Assiut Vet. Med. J. Vol. 36 No. 72, January 1997

Dept of Fish health, Central lab.
for Aquaculture, Abbassa, Abu-Hammad, Sharkia, Egypt

FLAVOBACTERIOSIS AMONG MUGIL CEPHALUS IN LAKE TEMSAH

(With One Table and One Figure)

By
A.S. DIAB

(Received at 18/11/1996)

مرض الفلافوبكتيريا بين أسماك البورى فى بحيرة التمساح

أحمد دياب

قامت الدراسة بفحص ١٠٠ سمكة بورى مريضة وحديثة النفوق تم جمعها من احدى المزارع البحريه ببحيرة التمساح بلاسماعيلية وتبين من الفحص الاكينيكى وجود اختناقات وتقرحات وخاصة حول منطقة الرأس بالاضافة الى مناطق الفم والغطاء الخيشومى، كما أثبتت الصفة التشريحية وجود احتقان فى معظم الأعضاء الداخلية. وبالفحص البكتيرى تم عزل ميكروب الفلافوبكتيريا، كما تم عمل عدوى صناعية عن طريق الحقن العضلى والحقن البريتونى فى أسماك البورى السليمة ظاهريا بنفس الميكروب العزول ووجد أن الميكروب مرضى وقادر على احداث العدوى وذلك فى حالة الحقن العضلى عنها فى حالة الحقن البريتونى.

SUMMARY

A total of 100 moribund and freshly dead cultured marine Mullet (Mugil cephalus), from a marine farm at Lake Temsah, was subjected to clinical and postmortem examinations. Samples were also aseptically taken for bacteriological examination. Haemorrhages and ulcers mainly at the head region beside eyes, gills, lips and operculia were observed. The majority of the affected fish showed congestion and petechiae in most of the internal organs. The isolated organisms were identified as flavobacterium spp. The isolated strain was found to be pathogenic when injected I.M. in healthy fish while I/P injection was less pathogenic.

Key words: *Mugil cephalus-Flavobacter-Lake Temsah.*

INTRODUCTION

Flavobacterium are normally present in water (Post, 1987) and can enter susceptible fishes through external wounds. The Flavobacteria cause disease in fresh water and marine fishes. The high mortality of affected fish may be attributed to the production of neuro-muscular toxins produced by Flavobacterium spp.

Regarding the identification of Flavobacterium spp. to genus only, Post (1987) and Eissa (1993) recorded that it was difficult to classify it due to inconsistencies of biochemical characters and suggested its name as Flavboacterium spp.

Boonyaratpalin et al. (1983) and Shamsudin (1986) showed that Flavobacterium spp. was found to be potentially pathogenic.

MATERIAL and METHODS

A) Fish:

A total of 100 moribund and freshly dead cultured marine Mullet fish *Mugil cephalus* was collected from a marine farm at Lake Temsah in Ismailia Governorate after taking the history of the disease.

B) Clinical and postmortem exmaminations:

All specimens were subjected to clinical and postmortem examinations where any external or internal gross lesions were observed according to the methods described by Schaperclaus et al. (1992).

C) Bacteriological examinations:

Samples were taken aspetically for bacteriological examination from skin lesions, gills, eyes, liver and spleen. They were inoculated into peptone water containing NaC1 3% and incubated at 25°C ± 1°C for 24-48 hours accordnig to Munn (1978).

Loopfuls from the inoculated tubes were subcultured on various selective media. Brain Heart Infusion agar (BHI), Rimler and Shotts (RS) and Trypticase Soya agar (TSA) with addition of 3% NaCI. The media were incubated at 25 ± 1°C for 48 hours accordnig to Austin (1988). Morphological and biochemical identification of suspected colonies were adopted after Plumb (1994).

Experimental infection:

The recovered bacterial isolates were examined for its ability to produce the disease in apparently healthy Mullet fish under experimental conditions. 40 fish were divided into four equal groups and each group was

kept in a well prepared aquarium and thermostatically controlled for adjusting water temperature at 25±1°C. After acclimatization for one week on salt water, the first group was inoculated intramuscularly (I/M) with 0.5ml of 24 hours broth culture containing about 10^7/ml viable bacteira while the second group was inoculated intraperitoneally (I/P) with the same dose according to the method described by Lucky (1977). The third and fourth groups were left as controls and infected with 0.5 ml sterile broth I/P & I/M respectively.

All the groups were observed daily for two weeks for clinical signs and any abnormalities. Reisolation of the inocualted bacterial pathogen was done.

RESULTS and DISCUSSION

Results of clinical examination:

Diseased Mullets *Mugil cephalus* fish suffered from lethargy, inability for swimming, gasping, stop feeding, inactive and lack the escape reflex. They revealed open irregular and haemorrhagic ulcers mainly at the head region beside eyes, gills, lips and operculi accompanied with erythema in the mouth and within the operculi (Fig. 1).

Results of postmortem examination:

Affected fish showed congestion and petechiae in most of the internal organs and musculature.

Results of Bacteriological examination:

A) The bacterial isolates from liver and kidneys revealed yellowish orange pigmented colonies. A smear revealed non motile long bacillus and was Gram negative on staining with Gram. The isolates gave positive results with catalase oxidase and nitrate reduction and were negative with indole, urea hydrolysis and oxidative and fermentative (O/F test), while was variable with hydrogen sulphide production test.

Based on the bacteriological examinations, these strains were identified as Flavobacterium spp.

B) Regarding the experimental infection, the I/M infected fish showed the same clinical signs and P.M. lesions observed in natural infection and showed 70% mortality rate. While, I/P infected ones revealed only erythema at the site of injection with mild congestion in the internal organs and gave only 30% mortality rate. Flavobacterium spp. was reisolated again from liver and kidneys of all freshly dead and clinically diseased fish (Table. 1).

Table. 1: Pathogenicity test in healthy Mullet fish with flavobacterium spp.

Group	Number	Route of inoculation	Dose	Mortality	
				Rate	%
1	10	I/P	0.5 ml X 10^7	3/10	30%
2	10	I/M	0.5 ml X 10^7	7/10	70%
3	10	I/P	0.5 ml sterile broth	0/10	0.0%
4	10	I/M	0.5 ml sterile broth	0/10	0.0%

The morphological features of the isolated organism in thise study agree with those of Post (1987), Eissa (1993) and Stoskopf (1993) who mentioned that the bacteria produce yellow orange or brown pigmented colonies on solid media, Gram-negative non-spore forming bacteria, ranging from coccobacilli to long, slender rod shaped organisms; they may be motile or non-motile.

The results of clinical examination and postmortem findings are similar to those mentioned by Senieszko and Axelrod (1971), Post (1987), Eissa (1993) and Stoskopf (1993).

The post mortem findings may be due to toxin production which causes, aimless swimming, disturbance of equilibrium, spasms, convulsions, finally paralysis and death (Meyer et al., 1959).

The clinical signs and postmortem lesions observed in the experimentally infected fish as well as the the reisolation of the flavobacterium spp. from these fish indicated that this organism caused the disease. The I/M route was more effective than the I/P route.

Shamsudin (1986) isolated from bighead carp (*Aristichths nobilis)* and grass carp (*Ctenopharngodon idella)* Gram-negative rods of the genus Flavobacterium which was found by other workers to be potentially pathogenic to fish.

ACKNOWLEDGMENT

The author received the fund from France through the Ministry of Agriculture to Marine and Shrimp diseases project.

REFERENCES

*Austin, B. (1988):*Marine microbiology. Cambridge Univ. Press. USA. 1st Ed. 118-120.

Boonyaratpalin, S.H.; Roengprach, S.; Direkbusarakam, J.; Kesornchan and Supamart, K. (1983): Diseases of fishes caused by bacteria. Thai. Fish. Ga2, 36: 247-255.

Eissa, L.A.M. (1993): Role of Borcelian crabs as enemies of Marine cultured mullet in lake Temsah. Zag. Vet. J. 21, 926-935.

Lucky, Z. (1977): Method for the diagnosis of fish diseases. Amerind Publishing Co. PVT Ltd, New Delhi, Bombay.

Meyer, S.P.; Baslow, M.H.; Bein, S.J. and Marks, C.E. (1959): Studies of Flavobacterium pisticida Bein I. Growth, Toxicity, and Ecological consideration. J. Bacteriol. 78: 225.

Munn, C.B. (1978): Haemolysin production by *Vibrio anguillarum* FEMS. Microbiology Let. 3: 265-268.

Pulmb, J.A. (1994): Health Maintenance of cultured fishes: principal microbial diseases CRC press, BoCa Raton, Fl. USA.

Post, G. (1987): Textbook of fish health. T.F.H. Publ., Ltd., 2nd Ed.

Schaperclaus, W.; Kulow, H. and Schreckenback, K.C. (1992): Fish diseases. Vol. I.A.A.

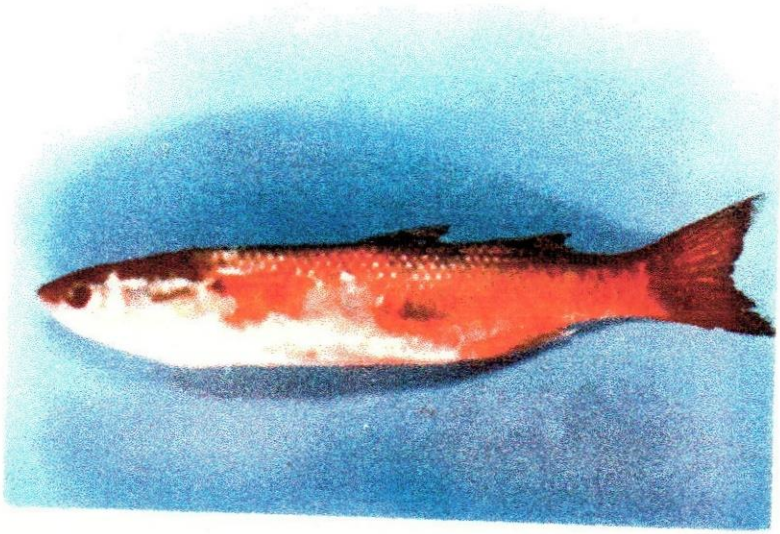

Fig. 1: Showing erythema allover the external body surface of Mullet fish *M.cephalus*