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Dept. of Poultry and Fish Diseases,
Fac. Vet. Med., Suez Canal University.

THE ROLE OF DOMESTIC WASTE WATER BACTERIAL POLLUTION ON THE HISTAMINE PRODUCTION IN SOME FISHES AND IMMUNE RESPONSE OF CATFISH (*CLARIAS LAZERA*)

(With 3 Tables)

By

A.F. BADRAN and M.M. HUSSEIN*

*: Dept. of Hygiene, Fav. Vet. Med., Suez Canal University

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دور التلوث البكتيري بمياه الصرف الصحي على إنتاج الهستامين في بعض الأسماك ودراسة تأثيره على الجهاز المناعي للأسماك القطبة (قرموط اللازيرا)

أحمد فكري بدران ، محمود محمد حسين

يعتبر التلوث بمياه الصرف الصحي من أهم المشاكل البيئية التي تهدر صناعة الأسماك لذلك أجـريت هـذه الدراسة على عدد ٢٣٠ سمكة مجمعة من نزعة الإسماعيلية وبحيرة التمساح ومزارع الأسماك الخاصة لربط مدى تأثير البكتريا الموجودة في المياه على تكوين الهسامين فـي الأسـماك وتم عزل الميكروبات الآتية أشيرشيا كولاى -كلبسيلا – بروتيس انتيروبكتر ايروجينز والستروبكتر وتم تحديد نسبة الهستامين في الأسماك وقد وصل إلى أعلى نسبة في الصـيف ٦ملـليجرام/١٠٠جـرام مـن وزن السمك ووصل إلى أدنى مستوى ٠,٠٥٥ في القراميط وتم دراسة قدرة هذه الميكروبات على إحداث المرض في الأسماك وأيضا تم دراسة تأثير الهستامين على الجهاز المناعي لأسماك القراميط ووجد أنه له تأثير مثبط للمناعة تحت ظروف مختلفة.

SUMMARY

Water pollution is considered as one of the major environmental problems. The domestic wastes either partially treated or non-treated are being discharged into surface water. Therefore, a total of 230 freshly caught of fish and water samples were collected from Ismailia Canal,

Lake Temsah and a private fish farm during different seasons to correlate between pollution with domestic waste water and histamine production in fish. The obtained results revealed the isolation of *E.coli, Citrobacter, amatonaticus klebsiella pneumoniae, Proteus mirabilis* and *Enterobacter aerogenes* from both water and fish. These organisms were might responsible for histamine formation in fish stored at room temperature for 6-8 hours, which reached maximum level in summer season as (6mg/100gram fish body weight) and lowest level in winter season. (055mg/100gram fish body weight). The pathogenicity of the bacterial isolates to fish was studied. Regarding the effect of histamine on the immune response, the results obtained indicated that histamine had immune suppressor effect on fish immunized by *E. aerogenes* under different treatments.

Key words: *Waste water, Bacterial pollution, Histamine production, Catfish/*

INTRODUCTION

Histamine has been implicated as the causative agent in scombroid fish poisoning which is sometimes associated with consumption of Tuna, mackerel and other fishes. The musculature of fish normally contains large amounts of free histidine, which can be decarboxylated to histamine by certain bacteria. Numerous of these histamine decarboxylating bacteria have been isolated from decomposed and fresh scombroid fish. Such bacteria mainly belong to mesophilic members of Enterobacteriaceae and others belong to vibrio, lactobacillus and clostridium species (Yashinaga and Frank, 1982).

Nowadays, pollution with domestic wastewater is most common in River Nile and their tributaries. Moreover, most of earthen ponds used as fish farms at Sharkia, governorate are supplied by such water containing bacteria, which may cause fish infection.

This study was planned to:

1- Investigate the predominant Enterobacteriaceae in some fish species and its responsibility on histamine formation in fish flesh.
2- Study the relationship between water pollution with sewage and histamine production in the examined fish.
3- Clarify the role of histamine on the immune response of fish.

MATERIAL AND METHODS

I- Fishes:

A total of 230 freshly caught fishes was used throughout this investigation for bacteriological, chemical and immunological studies collected from Ismailia Canal, Lake Temsah and a freshwater fish farm.

For bacteriological and histamine determination, a total of 120 fish was used and represented by 40 Mullet (*Mugil cephalus*) of 250 ±10 gram body weight, 40 Nile Tilapia (*Oreochromis niloticus*) weighted 100 ± 5 gram and 40 Armout catfish (*Clarias lazera*). The samples were collected throughout different seasons (10 of each species/season).

For pathogencity test, 60 Nile Tilapia of 80 ± 5 grams body weight were used. For immunological studies, 60 Armout catfish of 50 ± 5 grams body weight were used.

II- Water samples:

The water samples were collected throughout different seasons using sterile one liter glass bottle from Ismailia Canal, fish farm and Lake Temsah (brackish water). The sample was put in an icebox for bacteriological examination according to the procedure suggested by the American Public Health Association (1985).

1- Preparation of fish homogenate:

The fish homogenate was prepared by taking a sample from the dorsal musculature, homogenized for 10 minutes in 0.1 peptone phosphate buffer solution pH 7.0 (1:9 w/v).

As regards the water sample the determination of the Most Probable Number technique and the types of the coliforms were carried according to the standard of the American Public Health Association (1985).

2- Isolation of histidine decarboxylase producing bacteria:

These bacteria which are related to family Enterobacteriaceae were isolated by inoculating 1ml of water sample or fish homogenate into 10ml of Enterobacteriaceae enrichment broth (E.E.B) and incubated at 35°C ±1 for 24 hours. The E.E.B culture was diluted in 0.1% peptone water and one ml of the diluted media was used for inoculating duplicate pourplates of Niven's media, which were incubated at 35°C for± 24 hours. The Amino Acid producing bacteria on the mentioned media were characterized by the production of purple colonies with purple haloes, which were further identified according to scheme mentioned by Krieg and Holt (1984).

3- Histamine Determination:

Histamine was determined in fish homogenate by high performance liquid chromatography (HPLC) as described by Mietz and Karmas (1977). This was done on fresh caught fish as well as after being kept at room temperature for 6-8 hours.

4- Pathogenicity Test:

The pathogenicity of the bacterial isolates was performed for selecting the bacterin preparation isolates. Five pure bacterial isolates represented as *E. coli, Enterobacter aerogenes, Citrobacter amatonaticus, Klebsiella pneumoniae and Proteus mirabilis* were used for preparation of bacterial suspension using 24h culture from TSA incubated at 35°C±1 in sterile phosphate buffer solution for fish inoculation according to Badran (1987). The bacterial concentration was estimated to be between 3.0×10^8 and 1.3×10^4 colony forming unit/ml. Six groups of Nile Tilapia each contained 10 fish with a mean body weight of 50 ± 5g were used. The fish were maintained under investigation for 15 days before challenge for acclimatization. Each one of five groups was inoculated (I/P) by one of the five bacterial isolates using 0.5mg (bacterial cells by wet weight) as inoculum for each fish. The sixth control group received 1.0mg phosphate buffer saline (PBS) by I/P inoculation.

5- Immunological Tests:

The *Enterobacter aerogenes* strain was chosen as the most virulent strain and was used in bacterin preparation according to the method described by Badran (1987). The prepared bacterin was diluted with an equal volume of PBS solution (injectable bacterin). Sixty Armout catfishes of 80 ±5grams-body weight were immunized with prepared bacterin. Forty fish were divided equally into 4 groups. The fish were placed in glass aquaria supplied with dechlorinated tap water and fed on commercial fish diet. Fish of group (1) were injected by 0.2ml of PBS and kept as control. Fish of group (2) were injected with 0.2ml of the injectable bacterin fish of group (3) were injected with 0.2ml of original bacterin diluted with equal volume of 0.1% histamine solution. Fish of group (4) were injected with 0.2ml of bacterin and kept in an aquarium to which histamine has been added to the aquarium water at the level of 100mg/L. The level of histamine in injectable solution or that added to aquarium water was determined according to the result of histamine level in fish muscles.

The other 20 fish were divided into 4 groups each containing 5 fish. The fish were placed in glass aquaria under the same conditions of the previous 4 groups to compensate the accidental deaths during the experiment. After immunization weekly samples (2 *Claris lazera*) were taken from each group for blood collection according to Lied and Breakkan (1975). The natural and induced immune responses were determined by the micro agglutination test.

RESULTS

Table 1: Bacterial* density in examined water samples in different seasons.

Season	Water sample	Isamila Canal MPN	Lake Temsah MPN	Fish Farm MPN
Spring	3	$1.2x10^2$	$4.2x10^3$	$3.1x10^4$
Summer	3	$1.5x10^2$	$4.4x10^3$	$3.3x10^4$
Autumn	3	$0.9x10^2$	$3.6x10^3$	$2.4x10^4$
Winter	3	$0.7x10^2$	$3.2x10^3$	$2.7x10^4$

* Coliform was isolated from all examined samples.

The average Coliform counts of water samples collected from different sources are shown in table (1). The results revealed that water sample collected from fish farm had the highest level especially in summer season.

The results of bacterial examination of water samples and fresh caught fish samples revealed isolation of bacterial strains which according to their biochemical reactions were identified *as E. coli, Klebsiela pneumoniae, Citrobacter amaloticus, Enterobacter aerogenes and Proteus mirabilis* (Krieg and Holt, 1983).

Fish subjected to the pathogenicity test showedthe same clinical signs and P.M lesions of the natural infection. The injected organisms were reisolated from the experimentall diseased fish.

The result of histamine determination revealed that all fresh fish samples were free from histamine. Table (2) illustrated the average amount of histamine in fresh samples at different seasons. From this table, Nile Tilapia from the fish farm showed the highest content of histamine particularly through summer season (6.0mg/100g of fish body weight) while the lowest content was recorded in *C. lazera* caught from Ismalia Canal (0.055mg/100g fish body weight).

Table 2: The amount of histamine in fish samples at different seasons.

Season	Source of fish sample	Histamine mg/100g body weight*		
		O. niloticus	C. lazera	M. cephalus
Spring	Ismailia Canal	-	0.70	-
	Lake Temsah	-	-	3.90
	Fish farm	5.9	-	-
Summer	Ismailia Canal	-	0.70	-
	Lake Temsah	-	-	4.0
	Fish farm	6.0	-	-
Autumn	Ismailia Canal	-	0.068	-
	Lake Temsah	-	-	3.98
	Fish farm	5.8	-	-
Winter	Ismailia Canal	-	0.055	-
	Lake Temsah	-	-	3.25
	Fish farm	5.2	-	-

* The histamine content was determined after keeping the fish for 6-8 hours at room temperature.

Table 3 : Antibody response of Armout catfish against E. aerogenes bacterin under different treatments.

Group number	Treatment	Antibody titer (by log 2)			
		1st week	2nd week	3rd week	4th week
1	I/P with sterile phosphate buffer saline solution	2	2	2	2
2	I/P with injectable bacterin	4.5	8.5	11	14
3	I/P with original bacterin diluted with same volume of 0.1% histamine solution	3	4	4.5	4
4	I/P with bacterin with addition of histamine to aquarium water	3	6	9	7

The immune response of Armout catfish against *E. aerogenes* bacterin is given in Table (3). The results revealed that the fish inoculated with sterile PBS solution had antibody titer of 2(by log 2) at one week post inoculation and remained as such during the experimental period. The antibody response in fish immunized by I/P injection of *E. aerogenes* bacterin was detected at a high level at one week post immunization (4.5) and reached 14 at 4th week post immunization the antibiody liters in sera of fish immunized by 9/P inoculation of 0.2ml of original bacterin diluted with histamine solution were 3 and 4 at one and four weeks post immunization. While the antibody titers of fish injected with bacterin with addition of histamine to aquarium water were 3 and 7 at one and four weeks post immunization respectively.

DISCUSSION

The result of the present study revealed that the presence of Coliform especially *E. coli* in the water samples indicates faecal contamination of water collected from Ismailia Canal, Lake Temsah and a special fish farm. Besides, water collected from fish farm contained the highest level of coliform count than the other two sources. This may be due to the fact that 25% of fish farms water is domestic wastewater originating from highly polluted water of Bahr El Baker. In addition, pumping the domestic wastewater of Ismailia City to Lake Temsah represents the main source of pollution. Such waste water is the potential source of the member of Enterobacteriaceae (Badran, 1994) and consequently would carry a variety of fish pathogen such as Aeromonas hydrophila (Shread et al., 1981), Edwardsiella trada (Badran, 1993) and Streptococcous species (Badran, 1994). The present results revealed that the water environment with its bacterial population especially Enterobacteriaceae played an important role in the histamine content in fish. since the recorded levels of histamine in fish muscle corresponded with the total number of Entreobacteriaceae recorded in the 3 types of water samples. This was parallel with the observation of Rohani et al. (1985) who reported that histamine production in fish was due to histamine decarboxylase positive bacteria rather than the action of all kinds of bacteria causing spoilage. Niven et al. (1981) and Reilly and Santos (1985) also reported that the bacteria responsible for histamine production in Bollet Mackerel were almost belonging to the family Enterobacteriacease. Moreover, Sakabe (1973a) and Taylor et al. (1979) found that *Klebsiella pneumonia, Proteus morganin Esherichia sp.* and

Clostridium sp. were among the histidine decarboxylase positive bacteria. The result of the present investigation revealed that all samples of freshly caught fish were free from histamine which agrees with the findings of Frank et al. (1981) who reported that fresh skip jack tuna contained free histamine in most cases. However, keeping fish at room temperature (25-35°C) for 6-8h led to the formation of histamine in fish in different concentrations depending on the species and water environment from which fish had been caught. Tilapia caught from fish farm, lake Temsah and Ismailia canal showed histamine concentration of 5.9, 6, 5.8 and 5.2mg/100gram of fish respectively. These variations may be attributed to the degree of water pollution and accordingly to the number of amino acid producing bacteria in the fish muscle.

Immunolgical studies carried out through this investigation showed that unimmunized Armout catfish had a natural antibody specific to *Enterobacter aerogenes*. The level of natural antibody (2 by log2) may be attributed to the contact of *Clarias lazera* with *E. aerogenes* present in fresh water as a result of sewage pollution. This conclusion agree with those reported by Hodgins et al. (1973) Hazen et al. (1981) and Badran (1991) who reported that natural agglutinins were produced from continuous contact of fish with the environmental microorganisms. The results also revealed that the bacterin prepared from *E. aerogenes* was antigenic in nature and able to stimulate the immune system of *Clarias lazera* to produce high levels of specific antibodies. The immune response of *Calrias lazera* against *E. aerogenes* bacterin is similar to those reported by many authors in other fish species and against varied microorganisms. Collins et al. (1979) and Schachte (1978) reported that channel catfish showed strange antibody response to Enteric Redmouth bacteria, *Aeromonas hydrophila* and *Flexibacter columnaris*. Lamers and Parmentier (1985), Lamers and Van Muiswinkel (1986) and Ruangpan et al. (1986) found that formalin killed *Aeromonas hydrophila* evoked high antibody titer in carp than those of control group. The Egyptian fish species, Armout catfish and Nile Tilapia, produced high level of specific antibody against *Aermonas hydrophila Pseudomonas fluorescence* bacterins (Badran 1990, Badran et al., 1993 and Badran, 1994).

Regarding to the effect of histamine on the immune response of fish unfortunately there is no previous data dealing with this point. The available literature reported on the effect of cortizole on the specific and non-specific immune response of fish. Tatner (1990) recorded that the antibody response of rainbow trout immunized with *Aeromonas*

salmonicido bacterin was not affected by cortizole and the response was equal to that of the control. However, non specific immune response was reported by Maul and Schreck (1990) who found that the number of leukocytes in juvenile coho salmon increased significantly in the anterior kidney and decreased significantly in blood and spleen within 1 day after treatment with cortizole in feed and returned to the control level after 3 days of treatment. In the present study, the doses of histamine used either for I/P injection with the bacterin or added to aquarium water, were calculated from the results of histamine level in fish muscle. The result of histamine administration showed their immunosuppressive effect on the immune system of *Calrias lazera*. This was manifested by the low level of humoral antibody production particularly at one-week post immunization, which was slightly different from that of non-immunized *Clarias lazera*. The immunosuppressive effect of histamine injected with bacterin was more pronounced than that added to the aquarium water; this is attributed to the faster entrance of histamine into the circulation of fish by injection and consequently their quick action on the immune system in comparison with the gradual entrance of histamine added to the aquarium water.

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