</i> biotype C	<i>V. ordalii</i>	<i>V. damsela</i>	<i>V. vulnificus</i> group 2
80	27	33.75	16	8	5	3

Table 3: Recovery rate of Vibrio species among various organs of naturally infected *Mugil cephalus*

Vibrio species	Total isolates		Gills	%	Liver	%	Spleen	%	Kidneys	%
	No.	%								
<i>V. anguillarum</i> biotype C	16	59.25	3	18.75	7	43.75	4	25.00	2	12.50
<i>V. ordalii</i>	8	29.62	2	25.00	3	37.50	2	25.00	1	12.50
<i>V. damsela</i>	5	18.51	1	20.00	3	60.00	1	20.00	0	0.00
<i>V. vulnificus</i> group 2	3	11.11	0	0.00	2	66.66	1	33.33	0	0.00
Total	32		6	18.75	15	46.87	8	25.00	3	9.37

Table 4: Results of I/P experimental infection of *Vibrio* species in *Mugil cephalus*.

Vibrio species	No. of used isolates	No. of inoculated fish/isolate	Total No. of inoculated fish	Total mortalities	
				No.	%
<i>V. anguillarum</i> biotype C	2	5	10	8	80.00
<i>V. ordalii</i>	2	5	10	7	70.00
<i>V. damsela</i>	2	5	10	6	60.00
<i>V. vulnificus</i> group 2	2	5	10	4	40.00
total	8		40	25	62.50

Table 5: Sensitivity of the isolated *Vibrio* species to different antibiograms

Antibiogramme	Disc concentration	<i>V. anguillarum</i> biotype C	<i>V. ordalii</i>	<i>V. damsela</i>	<i>V. vulnificus</i> group 2
Amoxycillin	10 ug	R	R	S	S
Ampicillin	10 ug	R	R	R	R
Enrofloxacin	10 ug	S	S	S	S
Colistin sulphate	10 ug	S	S	S	S
Nitrofurantion	300 ug	S	S	S	S
Cefotaxime	30 ug	S	S	S	S
Oxytetracycline	30 ug	S	S	S	S
Erythromycin	15 ug	R	S	S	S
Lincomycin	2 ug	R	R	R	R
Tetracycline	30 ug	S	S	S	S

S: sensitivity of the studies isolated of each vibrio species to antibiogramme was > 50%.

R: Sensitivity of the studies isolates of each vibrio species to antibiogram was < 50%.

DISCUSSION

Vibrio spp. is a natural inhabitant of the fish. These organisms are considered food borne pathogens able to contaminate the fish causing world health problems and economic loss in fish industry.

Not all strains of vibrio are considered pathogenic strains except that produce thermostable direct hemolysin (Bag *et al.*, 1999).

The data presented in Table (2) indicated that the prevalence of vibrio species in *Mugil cephalus* was 33.75%. The obtained prevalence are nearly similar with reported by Abd El-Gaber *et al.*, (1997) who isolated vibrio species 37.50% from *Mugil cephalus*. In this study, recovered vibrio species were *V. anguillarum* biotype C 16 (59.25%), *V. ordalii* 8(29.62%), *V. damsela* 5 (18.51%) and *V. vulnificus* groups 2, 3 (11.11%). On the other hand, Abd El-Gaber *et al.*, (1997) isolated *V. anguillarum* biotype C. (34.37%), *V. ordalii* (28.12%), *V. damsela* (21.87%) and *V. vulnificus* group 2 (15.62%) from *Mugil cephalus* fish.

In the present study *V. anguillarum* which constituted the highest prevalence rate 16(59.25%) was recovered from *Mugil cephalus* as shown in Tables (2) and (3). This is in nearly agreement with most other studies Muroga and Egusa 1988; Rock and Nelson 2006 and Chai-Yingmei *et al.*, 2006). Such results were high as compared with those reported by Abd El-Gaber *et al.*, 1997) who isolated *V. anguillarum* biotype C 5 (15.62%) from *Mugil cephalus* while high results were recorded by Moustafa *et al.*, (1990) who isolated *V. anguillarum* type A from 74.00% of Mullet fish (*Mugil cephalus*).

The highest isolation rate could be attributed to environmental stresses particularly high water temperature, organically polluted water, high salinity and poor hygiene and handling resulting in depression of one or several defensive mechanisms (Ellis, 1981). This finding was supported by Moustafa *et al.*, (1990) who recorded that water pollution and high salinity were the major stress factors for occurrence of vibriosis among fishes.

Concerning the site of isolation from *Mugil cephalus* fish, vibrios were high from liver 15 isolates (46.87%) followed by spleen 8 isolates (25.00%), gills 6 isolates (18.75%) and kidneys 3 isolates (9.37%) Table (3). On the other hand, Abd El Gaber *et al.*, (1997) isolated Vibrios from liver, spleen, kidneys and gills of *Mugil cephalus* fish with percentage of 35.00 , 37.50, 30.00 and 22.50% respectively.

Regarding to the experimental infection of *Mugil cephalus* with different vibrio species Table (4), exhibited a septicemic picture within one week post intraperitoneal injection where 62.50% of inoculated fish were dead. Mortality rate ranged from 40.00% with *V. vulnificus* gp. 2 to 80.00% with *V. anguillarum* biotype C. while *V. ordalii* produced 70.00% mortalities and *V. damsela* 60.00% These findings were supported by Abd El-Gaber *et al.*, (1997) who recorded 70% mortalities

among *O. niloticus* with different *Vibrio* species. El-Bouhy *et al.*, (1990) found 80% mortalities was reported after interperitoneal infection of Nile catfish with *V. anguillarum* and Amany *et al.*, (2000) recorded 90% mortalities among *C. lazera* post. interaperitoneal infection of *V. anguillarum*.

Moreover, clinical signs and gross lesions induced by *Vibrios* in the present study were nearly similar to those observed in Abd El Gaber *et al.*, (1997). Nearly similar observations were also recorded in other fish species which had Vibriosis Austin and Austin (1989); Lavilla pitogo *et al.*, (1992) and Schaperclaus *et al.*, (1992).

As shown in Table (5), *Vibrio* species were sensitive to Enrofloxacin, Colistin sulphate, Nitrofuratoin, Oxytetra-cycline, Tetracycline and Cefotaxime. They resistance to Lincomycin and Erythromycin. These results nearly agreed with that recorded by different authors (Balsgaard and Bjerregaard 1991; Austin and Austin 1993; Shaahan *et al.* 1995; Yonis *et al.*, 1997; Abd El Gaber *et al.*, 1997; Zeinab Soliman 1999 and Amany *et al.*, 2000) who found that the isolated *V. anguillarum* strain was sensitive to Ampicillin and Chlormphenicol, while it was resistant to Erythromycin Joklik *et al.* (1992) and Stephens *et al.* (2006) recorded that most isolates of *V. damsela* were sensitive to Tetracycline.

It was concluded that *Vibrio* species existed in the examined fishes in varying percentages. *V. anguillarum* is the most serious pathogen of freshwater fish (*M. cephalus*) and it was highly pathogenic when injected in healthy ones and prevent the infections by Good handling, hygienic measurement and prevention the source of water pollution, also administration of the effective drug to fish should be carefully controlled on Vibriosis.

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