STUDIES ON SOME BACTERIAL AND FUNGAL PATHOGENS AMONG EELS (ANGUILLA ANGUILLA)

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ABSTRACT

A total number of 300 clinically and grossly diseased eels were collected from different commercial fish farms and subjected to clinical, postmortem, bacterial and fungal examinations. The prevalence of Aeromonas hydrophila, Pseudomonas fluorescens, Streptococcus faecalis, Vibrio anguillarium, Falvobacterium columnare, Vibrio alginolyticus, Lactobacillus species, Saprolegnia parasitica and Ichthyophonus hoferi were 33.33, 16. 67, 13, 12.33, 10.33, 9.33, 3, 1.67 and 1% respectively among the examined fish. The clinical signs and postmortem changes associated with each infection were recorded. Generally, the clinical signs and postmortem changes associated with bacterial infections were loss or derrease of feed intake, increase in mucus secretion, corneal opacity, exophthalmia, haemorrhage in different parts of the body and congestion of the internal organs. Saprolegnia infected fish showed whitish cotton-like growths. The pathogenicity of A. hydrophila, P. fluorescens, S. faecalis and V. anguillarum were tested by experimental infection in eels and showed high mortality rate among the inoculated fish. They were found to be sensitive to Ciprofloxacin. Also, Ciprofloxacin has a potential agent for the management of A. hydrophila infection in eels. Clove oil was effective in inhibiting bacterial growth.

INTRODUCTION

Aquaculture industry is of increasing importance in Egypt. The eel is one of the most mysterious of fish which requires continued research with scientific and technical developments and innovation (McKinnon, 2006). Their commercial production is rapidly expanding all over the world (Guo et al., 2005).

The introduction of a new aquatic animal to the species composition is necessary for development of aquaculture on both subsistence and commercial levels. However, such changes increase the probability of introducing new pathogens (Woo and Bruno, 1999).

Infectious diseases of cultured fish are among the most notable constraints on the expansion and development of aquaculture (**Woo and Bruno, 1999 and Plumb, 1999**). Bacterial diseases were shown to be a definite problem in culturing the eel (**Davis and Hayasaka, 1983; Toranzo et al., 2005 and Foaz et al., 2006**). Fungal diseases are of considerable concern and associated with heavy mortalities (**Lategan et al., 2004**).

However, to our knowledge there are little studies concerning the anguilliculture diseases caused by bacteria and fungal agents in eels. In the current investigation, I have monitored the susceptibility to bacterial or fungal pathogens of eel cultured in commercial aquaculture facilities under Egyptian conditions.

MATERIAL AND METHODS

Naturally infected fish:

A total number of 300 clinically and grossly diseased eels (Anguilla anguilla) were collected from different aquaculture facilities suffered from heavy mortalities in Egypt, transported to the laboratory and examined for bacterial and mycotic pathogens.

The collected eels were subjected to clinical and postmortem examinations according to the methods described by **Lucky (1977) and Schaperclaus et al., (1992)**.

Experimental fish:

A total number of 80 healthy eels were obtained from freshwater commercial fish farms and maintained to well prepared glass aquaria containing aerated and declorinated tap water under natural photoperiod and polypropylene tube serving as hiding place for acclimatization to be used in artificial infection and treatment trial. The exchange rate of the water was about one third of the aquaria per day.

Bacteriological examination:

Samples were taken under complete aseptic condition from the affected areas of skin, gills and internal organs (liver and kidney) and inoculated into brain heart infusion agar and incubated at 22 C. Purified isolates were identified according to standard biochemical tests (**Bergey et al., 1984; Schaperclaus et al., 1992 and Austin and Austin, 1993**).

Mycological examination:

Mycological examination was performed according to the method described by **Robert (1982)**.

Experimental infection:

The pathogenicity of the isolated bacteria was confirmed by dividing 50 eels into 5 equal groups. The first, second, third and fourth groups were injected with A. hydrophila, P. fluorescens, S. faecalis and V. anguillarum, at a dose of 0.2 ml of saline containing 10^8cells/ml intraperitoneally (i.p.) respectively. The fifth group was served as a control group. Fish were observed daily for clinical signs abnormalities and mortalities. The pathogens were reisolated as a pure culture from the freshly dead fish **(Amaro et al., 1992)**.

Sensitivity test:

The isolated strains were tested for antibiotic sensitivity using the disc diffusion method **(Carter and Cole, 1990)**. In-vitro sensitivity of the isolated strains against clove oil was performed. Minimal inhibitory concentration (MIC) was performed for clove oil as described by **Davis et al., (2003)**.

Laboratory evaluation of Ciprofloxacin (Cf):

A total number of 30 apparently healthy eels ere divided into three equal groups. The first group was subjected to a treatment. 1.6 g Cf was dissolved in ounce of 36% acetic acid to accelerate dissolution and then added to the water aquarium to give a final concentration of 10 µg/ml of Cf and injected i.p. with A. hydrophila. Eels were kept for 48 hr under exposure to medicated water without changing of water **(Guo et al., 2005)**. The second group was challenged with A. hydrophila and kept without any treatment. The third group was left as a control. All fish were kept under observation for any clinical abnormalities, mortalities and for the presence of A. hydrophila for 20 days.

RESULTS

The isolated bacterial pathogens with its prevalence rates were Aeromonas hydrophila (33.33%), Pseudomonas fluorescens (18. 67%), Streptococcus faecalis (13%), Vibrio anguillarium (12.33%), Falvobacterium columnare (10.33%), Vibrio vulnificus (9.33%) and Lactobacillus species (3%) (Table, 1). Regarding to fungal infections Saprolegnia parasitica was

recorded as secondary bacterial invasion in a percentage of 4.67% and Ichthyophonus hoferi 1% (Table, 2).

Motile Aeromonas septicemia:

MAS infected eel showed loss of balance, excessive mucus secretion, loss of appetite, sluggish swimming, dullness, skin erosion and ulcer. Petechial hemorrhages on the external surface and exophthalmia were also observed. Prolapse and congestion of the vent was noticed. Gills were congested or pale anemic and covered with heavy layer of mucus. Internally, a generalized hyperemic appearance and yellowish ascetic fluid were noticed. The liver varied from yellow to dark brown in color with necrotic foci in some cases and in association with over distended gall bladder. The digestive tract was void of feed (Photograph, 1).

Aeromonas hydrophila appeared to be Gram -ve, motile short rods and positive in cytochrome oxidase test (Table, 3).

Pseudomonadiasis :

Pseudomonas fluorescens was isolated as a causative agent of Pseudomonadiasis. The diseased fish showed slow swimming at the water surface, loss of appetite, petechial hemorrhages in abdominal wall, fins and tail, intestinal prolapse and fin rot. Congested or pale gills were noticed. Internally, liver hemorrhages, enlarged spleen and kidney with ascites (Photograph, 2). Ps. fluorescens was identified according to its morphological culture and biochemical characters (Table, 3).

Streptococosis :

Streptococcus faecalis was isolated from heavy mortalities among eels. Infected fish exhibited erratic swimming, loss of appetite, lethargy, no escape reflex, hemorrhages on different parts of skin, ulcer and corneal opacity. The most striking clinical signs shown by the diseased eels were uni-or-bilateral exophthalmia with accumulation of hemorrhagic fluid around the eyes (pop eye) and ventral petechial hemorrhages. Internal examination revealed bloody tinged ascitic fluid, enlargement and congestion of liver, spleen and kidney and no feed was detected in the stomach and intestine (Photograph, 3 & 4). Streptococcus faecalis was Gram positive cocci, non-motile, and catalase negative (Table, 3).

Vibriosis :

Fish affected with vibriosis showed a generalized septicemia with diffuse haemorrhages on the base of fins and corneal opacity. Moribund fish were anorexic with pale gills which reflect severe anemia. Internal examination of fish revealed no feed in the stomach. Internally, numerous petechial haemorrhages were observed on the walls of the abdominal cavity and on the intestinal and swimbladder surfaces. A moderate amount of fluid was present in the coelomic cavity and the spleen was much enlarged and congested. Gall bladder was distended with bile. The kidney was markedly softened and severely congested (Photograph, 5 &6). Vibrio vulnificus and Vibrio anguillarium are short Gram negative rods (Table, 3).

Columnaris disease :

The affected fish showed loss of appetite, loss of balance, eroded and hemorrhagic mouth, excessive mucus secretion, ulcerative skin lesions, frayed fins and tail rot and respiratory distress (Photograph, 7). In some cases, congested kidneys were noticed on postmortem examination.

By microscopical examinations of wet mounts obtained from gills or lesion, accumulations of long rods of gram-negative bacteria (Falvobactertum columnare) were detected (Table, 3).

Lactobacillus species infection :

Lactobacillus species infected fish showed dark pigmentation, hemorrhage on different parts of the body, eye cataract and fin rot. The postmortem examination revealed yellowish ascetic fluid and enlargement and congestion of the internal organs (Photograph, 8). Lactobacillus species is Gram positive cocci, cocco-bacilli and bacilli in shape and unable to produce catalase (Table, 3).

Saprolegniosis :

Saprolegnia parasitica had recognized as a causative agent of Saprolegniosis led to heavy mortality during acclimatization in fresh water and as a subsequent to Falvobacterium columnare infection. Clinically, the infected fish had whitish cotton-like growths on skin, fins, gills and around the mouth, excessive mucus secretion and rubbing against hard objects. Also, loss of appetite, loss of equilibrium, lethargy, respiratory distress, erythema, ulcer and loss of response to the external stimuli were observed (Photograph, 9).

Ichthyophoniasis :

No clinical signs abnormalities were recorded among the naturally infected eel with Ichthyophonus hoferi. Postmortem examination of dead fish revealed the presence of grayish white nodules on enlarged and congested kidney. Ichthyophonus hoferi was only isolated from eel kidney. Hyphal growth was recorded in the inoculated test tubes containing minimum essential media either at pH 3.5 or 7 (Photograph, 10). Microscopically, keel formation appeared at 24 hr. and spherical hyphal terminal bodies were formed after 10 days of inoculation.

Experimentally infected eels with different pathogenic bacteria (A. hydrophila, P. fluorescens, S. faecalis and V. anguilarum) showed similar clinical signs and postmortem changes to the naturally infected one. The mortality patterns among the artificially infected eel by i.p. were shown in table (4). Re-isolation of the injected bacterial pathogens was performed from all dead and experimentally diseased fish. On the other hand, no mortality was recorded in control group.

A number of compounds were shown to be effective in vitro against A. hydrophila P. fluorescens, Streptococcus faecalis and V. anguilarum (Table, 5). One of these, the antibiotic Ciprofloxacin, is the drug of choice for treatment of different bacterial diseases.

The results of the investigation demonstrated the potential therapeutical application of Clove oil as a herbal agent against the tested bacterial pathogens in eels (Photograph, 11). The MIC values of clove oil to A. hydrophila, P. fluorescens, S. faecalis and V. anguilarum were 0.015, 0.0075, 0.0075 and 0.02 _l.

Regarding to the laboratory trial for the treatment of fish artificially infected with A. hydrophila with use of Cf at concentration of 10 µg/ml, our result showed that Cf was effective against MAS. The clinical signs were disappeared and the treated fish returned to the normal state of health. A. hydrophila was not isolated from the treated fish. Table (6) showing the mortality rate among the treated and non-treated groups.

DISCUSSION

Aquaculture industry in Egypt has been growing rapidly in the past decade. It plays an important role in the development, a source for export earning, and has been a leading sector in economic growth **(McKinnon, 2006)**. Outbreaks of disease have become a critical factor which has hampered the development of aquaculture in many countries leading to significant economic losses of cultured fish which inhibits the expansion of aquaculture **(Austin and Austin, 1993 and Toranzo et al., 2005)**.

Fish are susceptible to a wide variety of bacterial pathogens. Generally, eels contract the

same types of bacterial diseases as do other warm water fishes **(Plumb, 1999)**. A higher incidence of bacterial pathogens and/or disease occurred during the process of culling and in older eels during warm months. The primary aetiological agent of disease of cultured eels was A. hydrophila. Other potential pathogens isolated included A. salmonicida, Vibrio spp. and Pseudomonas spp. **(Davis and Hayasaka, 1983)**. The culture of eels depends upon the collection of wild glass eels and elvers. This could result from added stress and possible abrasions occurring during the culling process **(Davis and Hayasaka, 1983)**. Fish farms provide ideal conditions for the incidence of the diseases **(Woo and Bruno, 1999)**.

Motile Aeromonas septicemia is a significant bacterial septicaemia caused by A. hydrophila. The organism appears to have a wide geographical distribution since it is found in many countries **(Plumb, 1999)**. Aeromonas hydrophila causes a haemorrhagic septicaemia in fish.

Internal examination revealed no feed in the stomach and intestine and generalized hyperemia. Similar observations were recorded by **Egusa, (1978); Davis and Hayasaka, (1983); Plumb, (1999); Toranzo et al., (2005) and Yavuzcan et al., (2005)**.

Pseudomonadiasis is one of the major bacterial pathogens causing hemorrhagic petechia in the skin and internal organs. Similar results were recorded by **Egusa, (1978); Davis and Hayasaka, (1983); Stewart et al., (1983); Plumb, (1999) and Toranzo et al., (2005)**.

Streptococcal disease in fish was first reported in 1957, affecting cultured rainbow trout in Japan **(Hoshina et al., 1958)**. Streptococcal infections of fish are considered an emerging serious disease affecting a variety of wild and cultured fish throughout the world. Diseased fish showed loss of appetite, lethargy and hemorrhage on different parts of skin, ulcer, exophthalmia and congestion of the internal organs. Similar picture was previously described by **El-Refaee, (2005); Toranzo et al., (2005) and El-Ashram and Abd El-Rahman, (2006)**. Streptococosis are considered also as potential zoonotic agents capable to cause disease in humans **(El-Refaee, 2005)**.

V. vulnificus and V. anguillarium are pathogenic gram-negative bacteria which represents the main infectious disease of eels causing significant external lesions including ulcer, necrosis, exophthalmia which make fish unmarketable, high mortality rates and causing important economic losses **(Austin and Austin, 1993)**. Fish affected with vibriosis showed a generalized septicemia, corneal opacity and exophthalmia. Moribund fish were anorexic and anemic. Such findings were met by **McCarthy (1976); Egusa (1978); Davis and Hayasaka, (1983); Shaaban et al., (1995); Plumb, (1999); Abd El-Rahman and El-Ashram, (2005); Toranzo et al., (2005) and Fouz et al., (2006)**. **McCarthy (1976)** isolated V. anguillarum from eels reared in freshwater farms with the same clinical signs and p.m. lesions. Vibriosis infections could represent a seri-

ous risk for public health **(Abd El-Rahman and El-Ashram, 2005)**.

The affected fish with Columnaris disease had eroded and hemorrhagic mouth, ulcerative skin, frayed fins and tail rot. These results were gone hand in hand with those mentioned by **Austin and Austin, (1993); Plumb, (1999) and Toranzo et al., (2005)**.

Diseased eels showed clinical signs and postmortem changes that were typical of Lactobacillus species infection **(Abd El-Rahman, 2003 and Toranzo et al., 2005)**.

Similar morphological and biochemical reactions of the isolated bacterial pathogens were reported by **Austin and Austin, (1993); Shaaban et al., (1995); Plumb, (1999); Abd El-Rahman, (2003) and El-Refaee, (2005)**.

Saprolegniosis is a serious winter disease that occurs in fresh-water farmed fish **(Lategan et al., 2004)**. Fungal filament become first evident on the snout or the tail region of diseased eels followed by necrosis and eventually ulceration **(Egusa, 1978 and Lategan et al., 2004)**. However, stress suffered by the eels during the necessary management practices of grading and handling, particularly when water temperatures fall, leads to outbreaks of saprolegniosis in the eel ponds **(Lategan et al., 2004)**.

Ichthyophoniasis is a granulomatus systemic fungal disease, occurs in both freshwater and marine fishes **(Henfy, 2002)**. Ichthyophonus hoferi was isolated from kidney showing grayish white nodules and congestion. Similar results were obtained by **Schaperclaus et al., (1992) and Henfy, (2002)**.

Outbreaks of diseases are usually accompanied with stress factors as overcrowding, sudden change in temperature, pollution, handling and nutritional status **(Plumb, 1999; Woo and Bruno, 1999 and Yavuzcan et al., 2005)**.

With development of today's intensive aquaculture industry, large amounts of antibiotics and synthetic antibacterial agents are used in eel's farms to prevent and treat infectious diseases. Ciprofloxacin is currently the most used clinical antibiotic in the world **(Guo et al., 2005)**. Ciprofloxacin showed potential as candidate compounds for in-vitro and in vivo trials. Such findings were recorded by **Abd El-Rahman and El-Ashram, (2005) and El-Refaee, (2005)**.

Clove oil is derived from the stems, leaves and buds of Eugenia caryophyllata tree, and its active ingredient is eugenol. It is safe, effective, inexpensive and anesthetic **(Walsh and Pease, 2002)**. Our result showed that clove oil had an inhibitory effect against different bacterial pathogens **(Keene et al., 1998)**. Clove oil and eugenol are listed by the US Food and Drug administration (1978) as safe in human when used at level not exceeding 1500 p.p.m.

In conclusion, it is obvious from this study that it is important to perform extensive monitor-

ing of fish pathogenic bacteria in order to obtain increasing knowledge of the most important diseases which occur in fish farms. Also, to evaluate the most suitable method for prevention and control of diseases.

Table (1): The prevalence rate of the isolated bacterial pathogens.

Bacterial pathogen	Number of examined fish	Number of diseased fish	Percentage of infection
A. hydrophila	300	100	33.33
P. fluorescens	300	56	18.67
S. faecalis	300	39	13
V. anguilarum	300	37	12.33
F. columnare	300	31	10.33
V. vulnificus	300	28	9.33
Lactobacillus	300	9	3

Table (2): The prevalence rate of the isolated fungi.

Fungus	Number of examined fish	Number of diseased fish	Percentage of infection
S. parasitica	300	14	4.67
I. hoferi	300	3	1

Table (3): Morphological and biochemical reactions of the isolated bacterial pathogens from naturally infected eels.

Items	*A. hydrophila*	*P. fluorescens*	*S. faecalis*	*V. anguillarum*	*F. columnaris*	*V. vulnificus*	*Lactobacillus* species
Gram-stain	-ve	-ve	+ve	-ve	-ve	-ve	+ve
Shape	coccobacilli	Bacilli	cocci	Bacilli	Bacilli	Curved Bacilli	Bacilli cocci
Arrangement	single	single	Single short chain	single	single	Single short chain	Single short chain
Oxidase	+	+	-	+	+	+	+
Catalase	+	+	-	+	+	+	D
O/F	F	O	F	F	O/-	F	O/-
Motility	+	+	-	+	+	+	-
Indol	+	-	-	+		-	-
V.P.	+	-	-	+	-	-	-
M.R.	+	+	+	+	-	+	+
H2S	-	-	-	-	+	.	-
Citrate	+	+	+			.D	-
Starch	-	+		+	-	-	-
Gelatin	+	+	-	-	+	v	-
Acid from: glucose	+	+	+	+	D	+	+
Sucrose	+	+	+	+	-	-	+
Fructose	+	+	+	D	-	+	+
Salicin	-	-	-	-	-	+	D
Arabinose	D	+	-	+	-	-	+
Lactose	-	D	-	-	-	+	-
Galactose	+		D	-	-	+	-
Sorbitol	-	+	+	+	-	+	+
Xylose	-	+	+	D	-	-	-
Trehalose		+	+	D	-	-	D
Manitol	-	+	+	+	-	-	+
Maltose	+	+	+	+	-	+	D
Glycerol	-	+	+	-	-	D	-
inisitol	-	+	+	-	-	+	+
Nitrate.	+		-	+	+	-	-
Arginine hyd.	+	+	+	+	+	+	+
Dec. of Lycin	-	-	-	-	-	+	-
Ornithine	+	+	-	-	-	-	-
Growth on Nacl 0.0%	+	+	+	-	+	+	+
7%	-	-	-	+	-	+	-
37 °C	+	+	+	+	D	+	-

Table (4): Pathogenicity of *A. hydrophila, P. fluorescens, Streptococcus faecalis* and *Vibrio anguilarum* among artificially infected eels.

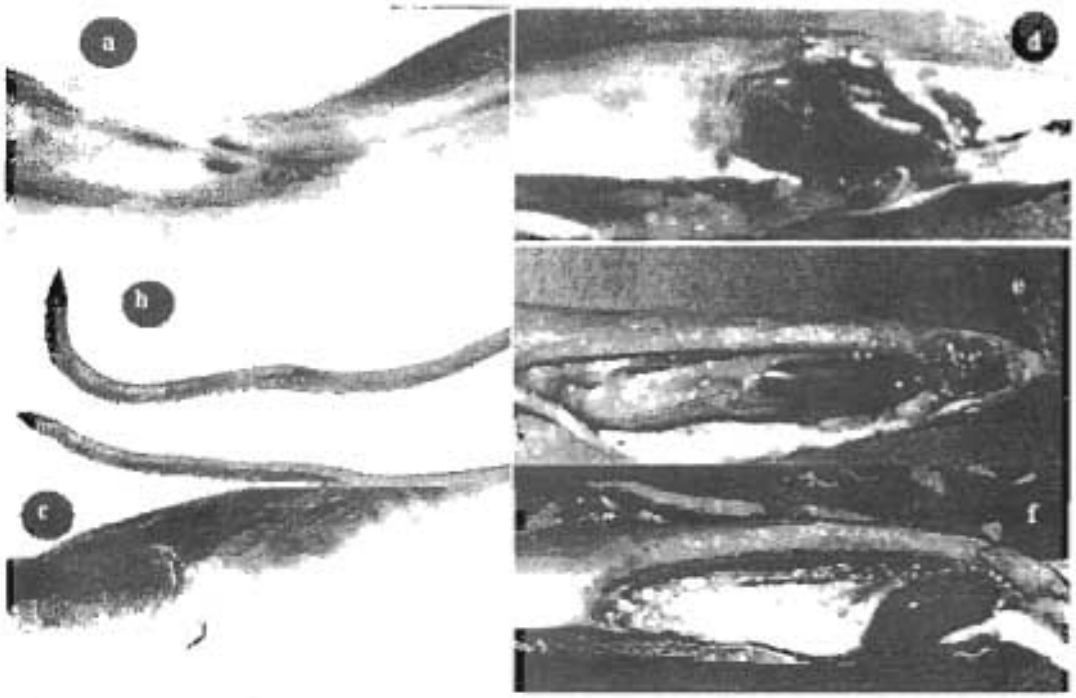
Fish group	Bacterial pathogens	Route of inoculation	Number of infected fish	Number of dead fish	Mortality rate
I	*A. hydrophila*	I/P	10	10	100
II	*P. fluorescens*	I/P	10	7	70
III	*S. faecalis*	I/P	10	9	90
IV	*V. anguilarum*	I/P	10	10	100
V	Control	I/P	10	0	0

Table (5) Sensitivity of some bacterial isolates to different antibiograms.

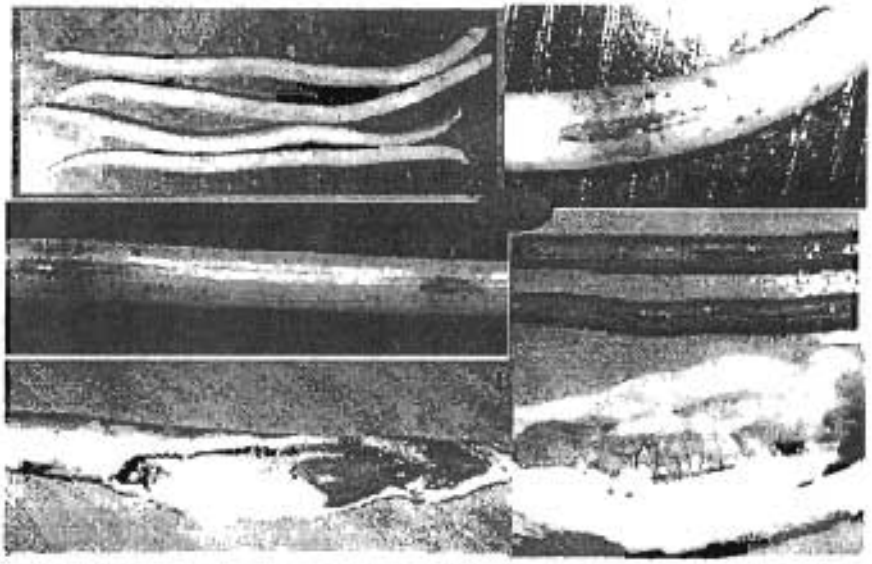
Antibiotic against	symbol	Concentration (mcg)	Susceptible zone (mm)	*A. hydrophila*		Pseudomonas spp.		*Vibrio spp*		*Streptococcus sp.*	
				Inhibition zone (mm)	Sensitivity reaction	Inhibition zone (mm	Sensitivity reaction	Inhibition zone (mm	Sensitivity reaction	Inhibition zone (mm	Sensitivity reaction
Ampicillin	AM	10	≥29	19	R	7	R	8	R	12	R
Ciprofloxacin	CIP	5	≥21	32	S	27	S	30	S	15	R
Erthromycine	E	15	≥18	22	S	8	R	10	R	24	S
Kanamycin	K	30	≥18	14	R	18	S	11	R	10	R
Nalidixic acid	NA	30	≥19	26	S	12	R	25	S	6	R
sulphonamycin	P	10	22-29	0.0	R	0.0	R	0.0	R	14	R
Streptomycin	S	10	≥15	10	R	19	S	10	R	16	S
Trimethoprim + sulphamethoxazol	Sxt	1.25/ 23.75	≥16	25	S	18	S	20	S	0.0	R
Tetracycline	TE	30	≥19	22	S	15	R	20	S	22	S
Vancomycin	VA	30	≥12	0.0	R	0.0	R	0.0	R	20	S

Table (6): Laboratory efficacy of Ciprofloxacin for the control of *A. hydrophila* infection.

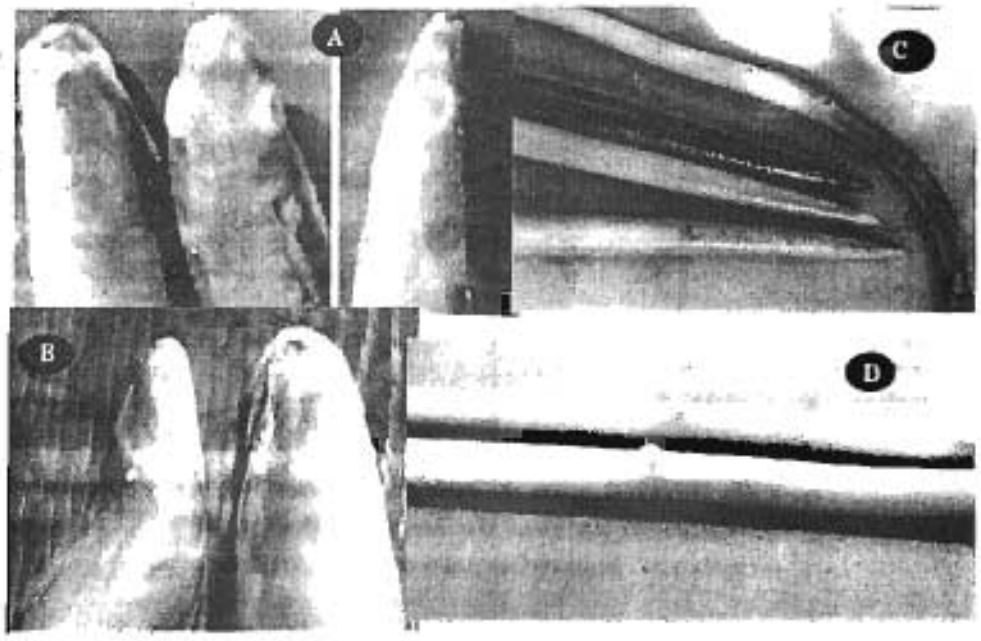
Fish group	Number of fish	Number of dead fish	Mortality rate
Treated and infected	10	2	20
Non-treated and infected	10	10	100
Control	10	0	0



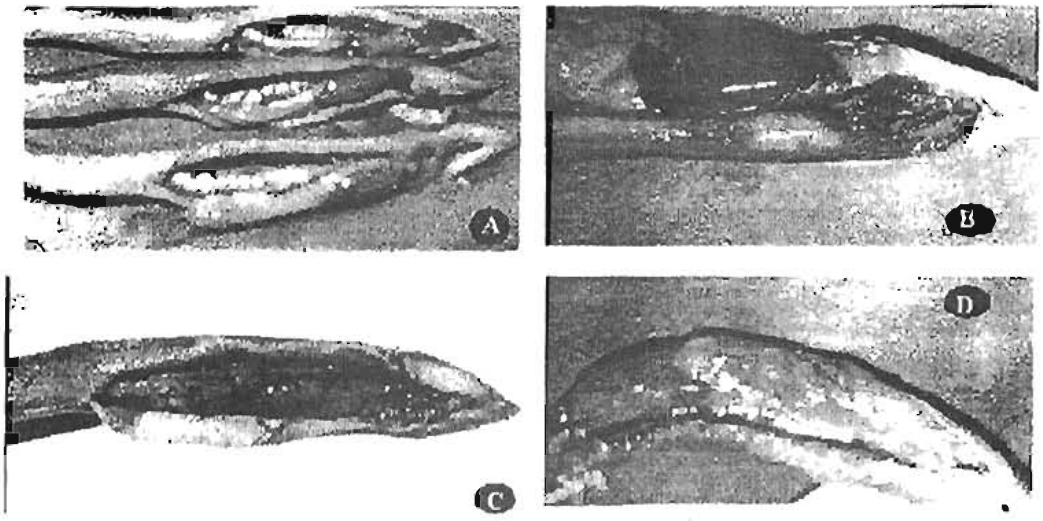
Photograph (1): Eels infected with *A. hydrophila* showing severe hemorrhages on different parts of the body (A, B&C). (D, E&F) Congestion of gills and internal organs with empty intestine.



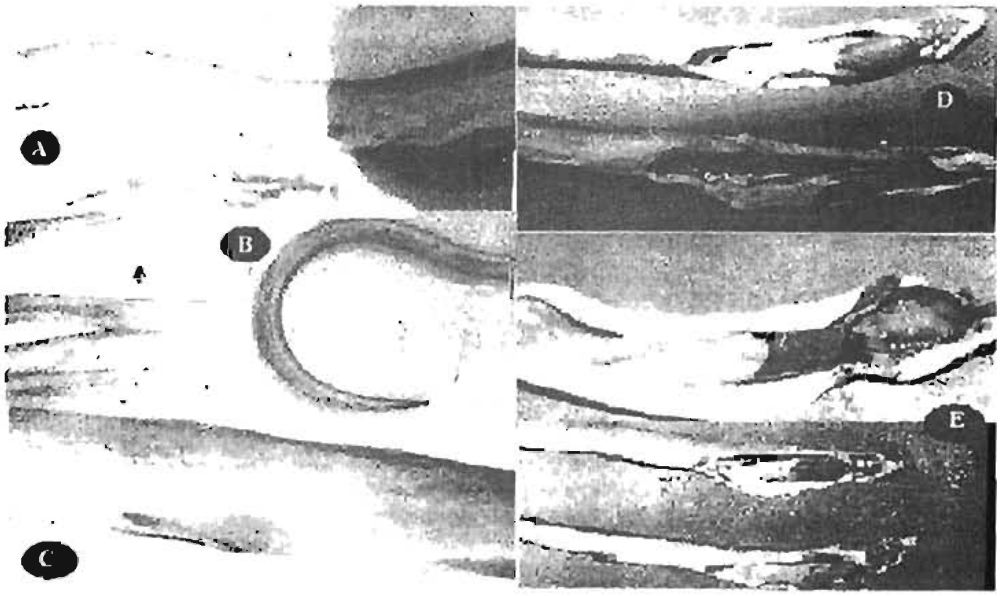
Photograph (2): Eel fish suffered from Pseudomonas septicemia. (A) Petechial hemorrhages and hemorrhages on different parts of the body especially abdominal part. (B) Congestion in the internal organs.



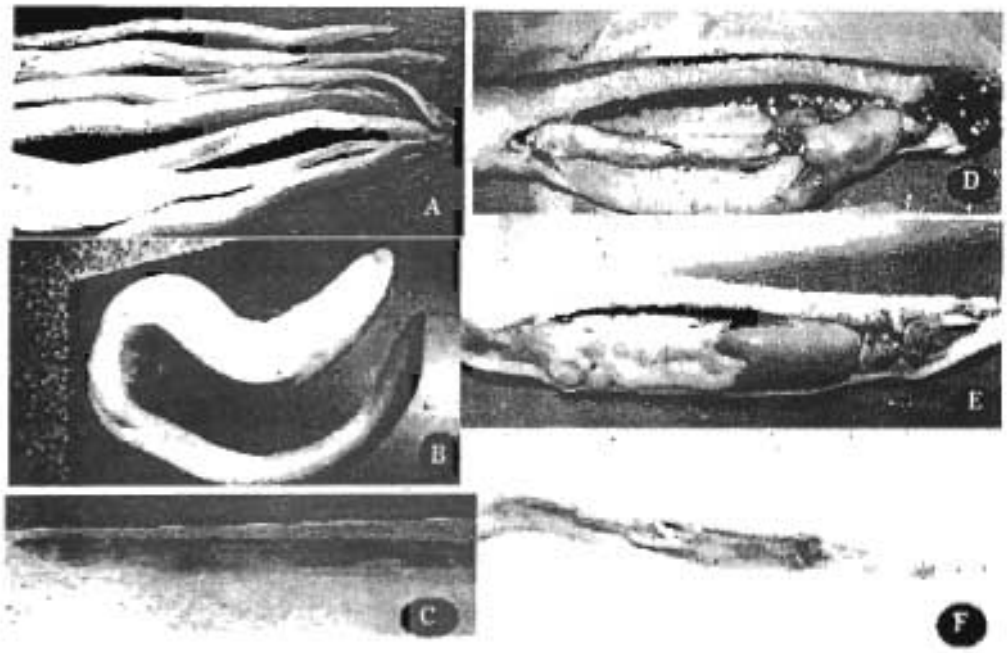
Photograph (3): Eels infected with *S. faecalis*. (A): Exophthalmia and eye cataract. (B): Congested eye. (C): Slight hemorrhages on skin and dark coloration. (D): Intestinal prolapse.



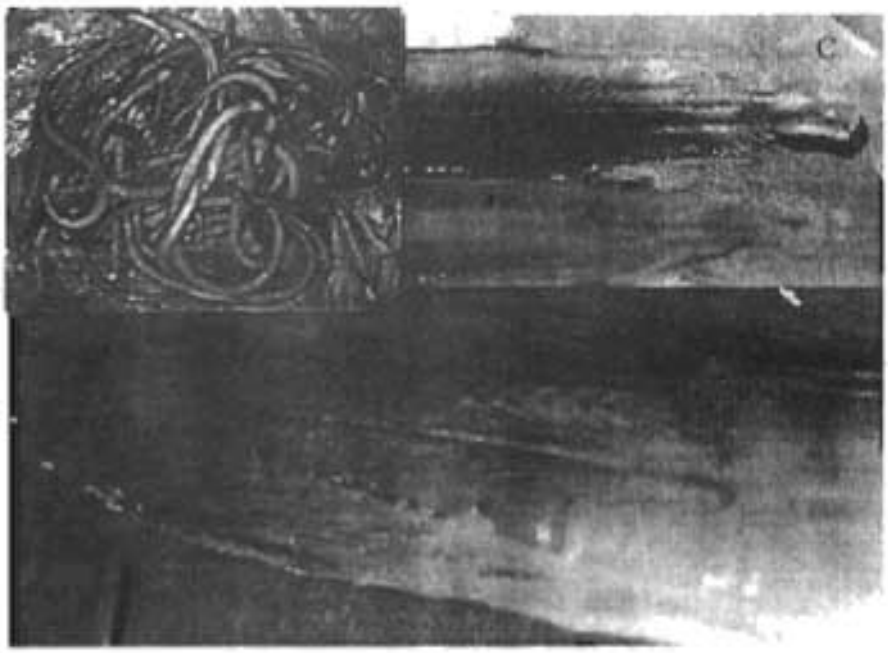
Photograph (4): Postmortem changes associated with *S. faecalis*. (A): Congested gills and pale liver. (B): Congested liver with yellow patches. (C): Congestion in the internal organs. (D): Liver showing pin head white foci.



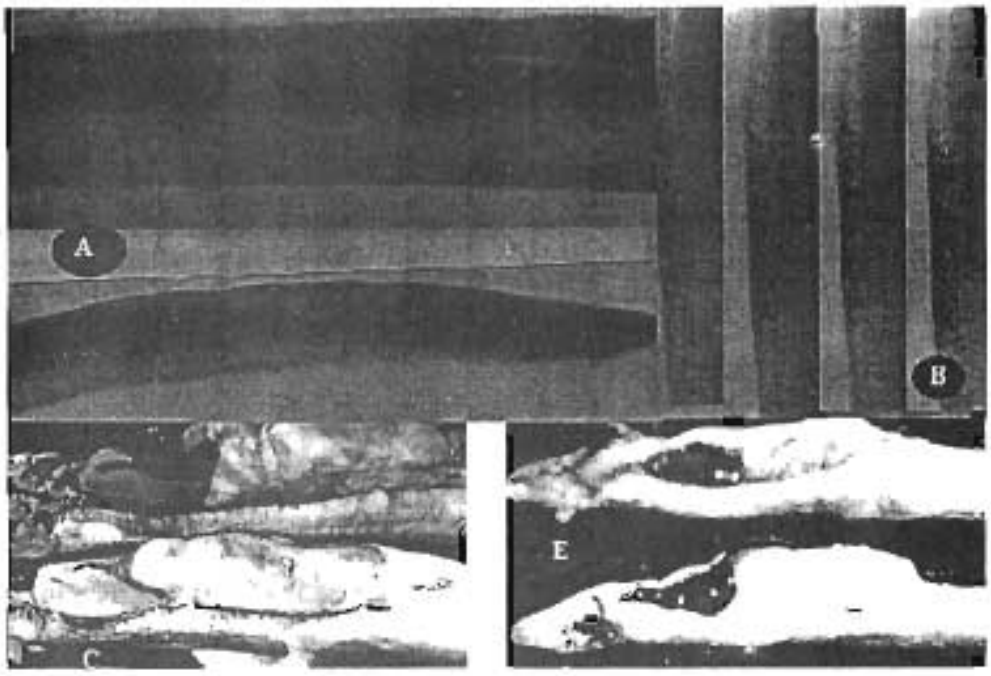
Photograph (5): Eel fish infected with *V. anguillarum*. (A, B&C): Hemorrhages on different parts of body and inflamed anal opening. (D&E): Congested gills and dark congested liver.



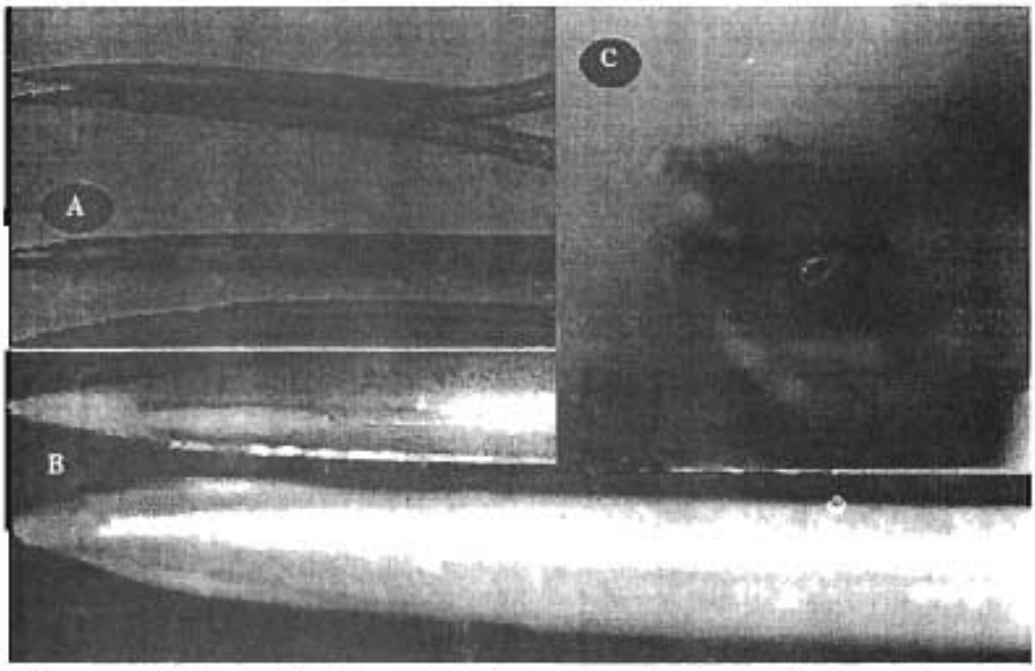
Photograph (6): Eel naturally infected with *V. vulnificus*. (A, B&C): Hemorrhages on different parts of body and inflamed anal opening. (D): Congested gills and yellowish liver. (E&F): congested internal organs.



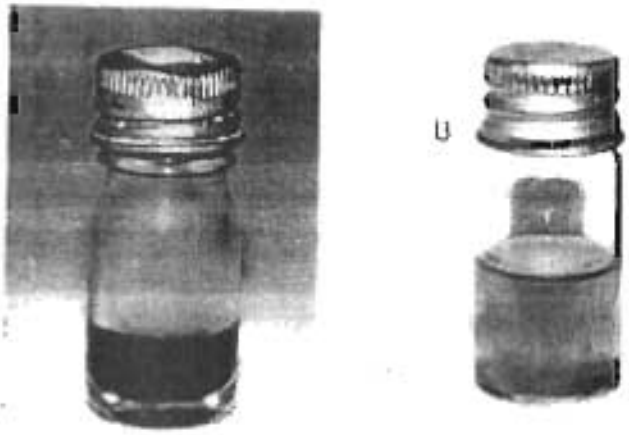
Photograph (7): Eel fish suffered from *F. columnare* infection. (A): Mass mortality. (B): Ulcer. (C): Tail and fin rot and appearance of vertebral column.



Photograph (8): Eel fish suffered from Lactobacillosis. Dark black coloration allover the body (A) on the dorsal part (B). (C&E): Congested and hemorrhagic liver.



Photograph (9): Eel suffered from Saprolegniosis. (A&B): fungal growth on fins and tails. (C): Fungal growth on the mouth.



Photograph (10): Culture of *J. hoferi* on MEM-10 showing hyphal growth at pH 7.0 (A) and pH 3.5 (B).

Photograph (11): In-vitro sensitivity test showing inhibition zone due to clove oil.

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الملخص العربى

دراسات على بعض الممرضات البكتيرية والفطرية التى تصيب أسماك الشعبان

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تم إجراء هذه الدراسة على عدد ٣٠٠ سمكة مريضة من أسماك الشعبان المرباة فى المزارع السمكية لعمل حصر للمشاكل البكتيرية والفطرية التى تواجه إستزراع أسماك الشعبان. تم عزل بعض العشرات البكتيرية السالبة والموجبة الجرام من هذه الأسماك. كانت نسبة الإصابة الكلية لكل من الأيروموناس هيدروفيلا والسودوموناس فلوروسنس والأستربتوكوكس فيكالبز والفيبريو انجيوليرم والفلافوبكتيريا كولنسارز والفيبريو فيليفكس والكترياسيليس والصيروجنيا بارازيتيكا والأكينيتوناس هوفرى هى كالتالى 33.33 و 18.67 و 13 و 12.33 و 10.33 و 9.33 و 3 و 4.67 و 1٪ على التوالى. وتتمثل العلامات المرضية للأسماك المصابة طبيعياً بالبكتيريا فى قفقان الشهية، وزيادة فى إفراز المخاط، وجود أنزفة على الجلد وعلى أماكن متفرقة من جسم الأسماك، عتامة فى العين وجحوظ فى العينين، وتشكل الصفة التشريحية لهذه الأسماك فى إحتقان للأعضاء الداخلية. أما الأسماك المصابة بفطر الصيروجنيا فلقد ظهر عليها نموات قطنية على السطح الخارجى، تم إجراء العدوى الصناعية لبكتريات الأيروموناس هيدروفيلا والسودوموناس فلوروسنس والأستربتوكوكس فيكالبز والفيبريو انجيوليرم حيث إرتفعت معدلات النفوق بين الأسماك العدية، أثبت إختبار الحساسية أن للسيروفلكساسين تأثير قوى كمضاد حيوى، كذلك استطاع علاج الأسماك المصابة إصطناعياً بالأيروموناس هيدروفيلا. أثبتت الدراسة أن لزيت القرنفل تأثير جيد فى منع نمو البكتيريا.