

**CONTRIBUTION ON *PSEUDOMONONAS ANGUILLISEPTICA*  
CAUSING *PSEUDOMONONAS SEPTICEMIA* AMONG  
CULTURED EEL (*ANGUILLA ANGUILLA*)**

**Mohamed .E. Abou El Atta and Ahmed El- Refaee**

*Fish Health and Management Department, Central Laboratory For  
Aquaculture Research, Agricultural Research Center, Egypt*

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**Abstract**

*Pseudomonas anguilliseptica* was isolated from naturally infected eel (*Anguilla anguilla*), showing septicemic picture, clinically the infected eel, suffered from loss of appetite and loss of equilibrium, dullness, sluggish movement, petechial and diffused hemorrhage distributed in different parts of the body and mouth region so may be called the red mouth disease of eel, also, diffused hemorrhage distributed on the lateral and ventral parts of the body and base of the fins, congestion and reddening of the vent and protrusion of genital opening. Internally the infected eel, showed generalized septicemia or severe hyperemic appearance, congestion of the gills, enlarged liver and in some cases showed petechial hemorrhage on its surface, the gall bladder was distended with bile. Congestion of the kidney and spleen, and accumulation of blood tinged exudates in the abdominal cavity. A total 127 isolates from different organs of infected eel (*Anguilla anguilla*) and according to the morphological, biochemical characters and API 20, *Ps anguilliseptica* was identified. The higher percentage was from fins, gills, liver, kidney and spleen and with lower percentage from eye and blood. In experimental injection of eel with isolated *Ps anguilliseptica* in a dose 0.5 ml ( $5 \times 10^5$  CfU /ml) showed I/P route was more virulence than I/M rout. *Ps anguilliseptica* was sensitive to Ciprofloxacin, Oxytetracyclin and Nalidixic acid and resistant to Amikacin, Kanamycin and Colistin.

## INTRODUCTION

Aquaculture is playing an important role in boosting global fish production in meeting the rising demand for animal protein. Bacterial fish diseases constitute one of the major challenges facing sustainable aquaculture production. *Pseudomonas anguilliseptica* was originally isolated from pond cultured Japanese eel (*Anguilla japonica*) suffering from (Sekiten – byo) red spot disease Wakabayashi and Egusa (1972). Later on the infection occurred in eel farmed in Taiwan Kuo and Koy (1978); Stewart *et al.* (1983); Denmark Melergaard and Dulsgaard (1986) and France Michel *et al.* (1992). *Ps anguilliseptica* is an opportunistic pathogen for variety of fish species cultured in marine and brackish water world wide Daly (1999), it was isolated from black sea bream (*Aconthopagrus schlegeli*) Nakajima *et al.* (1983), from Salmonid fish Wiklund and Dalsgaard (1987). An increasing of fin fish farms have been suffering from the diseases in recent years, also *Ps anguilliseptica* was isolated from *Oreochromis niloticus* Saleh and Abd El Rhman (2008). Aquaculture health management is vital to successful industry. The lack of effective disease diagnosis, prevention and control are the chief limiting factors of the realization of highly stable fish production, so the current investigation was planned to throw light on *Ps anguilliseptica*, isolation, identification, pathogenicity, antibiogram and control of *Ps anguilliseptica* in cultured eel (*Anguilla anguilla*).

## MATERIALS AND METHODS

### Naturally infected eel:

A total number of seventy five moribund eels (*Anguilla anguilla*),  $25 \pm 2$  cm in length and 85gm in weight collected from culturing cement ponds in Abbassa, fish farm, Central laboratory for Aquaculture Research, Abbassa, Sharkia, Egypt) without regard to their symptoms, where mass mortalities were being observed during November 2009, the

moribund eels were subjected to clinical and postmortem examinations which mentioned by Amlacher (1970) and luky (1977).

### **Bacterial examination:**

Bacterial swabs were taken from moribund eels (*Anguilla anguilla*) under aseptic condition (Fin, gills, eye, kidney, liver, spleen and abdominal fluid, inoculated into tryptic soy borth (Difco) also blood samples were streaked into tryptic soy agar and pseudomonas base agar supplied with glycerol 1% -, then incubated at 25 °C for 24hrs, pure bacterial isolates were identified using culture characteristics, morphology, physiological and biochemical tests according to Austin and Austin (1993) also API 20 (Bio Merieux) as biochemical tests were done as confirmatory tests.

### **Pathogenicity test:**

A total number of 40 apparently healthy *Anguilla anguilla*,  $25 \pm 2$  cm in length and 85gm weight were distributed into 4 equal groups distributed in 4 glass aquaria (40×40×80) cm, 10 eel in every group, 0.5 ml dose of 24hrs broth culture from isolated *Ps anguilliseptica* ( $5 \times 10^5$  cfu/ml ) were given intramuscular (I/M) in back muscle and intra peritoneal (I/P) for first and second groups and the third and fourth group were injected with sterile broth I/m and I/P according to Wakabayshi and Egusa (1972), the injected eels were daily observed for abnormal clinical signs and mortalities and re-isolation of inoculated strain was done.

### **Sensitivity test:**

The antibiograms of isolated strains were done on Muller hinton's agar medium using the disc diffusion method using discs, Nalidixic Acid, Amikacin, Ciprofloxacin, Tetracyclin, Colistin and Kanamycin. The interpretation of zones of inhibition were estimated according to the limits given by Bio -Merieux (1994)

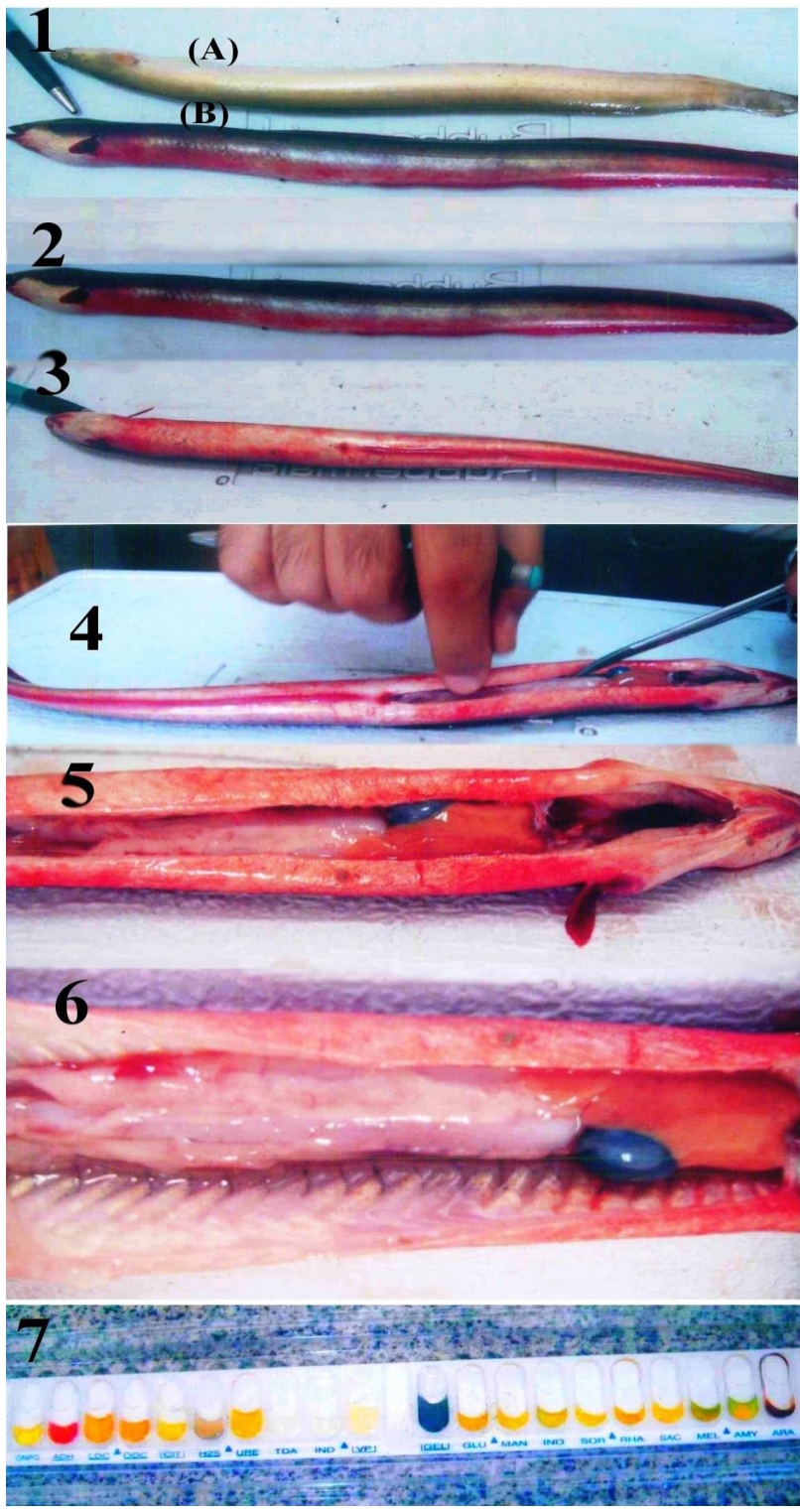
## RESULTS

### Clinical examination:

Infected eels (*Anguilla anguilla*), showed loss of appetite, dullness, loss of equilibrium, sluggish movement, petechial hemorrhage distributed on different parts of skin, mouth region (red mouth), opercula ventral side of the body (red spot disease), reddening of the fins, around the eyes, bilateral exophthalmia and in some cases suffering from opaqueness of the eyes ended with blindness, enlarged abdomen protrusion of the vent, diffused hemorrhage at lateral and ventral surface of the body, reddening of genital region as shown on fig (1), (2), (3). Internally the infected eels showed generalized septicemia or severe hyperemic appearance, gills might to be congested to pale or anemic, petechial hemorrhage in internal organs, accumulation of bloody tinged exudates in abdominal cavity) liver enlarged and the color varied from yellowish to dark brown color, sometime petechial hemorrhage occurred on liver surface in some cases, gall bladder very distended with bile, kidney and spleen were enlarged and congested as shown in fig (4), (5), (6).

### Bacterial isolation:

About one hundred and twenty seven pure cultures of bacterium were isolated from gills, fin, eyes, blood, liver, spleen and kidney of eels with clinical signs described before, based on the culture, characters on tryptic soy agar (glistening, light grey, entire, convex translucent and viscid), morphological characters (the bacteria were gram negative, motile) and physiological and biochemical characteristics are shown in table (1). According to morphological and biochemical tests and API20 the isolates were identified as *Pseudomonas anguilliseptica*, as shown in fig (7), also, some bacteria isolated than *Ps anguilliseptica*. *Ps anguilliseptica* was isolated from different organ of infected eel as shown in table (2) where a higher percentage was from fins 35 isolates (7.08%) and lowers isolates from blood 6, (4.72%).



**Fig. (1)- (A):** normal eel; **(B):** diseased eel.

**Fig. (2)** Dorsal; **Fig. (3)** Ventral view showed petechial hemorrhage distributed on different parts of skin, mouth region (red mouth), opercula ventral side of the body (red spot disease), reddening of the fins, around the eyes, bilateral exophthalmia

**Fig.(4): (5); (6)** showed severe hyperemic appearance, congested gills, petechial hemorrhage in internal organs, accumulation of bloody tinged exudates in abdominal cavity, enlarged and congested liver, distended gall bladder, kidney and spleen were enlarged and congested.

**Fig. (7)** API. Identification of the isolated bacteria.

**Table (1):** Morphological and biochemical characteristics of *Ps anguilliseptica* isolated from eel (*Anguilla anguilla*).

Test	Reaction	Test	Reaction
Gram staining		Growth 37°C	+ve
Morphology		Indol	-ve
Motility		V.P	+ve
Oxidase	+ve	M.R	+ve
Catalase	+ve	H <sub>2</sub> S production	-ve
Arginine hydrolysis	+ve	Nitrate Reduction	+ve
Gelatine liquification	+ve	Citrate utilization	+ve
Growth on 0% Nad	+ve	Sucrose fermentation	-ve
Growth on 5% Nad	+ve	Lactose fermentation	-ve
Growth on 10% Nad	-ve	Maltose fermentation	-ve
Growth at 5°C	+ve	Galactose fermentation	+ve
Growth 30°C	+ve	Glucose fermentation	+ve

**Table (2):** Distribution of *Ps*-anguilliseptica in different organs and tissues.

Organs Bacteria	Fins		Gills		eye		liver		spleen		kidney		Blood	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
<i>Ps anguilliseptica</i>	35	27.55	23	18.11	9	7.08	21	16.53	16	12.59	17	13.38	6	4.72

**Table (3):** Sensitivity of *Ps anguilliseptica* to different antibiotics.

Antibiotics	Code	Sensitivity concentration	Susceptible Zone	Inhibition Zone	Reaction
Nalidixic Acid	NA	30	$\geq 19$	8	+ve
Amikacin	AN	30	$\geq 17$		R
Ciprofloxacin	Cip	5	$\geq 21$	32	+++
Tetracyclin	Te	30	$\geq 19$	22	++
Colistin	CT	10mcg	$\geq 11$	8	R
Kanamycin	K	30	$\geq 18$	20	R

**Pathogenicity test:**

Experimentally infected eel (*Anguilla anguilla*), showed nearly similar clinical and postmortem signs of naturally infected eel as shown in Table (4). The I/P route of injection produced higher mortalities (90%) than I/M route (80%). Re-isolation of *Ps anguilliseptica* was succeeded from all dead and clinically diseased eels but not isolated from control groups which injected with sterile saline.

**Table (4):** Experimental and mortalities pattern among the artificial injected eel with *ps.anguilliseptica*.

Group	No. of eel	Injected material	Site	Dose	Lethal time	Mortality rate
1 <sup>st</sup>	10	<i>Ps. anguilliseptica</i>	I/M	0.5ml $5 \times 10^5$	6 <sup>th</sup> day	80%
2 <sup>nd</sup>	10	<i>Ps. anguilliseptica</i>	I/P	0.5ml $5 \times 10^5$	3 <sup>rd</sup> day	90%
3 <sup>rd</sup>	10	Sterile broth	I/M	0.5ml	survival	0%
4 <sup>th</sup>	10	Sterile broth	I/P	0.5ml	survival	0%

**Sensitivity test:**

Regarding the sensitivity of *Ps anguilliseptica* to different antibacterial agents, the *Ps anguilliseptica* was sensitive to Ciprofloxacin, which showed highly efficiency and higher inhibition zone as shown in fig (8) and table (3) followed by Oxytetracyclin, Nalidixic acid and resistant to Amikacin, Kanamycin and Colisitin.

**Fig.(8)** Antibiogram test.



## DISCUSSION

Sever economic losses in fish farming due to pseudomonas septicemia which caused by genus pseudomonades, *Ps anguilliseptica* infections have caused high mortalities on Japanese eel from since the disease was first recorded in (1971) Muroga and Nakajima (1981). The disease occurs at low temperature during the winter, the main clinical signs in diseased eel (*Anguilla anguilla*) were, loss of appetite, dullness, loss of equilibrium. Sluggish movement due to septicemia and eroded and hemorrhage of fins, petechial hemorrhage on the skin of the mouth region (reddish mouth), opercula, and ventral side of the body (red spot disease), these results were in accordance with the recording with Wakabayashi and Egusa (1972) which called the disease " Sekiten – bogu" which mean red spot disease also, was recorded by Haenen and Davidse (2001) and El Refaee *et al.* (2008). Hemorrhages at the base of the fins could be attributed to release of powerful bacterial proteolytic enzymes of *Ps anguilliseptica* which leads to electrolyte and protein loss together with disturbed blood circulation Amlacher (1970) and Morita, (1975). Internally the diseased eel (*Anguilla anguilla*) Suffering from sever hyperemic appearance, congestion of all internal organs, petechial hemorrhage on liver surface, enlarged gall bladder, accumulation of bloody fluid in abdominal cavity, these lesions recorded by Wakabayashi and Egusa (1972); Muroga and Nakajima (1981) Nakai and Muroga (1982); Biosca *et al.* (1991); Michel *et al.* (1992) and El Refaee *et al.* (2008). Congestion of internal organs and edema were seen to play a role in the enlargement of kidney spleen, liver, the over distended gall bladder could be attributed to enteritis and constriction of the common bile duct by periduct fibrosis, these results are conceited with those noticed by Miyazki and Egyuse (1997); Lonnstrom *et al.* (1994); Eillis *et al.* (1993); Berthe *et al.* (1995) and also recorded by Sakr and Abd El Rhman (2008).

The isolated bacteria according to culture, morphological, biochemical characterizes and API 20 identified as *Ps. anguilliseptica*, it was isolated with high percentage from fins, gills, liver, kidney spleen and low percentage from eyes and blood, these results agree with those recorded by Wakabayashi and Egusa, (1972); Haenen and Davides (2001), also Sakr and Abd El Rhamn (2008) isolated *Ps. anguilliseptica* from cultured *Oreochromis niloticus* reared in freshwater. The experimental infection to eel *Anguilla anguilla* showed nearly similar clinical and postmortem lesions and also I/P route of injection was more pathogenic than I/M route, these results mentioned by Wakabayashi and Egusa (1972); Berthe *et al.* (1995); Haenen and Davides (2001) and Sakr and Abd El Rhman (2008). The isolated *Ps. anguilliseptica* was sensitive to Ciprofloxacin which gave highly efficacy and higher inhibition zone; these results agree with that obtained by Sakr and Abd El Rhman (2008) which recorded that *Ps. anguilliseptica* isolated from *Oreochromis niloticus* was sensitive to Ciprofloxacin.

### CONCLUSIONS

From the results obtained from this experiment, *Ps. anguilliseptica* was isolated from infected eel *Anguilla Anguilla* and cause septicemia among cultured eel and so the disease called red mouth disease of eel; Ciprofloxacin 5µg was the most effective drug used in control of , *Ps. anguilliseptica*.

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## إسهامه على السودوموناس أنجويللي سببتكا المسببة للتسمم الدموي السودوموناسي في أسماك الثعبان المستزرعة

محمد السيد أبو العطا ، أحمد محمد عزت الرفاعي

قسم صحة الأسماك - المعمل المركزي لبحوث الثروة السمكية العباسية - مركز البحوث الزراعية  
- القاهرة - مصر .

تم إجراء هذه الدراسة على ميكروب السودوموناس أنجويللي سببتكا أحد المسببات لمرض التسمم الدموي السودوموناس في أسماك الثعبان المستزرعة حيث كانت الأعراض الإكلينيكية تمثل في فقدان الشهية والتوازن - فقدان الحركة والحركة المتخبطة. ظهور نقط نزيفية وأنزفه كانت منتشرة في مناطق مختلفة للجسم في منطقة الفم ولذلك سمى المرض بأسم (مرض الفم الأحمر) وكذلك عند قواعد الزعانف وعلى جانبي الجسم وكذلك منطقة البطن، إحمرار وتورم منطقة التناسل وفتحة الشرج. داخليا كانت أسماك الثعبان تعاني من إحتقان الخياشيم وأعراض التسمم الدموي- كبر حجم الكبد وإحتقان الكلى والطحال وإمتلاء الحويصلة المرارية بإفرازاتها ووجود سوائل مختلطة بدم في منطقة البطن. من الفحص البكتريولوجي لاسماك الثعبان المصابة تم عزل ١٢٧ عترة من السودوموناس حيث تم التعرف عليها مورفولوجيا وفسولوجيا وبيوكيميائيا وكذلك عن طريق API<sub>20</sub> حيث تم تعريفها الى السودوموناس أنجويللي سببتكا وتم عمل العدوى الصناعيه عن طريق الحقن البرويتونى والحقن العضلى حيث اثبتت التجربه ان الحقن البرويتونى للميكروب اكثر ضراوه من الحقن العضلى وتم عمل اختبار الحساسيه لميكروب السودوموناس أنجويللي سببتكا وتبين انها حساسه لكل من السيبروفلوكسا سين والتترا سيكلين ونالديكسك اسد بينما كانت مقاومه لكل من الاميكاسين والكولستين والكاناميسن.