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PATHOGENICITY OF PSEUDOMONAS AERUGINOSA TO NILE FISH

(With 1 Table & 2 Figs.)

By

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دراسة تجريبية لمعرفة القدرة المرضية لميكروب
السودوموناس ايرجينوزا للأسماك النيلية

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تم عزل ثلاثة عترات من ميكروب السودوموناس ايرجينوزا من الأحشاء الداخلية للأسماك والدواجن وكذلك من بول الانسان وقد تم دراسة القدرة المرضية للميكروب المعزول من المصادر المختلفة على عدد ٦٠ من أسماك البلطي بعد تقسيمها الى مجموعات كما يلي ووضعها في أحواض زجاجية سعة ١٠٨ لتر مزودة بمضخات هواء وتم التغذية بعليقة مكونة من ٦٠٪ ردة أرز، ٢٥٪ بذرة القطن المقشر ، ١٥٪ سمك ولحوم وقد تم تغير المياه يوميا وترك السمك لمدة ثلاثة أيام قبل الحقن لملائمة الأسماك مع جو المعمل والبيئة الموجود بها . واحتوت المجموعة الأولى على عدد ١٥ سمكة مقسمة الى ثلاث مجموعات في ثلاثة أحواض تم حقنها في العضل بميكروب السودوموناس أرجينوزا المعزول من الانسان على النحو التالي : عدد ٥ سمكه حقنت بمزرعة تحتوى على ١٠^٦ ميكروب ، عدد ٥ سمكه حقنت بمزرعة تحتوى على ١٠^٧ ميكروب ، عدد ٥ سمكه حقنت بمزرعة تحتوى على ١٠^٨ ميكروب . وكما احتوت المجموعة الثانية عدد ١٥ سمكة حقنت في العضل بالميكروب المعزول من الدواجن بنفس الجرعة والكمية في المجموعة الثانية. وكذلك حقنت المجموعة الثالثة بنفس الطريقة السابقة ولكن بميكروب معزول من الأسماك ، واخيرا تركت المجموعة الرابعة بدون حقن واستعملت كضوابط للتجربة . وقد أوضحت النتائج أن عدوى الأسماك بميكروب السودوموناس ايرجينوزا المعزول من المصادر المختلفة وبتركيزات مختلفة (١٠^٦ ، ١٠^٧ ، ١٠^٨ ميكروب) أدت الي موت الأسماك بنسبة ١٠٠٪ في مدة تتراوح مابين ١، ١٥ يوم حسب الجرعة المحقونة وبالكشف على الأسماك الميتة ووجد نزيف منتشر على أجزاء الجسم المختلفة .

SUMMARY

Intramuscular (i/m) inoculation of fish, Poultry & human strains of Ps. aeruginosa revealed that the three strains were pathogenic to fish and lead to 100% mortality within 1-15 days post inoculation and according to the dose used in infection. The clinical signs began to appear within few hours post inoculation. Died fishes showed different haemorrhagic patches distributed all over the body.

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INTRODUCTION

Many fishery workers have described Pseudomonad infections in warmwater, cold water aquarium and marine fishes.

A case of tail rot in gold fish was attributed to bacterium whose characteristics suggested it was a Pseudomonad (CONROY, 1963). When the bacterium was injected into healthy gold fish, petechiae and red spots developed on the fins.

KUBOTA and KAKAKUWA (1963) observed the occurrance of an infectious dermatitis caused by Pseudomonas species, which constitute an important cause of loss in marine fish forming operations. The infection was characterized principally by an inflammation and change of colour of the skin.

An epizootic among artificially propagated gold fish was apparently caused by a non motile, capsulated, Pseudomonad (BULLOCK, 1965). Sick fish listeleness exhibited, haemorrhages on the fins, skin and viscera and accumulation of bloody peritoneal fluids.

A study of the characteristics of the fish pathogenic pseudomonads indicate most infections are caused by Ps.fluourescence or very closely related species (BULLOCK, et al. 1965).

STEWART, et al. (1981) showed that in early 1981 a severe haemorrhagic disease broke out among cultured adult european eels Anguilla angiulla at Herriot-walt University. REd spot disease was diagnosed with Ps.angilliseptica isolated from diseased fish.

NAKAI and MUROGA (1982), could isolate two strains of Ps.anguilliseptica from red spot disease in eels in Scotland were identical to the Japanese strain No. 2 (K-type).

DAVIS and HAYASAKA (1983), reported that Pseudomonas species isolated from American eels considered as a potential pathogenes.

MOUSTAFA (1986) studied the relationship between virulence and route of inoculation (i/p, i/m and scarification) of Ps.flourescens in Nile fish and noticed that mortality rate reached to 100% within 1-3 days in fishes inoculated i/p, and 75% within 2-5 days in fishes inoculated i/m. Fish which were infected by scarification route survived the infection without any visible clinical signs of the disease except slight oedematus swelling at the site of scarification.

The aim of the present work is to study the pathogenicity of Ps.aeruginosa to fish. Moreover, to study the relationship between the source of the organism and its pathogenicity to fish.

MATERIAL and METHODS

Three strains of Ps.aeruginosa were isolated from internal organs of fish, poultry as well as human urine on Pseudomonas CN supplement SR 102.

The isolated strains were subjected to identification according to BUCHANAN and GIBBONS, 1974 and COWAN and STEEL, 1975 by pigment production, growth at 41°C not at 4°C, motility, O.F test, Casein hydrolysis, gelatine liquefaction and haemolysis on blood agar.

The strains were kept in semisolid agar and when need for inoculation were subcultured in nutrient broth 24 hours at 37°C inculation. Decimal dilutions were carried out in 0.9% sterile saline solutions and number of the organism per ml was determined by plate count method (CRUICKSHANK, 1965).

Experimental infection in fish:

A total number of 60 Tilapia nilotica were caught from the ponds with nylon seine according to MATHUR and RAMSAY (1974) and transported to the laboratory avoiding any damage.

Fish were kept under the laboratory condition at least for three days to adopt them to aquaria (MACEY, et al. 1974). The fish were divided into four groups, each group consisted 15 speciemens.

The aquaria used in the present experiment were belonging to 12 glass aquaria of 90 x 35 x 40 cm. Size of 108 litres capacity. 5 speciemens were reared in each glass aquarium. The aquaria were aerated continously by electric air pumps, RENA type 301 and the water was circulated continously during all the experimental period. The water of the aquaria was renewed partially every one day. All of the experiment were done under natural light.

Fish were fed on a ration contained a mixture of 60% rice bran, 25% decorticated cotton seed meal and 15% fish meal and meat meal (BISHAI, et al. 1972 and JAUNCEY and ROSS, 1982).

The number of the groups depended on the scheme of the experiments:

Group (1): 15 fish were inoculated i/m with Ps.aeruginosa isolated from human according to the following system:

a) 5 fish were inoculated by broth culture containing 10^4 per ml viable cells of Ps.aeruginosa.

b) 5 fish were inoculated by broth culture containing 10^6 per ml viable cells of Ps.aeruginosa.

c) 5 fish were inoculated by broth culture containing 10^8 per ml viable cells of Ps.aeruginosa.

Group (2): 15 fish were inoculated i/m by broth culture containing Ps.aeruginosa isolated from poultry and with the same by as group 1.

Group (3): 15 fish were inoculated i/m by broth culture containing Ps.aeruginosa isolated from fresh - water fish and with the same manner as group 1.

Group (4): 15 fish left without inoculation as a control. All groups were kept under observation for 15 days. Fish died were subjected to bacteriological and PM examination.

RESULTS

Results of experimental infection in fish (Tilapia nilotica):

Experimental results are recorded in Table (1).

Experimental infection of fish intramuscularly with various doses (10^4/ml, 10^6/ml & 10^8/ml) of human strain leading to 100% mortality at different period, 9, 11 & 7 day's post infection respectively.

Infection of fish with different dilution of poultry strain produced 100% mortality within period 15, 8 & 5 days post infection according to the dose used in infectino.

Ps. aeruginosa of fish strain resulted in 100% mortality within period 6-9 days post infection according to the dose used in infection.

Control group were survived during the period of the experiment (15 days).

Postmortem examination of fish died within 72 h post infection showed haemorrhagic patches all over the body particularly on dorsal & caudal fins and muscle of thoracic region, the haemorrhage extended deeply in the muscle (Figs. 1 & 2).

It is worths to mention that there was no relationship between the severity of gross lesions and the dose of the organism used in the infection i.e. infection of fish with 10^{-4}, 10^{-6} and 10^{-8}/ml Ps. aeruginosa resulted in similar gross lesions.

DISCUSSION

Although, there are many invistigators (CONROY, 1963; KUBOTA and KAKAKUWA, 1963; BULLOCK, 1965; BULLOCK, et al. 1965; STEWART, et al. 1981; NAKAI and MUROGA, 1982; DAVIS and HAYASAKA, 1983 and MOUSTAFA, 1986) who studied the pathogenicity of Pseudomonas spp. (Particularly ps. flourscens) to fish, yet there are no available literature which discuss the pathogenicity of Ps. aeruginosa to fish. Yet the results of the present study, proved that experimental infection of fish (Tilapia nilotica) with Ps. aeruginosa was highly pathogenic to fish.

Irrespective of the origin and dose of Ps. aeruginosa, fish were highly susceptible to different doses (10^4, 10^6 & 10^8/ml) of human, poultry and fish strains. Death occur 1-15 days post infection.

The clinical signs began to appear on fish within few hours post inoculation. Fish sinked down to the bottom of the aquaria, grouped together and listeleness.

Fish that died within 24-72 hours post infection showed different haemorrhagic patches distributed all over the body particularly on dorsal, caudal fins and muscle of thoracic region, haemorrhage extended deeply in the muscles.

Fish which were died 3-15 days post infection showed, roughness of the scale and detachment from some part of the skin.

All visceral organs were congested and the gall bladder was enlarged and distended with bile.

Ps. aeruginosa was reisolated from dead fish, control fish possesed no clinical signs and were still alive after 15 days (experiment period).

With respect to the origin of Ps. aeruginosa, the present study indicated that the organism is highly virulent to fish and produced lesions similar to that produced by the other species of Pseudomonacae.

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Table (1): Results of Pathogenicity test of fish, poultry & human strains of Ps. aeruginosa in Tilapia milotica.

Group No.	Route of infection	Dose/ml	Source of strain	No. of infected fish	Post infection mortality at days															Mortality		Survive	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	No.	%	No.	%
1	I/M	10^4/ml	Fish	5			1					4								5	100	0	0
			Poultry	5							1		2		1				1	5	100	0	0
			Human	5		2					1	1	1							5	100	0	0
2	I/M	10^6/ml	Fish	5	1	1						2	1							5	100	0	0
			Poultry	5	2		1				1	1								5	100	0	0
			Human	5	2	1	1								1					5	100	0	0
3	I/M	10^8/ml	Fish	5	2	1	1			1										5	100	0	0
			Poultry	5			1	3	1											5	100	0	0
			Human	5	1	2	1				1									5	100	0	0
4	Control	Control	--	5																0	0	5	100

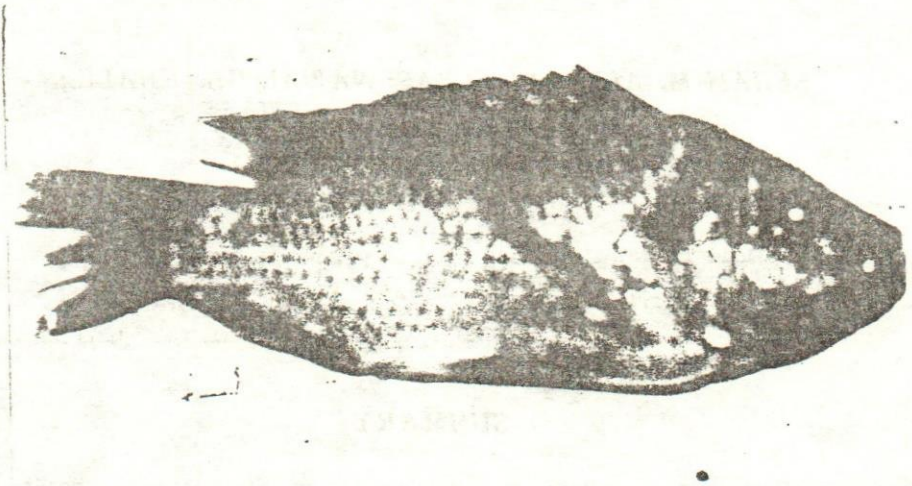

Fig. (1): Fish died within 48 hour post inoculation with 10^8/ml. fish strain. Ps. aeruginosa showing severe haemorrhage on the different parts.

Fig. (2): Fish died from poultry strain of Ps. aeruginosa showing signs of septicaemia.