Experimentally pathogenesis of *Aeromonas hydrophila* in freshwater Crayfish (*Astacus leptodactylus*) in iran

SamCookiyaei A.[1*]; Afsharnasab M.[2]; Razavilar V.[1]; Motalebi A. A.[2];Kakoolaki S.[2]; Asadpor Y.[2]; Yahyazade M. Y.[2];Nekuie Fard A.[2]

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Abstract

In this research the affect of *A. hydrophila* on pathogenesis of Crayfish *A. leptodactylus* was carried out. This study was designed in three groups as treatments and one group as control in triplicate with 20 Crayfish in each glass aquarium. The treatments have exposed to *A. hydrophila*. The concentrations of the bacteria in treatments were 3×10^{8}, 3×10^{6}, and 3×10^{4} $CFUmL^{-1}$ respectively. The untreated control group was disinfected by oxytetracyclin at concentration of 100 ppm for 24 hours. The haemolymph samples were withdrawn for measuring of THC and TPC within interval hours (2, 6, 12, 24, 48, 96, 144, 240 and 336). The Crayfish samples were then fixed in Davidson fixative. The results showed that the differences of THC value between treatment 4 (95.33±23.16) and control (1.13±.45) were significant ($P<0.05$) as well as control and treatments 3 (35.33±16.16) and 4 (95.33±23.16) during 2 initial hours. In case of THC the significant difference ($P<0.05$) was observed between control group (72.33±15.04) and treatment 3 (13.13±5.85) after 48 hours. During 240 hours, also the difference of THC value between control group (80.33±24.94) with treatment 2 (14.67±3.51) was significant ($P<0.05$). The finding of TPP value showed that there was no significant difference between control group and treatments in during mentioned times ($P<0.05$). The result of histopathology in heaptopancreas and gill sections showed that hemocyte aggregation and necrosis within pyknosis of nucleus. In lower concentrations of bacteria no pathological changes of heart were observed. In digestive tract no changes were appeared in treatments, but in concentration of 3×10^{8} CFU ml^{-1} a low aggregation of hemocytes was revealed.

Keywords: *Astacus leptodactylus*, *Aeromonas hydrophilia*, Total Hemocyte Count, Total Protein Concentration, Histopathology

1-Faculty of specialized veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

2-Iranian Fisheries Research organization, P.O. Box:14155-6116.Tehran ,Iran.

Corresponding author's email:Drsam.arash@gmail.com

Introduction

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The Crayfish are the largest freshwater crustacean with high ability against wide range of environment variables (Mc Mahon, 1976). The freshwater Crayfish *Astacus leptodactylus* is the one of the important species of Crayfish family that rearing it considerable in numerous countries (Holdich et al., 1997).Iran has an important role in export of *A. leptodactylus* to European countries. At the moment, Aras dam reservoir is a natural habitat of *A. leptodactylus* in Iran (Matinfar, 2007). Many pathogens can impact this species in natural habitat. Among these pathogen bacteria *Aeromonas, hydrophila*, *Citobacter spp*. *Flavobacterium spp*. play important roles in susceptibility of disease in Crayfish (Edgerton et al., 2002). Post (1989) and Zarrilla et al. (2003) presented a significant effect of bacterial pathogens on losing the yields in aquaculture. The research indicated that the numerous genera of bacterial species including both gram–negative (*Acinetobacter, Aeromonas, Citrobacter, Flavobacterium, pseudomonas and Vibrio*) and gram–positive (*Corynebacterium, Bacillus, Micrococcus and staphylococcus*) species isolated from freshwater Crayfish (Edgerton et al., 2002). These bacteria are responsible for heavy economic losses (high mortality and deterioration of quality) in throughout of the world (Groff and Lapatra , 2000; Karuna- Sagar et al., 2003). Studies demonstrated that disease is only occurred when the host effected by the stressor factors, such as high density, poor nutrition and so on (Quaglio et al., 2002).

Aeromonas spp, are responsible for wide range of diseases in poikilothremic and homeothermic animals (Khardori and Fainstein , 1988; Mathewson and Dupont , 1992; Austin and Autin , 1993). These bacteria are Gram- negative, oxidase positive bacill form, which are usually singly present, in pairs or in short chains (Blair et al., 1999) The main virulence factors of *Aeromonas spp* that pertaining to the pathogenecity of the disease are the expression of extra cellular proteins including exotoxins and exoenzymes , endotoxin, Lipopolysaccharide (LPS) layer , the presence of S-layers and fimbriae and the production of Capsular Layers (Blair et al., 1999). Motile *Aeromonadas* are adapted to the environments with a wide range of conductivity, turbidity, pH, salinity, and temperature (Hazen et al., 1987). Environmental and physiological parameter changes are most commonly associated with outbreak of disease under the intensive condition in fish pond. (Cipriano, 2001). Among *Aeromonas spp. A. hydrophila* is an important bacterial pathogen as it infects both fish and humans (Janda et al., 1996).This bacterium in fishes has been reported to occur from time to time in Asian countries including China, Phillipines, Thailand and India (Ebanks et al., 2004), and in fish culture results in decrease in production and economic losses. This species has been incriminated as the etiological agent in diseases of fish where it cause haemorhagic septicemia, and often aquatic and terrestrial animals (Asha et al., 2004).Several studies have described a wide variation in the pathogenicity of *A. hydrophila* in different fish species. This is mainly due to the heterogenicity of strains and differences in the adhesive and

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enterotoxic mechanisms responsible for causing infection in fish (Fang et al., 2004). Under poor conditions, such as high density, lack of foods or poor water quality, *Aeromoniasis* appears in Crayfish (Vey, 1977). Some studies indicated that Total Hemocyte Count (THC) and Total Protein Plasma (TPP) can be fluctuated due to crustacean diseases (Rodrigues and Lemoullac, 2000). Studies showed that hemocytes play a major role in crustacean immune system. In crustaceans, circulating hemocytes play an important role in defense system (jiravanichpaisal et al., 2006). First, they eliminate foreign particles in the hemocoel though phagocytosis, encapsulation and nodular aggregation. Secondly, hemocytes participate in wound healing by cellular clumping and begin coagulation processes though the release of factors required for plasma gelation (kakoolaki et al., 2010, 2011).Also Crayfish hemocytes play important roles in the initiation of several immune responses and production of antimicrobial peptides (Jiravanichpaisal et al., 2007). The reports indicated that hemocyte and hepatopancreas are crucial for the immune system in crustacean as it is the main production site for immune recognition molecules, initiales the humoral reaction, and takes part in the cellular reaction by some specialized cells and phagocytes (Gross et al., 2001). Until now, the pathogenicity of *A. hydrophila* has been reported in fish, human and Crayfish *pacifastacus leniusculus*. Therfore In this research that was implemented for the first time in the region, different Concentration of bacteria *A. hydrophila* on pathogenicity and differences of THC and TPP in Crayfish *A. leptodactylus* were demonstrated.

Material and methods

Adaptation and Collection of samples

Two – hundred and forty freshwater Crayfish, *A. leptodactylus* with average weight of 25-40g were purchased from Aras dam reservoir in West Azerbayjan province and transported to Iranian Artemia Research Center of Urmia city in September 2010. Before examination, the Crayfish were acclimated to the condition of the laboratory for ten days. During this time, Crayfish were fed once daily with commercial pellet (trout, Biomar and Blood warm). Water of Crayfish aquarium was changed daily. During the study, water temperature, pH and dissolved oxygen were daily monitored as 15 ± 1^0C, 7-8 and 5- 5.5ppm, Respectively.

Isolation of A. hydrophila

Many of infected Crayfish from Aras dam reservoir were used for isolation of bacteria. Haemolymph sample were collected from infected Crayfish with cutting their antennules and transferred to TSA medium (tryptic soy agar). Haemolymph specimens were then cultured on TSA for 24 h at 25^0C. After 24 h of incubation the many of colonies of bacteria grew in TSA. Eventually, *A. hydrophila* were identified based on biochemical test (Mohajeri et al., 2011). Also specific medium, Rimler Shotts Agar, were used to confirm the isolated bacteria. Cell densities were photometrically obtained at 590 nm wavelength (Saulnier et al., 2000), based on the Mcfarland

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standard and serially diluted to reach the target densities as follows.

Treatments and control group

The study was designed as three treatments, 3×10^4, 3×10^6 and 3×10^8 CFU ml^{-1} of the bacteria (named 2, 3 and 4) and one control (named 1) in triplicates. Twelve glass aquariums were divided to the groups. Twenty Crayfish were allocated to each aquarium. The control group was untreated but disinfected by oxytetracyclin antibiotic at concentration of 100 ppm for 24 h as well as the treatments. The treatments were exposed to *A. hydrophila* with no any water changes for 2 h. Crayfish samples were then washed with chlorinated tap water and transferred to the glass Aquariums.

Haemolymph Sampling

Haemolymph were then obtained within interval hours 2, 6, 12, 24, 48, 96, 144, 240 and 336 from the second-ventral segment of Crayfish using 25 gauge needle of 1 ml syringe. Haemolymph samples were pre-mixed 1 to 1 with an anticoagulant solution (Smith and Soderhall, 1983; Mohajeri et al., 2011) and transferred in individual gamma plastic tubes for measuring THC and TPP.

Total Hemocyte Count

A drop of the hemolymph mixture was transferred on a hemocytometer in order to measure THC using light microscope as mentioned by Jiang et al. (2004).

Total Plasma Protein

Haemolymph samples were centrifuged at 2900 × g at 4^0C for 10 minutes and the samples were measured according to a Hitachi 902 autoanalyzer by the Biuret-method at 520-560 nm wavelengths (Silverman and Christenson, 1996).

Histopathology

Tissues samples (heart, gill, haepatopancreas and digestive tract) were also collected at 2, 6, 12, 24, 48, 96, 144, 240 and 336 h after exposure. The Crayfish tissues were then fixed in Davidsons fixative for 48 h and transferred to 70% ethyl alcohol. After this time the tissue samples were processed for histhopathological study by Bell and Lightner (1988) method and sections were prepared for H&E staining and viewed under light microscopy.

Statistical analysis

A one – way ANOVA test was used to compare the differences of THC and TPP value among treatments and control group at confidence interval of 95% ($p< 0.05$). Multiple comparisons along with the Tukey HSD was conducted to show that at least a group has a significant difference with other groups while the P value indicated the value lower than 0.05. All Statistical tests were evaluated using the version. 18 SPSS computer software

Results

Total Hemocyte Count

The values of THC in different groups was listed in table 1 and figure 1 that showed significant increasing in the time of 2 h in treatment 3×10^8 CFU ml^{-1} in comparison to control group and treatments 3×10^6 and 3×10^4 CFU ml^{-1} ($p<0.05$). The value

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of THC for the treatment 3×10^6 CFU ml^{-1} at 48 h was significantly showed a difference in comparison to control group ($p<0.05$). For the Crayfish which had been transferred to 3×10^4 CFU ml^{-1} after 240 h, the value of THC was significantly decreased in comparison to control group ($p<0.05$). In remaining time, no significant difference were observed in THC value between treatments with each other and treatments with control ($p>0.05$).

Table1: THC values ($\times10^4$) in different groups and definite hours

	Times (h)								
	2	6	12	24	48	96	144	240	336
Control	34.00±17.34	90.00±45.78	119.00±72.38	43.33±23.02	72.33±15.04	39.67±19.50	28.00±7.21	80.33±24.94	42.00±33.18
3×10^4 ml^{-1}	28.33±11.37	42.33±25.77	48.67±2.30	31.00±15.39	20.67±5.50	37.00±9.84	30.67±25.69	*14.67±3.51	10.33±3.78
3×10^4 ml^6	35.33±16.16	54.67±15.04	37.67±35.36	25.33±4.50	*13.33±5.85	22.33±14.01	24.00±14.93	47.00±22.60	29.00±12.12
3×10^4 ml^8	*95.33±23.18	31.00±15.71	29.00±17.08	37.33±15.37	53.67±30.07	32.33±3.21	44.33±45.21	63.00±34.65	24.67±11.01

The values are given as mean ± SD
Asterisk sign have significantly indicated differences of THC in similar times

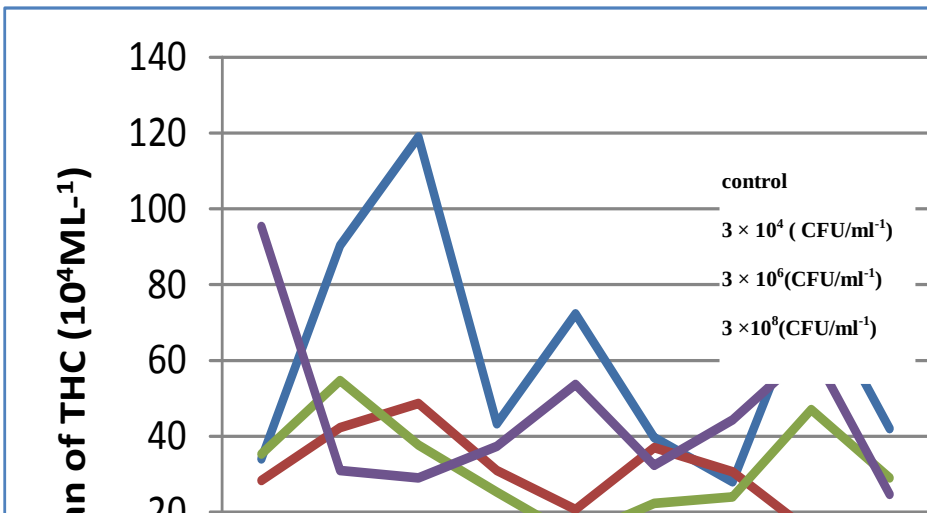


Figure 1: The effect of different concentration of bacteria on THC

Total Protein Plasma

The TPP values in different groups were listed in table 2 and figure 2 which showed no significant differences in those of TPP in different time during the study ($p > 0.05$). The lowest value of TPP reached 0.9 gr dl^{-1} in control group at 24 h while the highest value of TPP showed 1.96 gr dl^{-1} in control group at 6 h. According to sampling from 108 Crayfish, the lowest value of TPP was observed 0.5 gr dl^{-1} in

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control group and treatment 3 $\times 10^8$ CFU ml^{-1} at 336 h whilst at the same time, the highest value of TPP in control group reached 2.6 gr dl^{-1} .

Table 2: TPP (gr dl^{-1}) in different groups and definite hours

	Times (h)								
Groups	2	6	12	24	48	96	144	240	336
Control	1.13±045	1.961±0.6	1.90±0.00	1.46±0.70	1.33±0.58	1.23±0.55	1.63±0.75	0.90±0.10	1.00±0.50
3×10^4 ml^{-1}	1.50±3.00	1.36±0.80	1.63±0.15	1.86±0.40	1.70±0.20	1.56±0.56	1.30±0.70	1.06±0.28	1.56±0.25
3×10^6 ml^{-1}	1.50±0.05	1.43±0.47	1.50±0.60	1.53±0.58	1.30±0.26	1.40±0.20	1.23±0.55	1.46±0.64	1.04±0.52
3×10^8 ml^{-1}	1.36±0.28	1.70±0.26	1.23±0.68	1.83±0.45	1.50±0.34	1.86±0.05	1.80±0.52	1.00±0.10	0.96±0.41

The values are given as mean ± SD

Asterisk sign have significantly indicated differences of THC in similar times

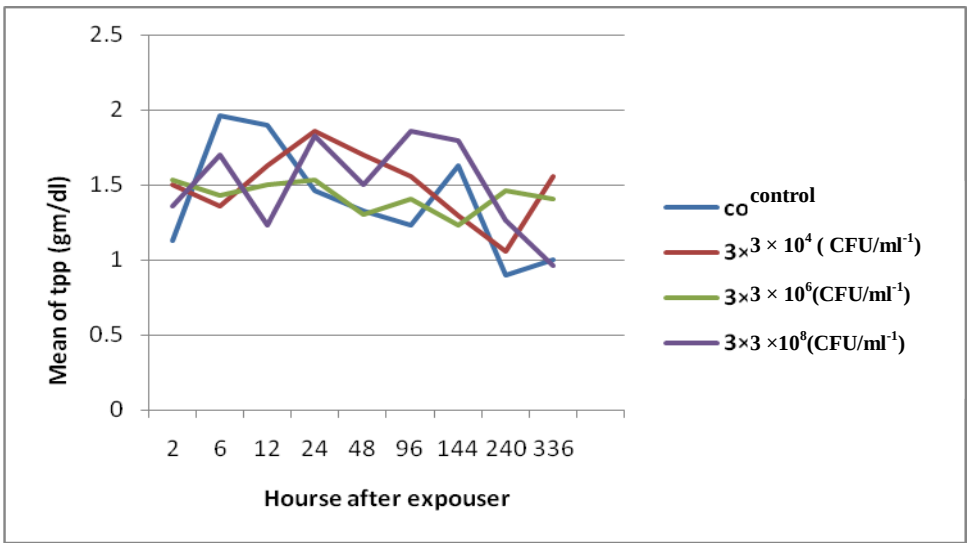


Figure 2: TPP fluctuations in different concentration of bacteria hours after exposure

Histopathology

In gill histopathology findings, hemocytes with degradation of necrotic and pyknotic nuclei were observed. This degradation with cell necrosis and aggregation, consequently, they specifically were observed in the treatment 3×10^8 CFU ml^{-1}(Fig.2-A). lower grades of pathologic evidences were seen in treatments 3×10^4 and 3×10^6 CFU ml^{-1} in comparison to that of 3×10^8 CFU ml^{-1} (Fig. 3-B, C) whilst not the same appearances were observed in gill tissue in control group (Fig.3-D).

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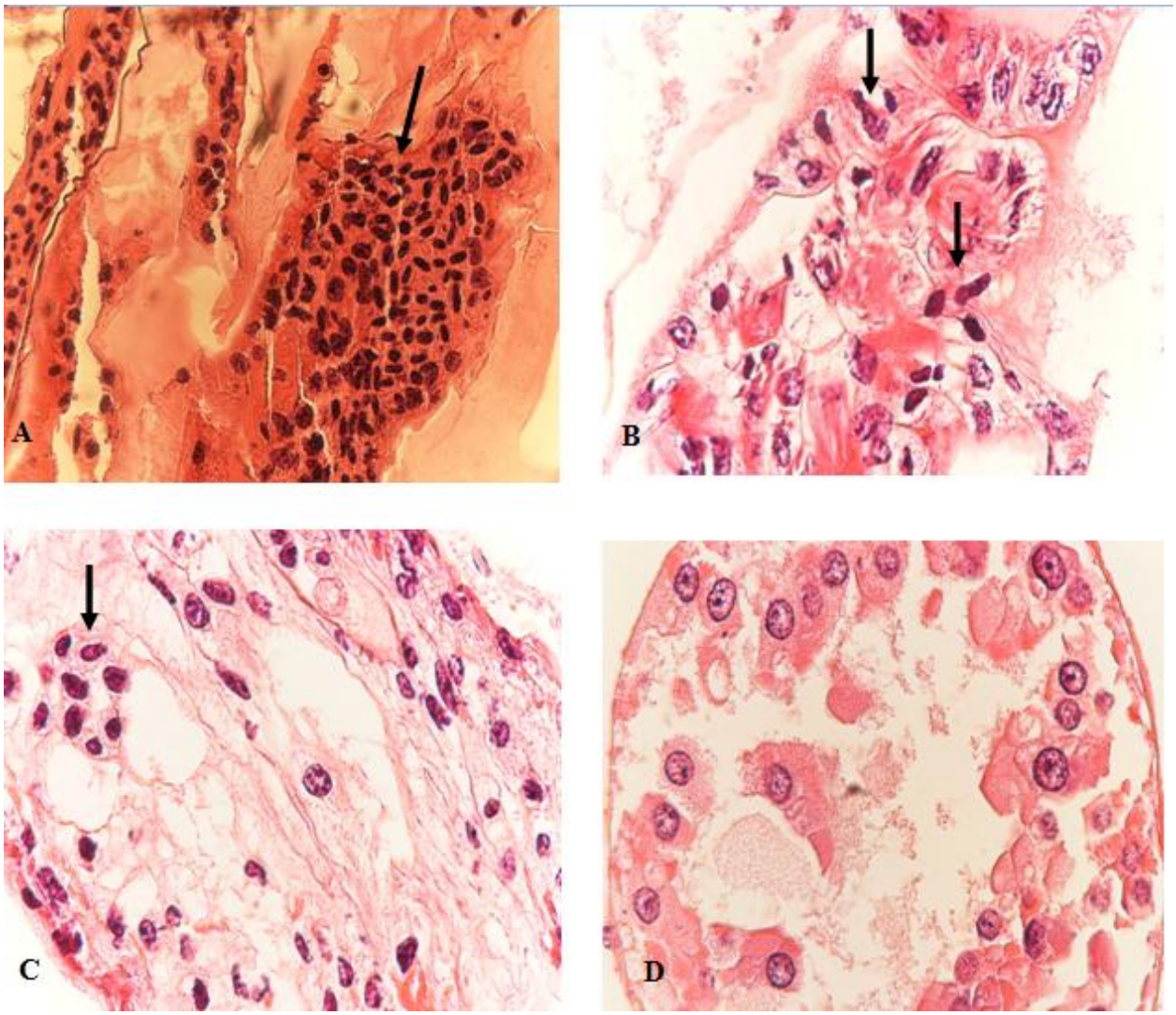


Figure 3: Pathological changes in lamella tissues owed to different level of *A. hydrophila*
A; Infected gill lamella (3×10^8 CFU ml^{-1}) 48 h after exposing to *A. hydrophila*; Severe cell necrosis and hemocyte aggregation (H&E ×440).
B and C: Infected gill lamella in the treatment 3×10^6(B) and 3×10^4 CFU ml^{-1} (C) 48 hours after exposure time; Mild necrosis and hemocyte aggregation (H&E× 880),
D: Infected gill lamella (without of bacteria *A. hydrophila* as control), 48 hours after exposure time; Normal cell appearances were observed with no any changes (H&E ×880)

In histopathology results, the massive aggregattion of hemocytes and pyknotic nuclei in haepatopancreas of the samples in treatment 3×10^8 CFU ml^{-1} were observed (Fig. 4-E) while the appearance of hemocyte aggregations in the treatments 3×10^6 and 3×10^4 CFU ml^{-1} were mild, in comparison to the former concentration.(Figs. 4-F, G). No any similar appearances were observed in control group (Fig. 4-H).

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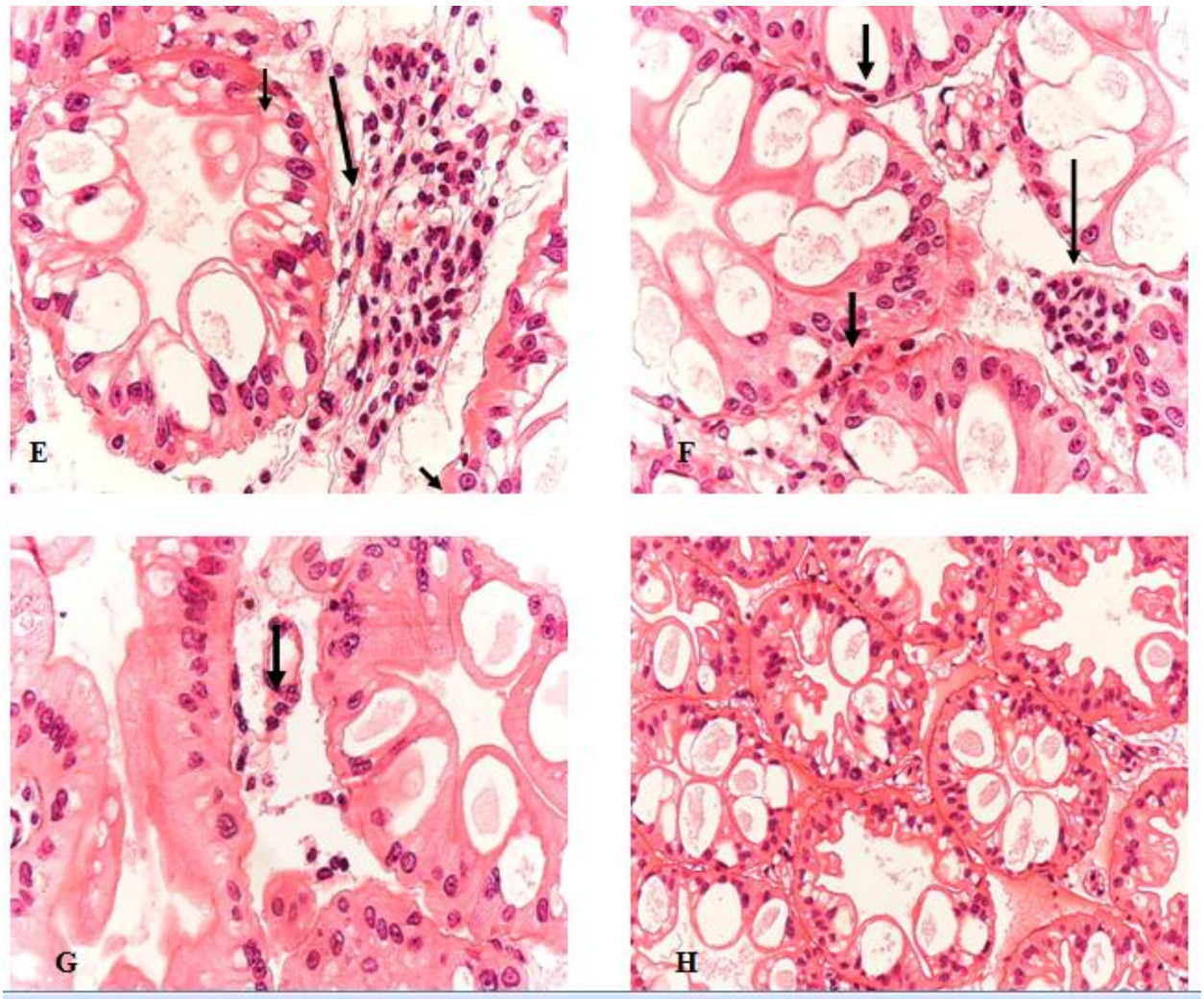


Figure 4: **Pathological changes in haepatopancreas tissues owed to different level of *A. hydrophila***

E; Infected haepatopancreas of Crayfish in treatment 3×10^{8} CFU ml^{-1} *A. hydrophila*; massive hemocytes aggregation are infiltrated (long arrow) and many of cells shows pyknosis (short arrows) (H&E ×880).

F and **G**; Infected haepatopancreas of Crayfish in 3×10^{6} CFU ml^{-1}(F) and 3×10^{4} CFU ml^{-1}(G);

hemocyte aggregation (long arrow) and pyknotic nuclei (small arrow) were observed. No differences were observed between 3×10^{6} and 3×10^{4} CFU ml^{-1} for histopathology changes (H&E ×880).

H; No changes were observed in cells of haepatopancreas in control group (H&E ×440).

Histopathological finding in heart and digestive system in Crayfish were infected with 3×10^{8} CFU ml^{-1} of *A.hydrophila* shows hemocyte aggregation and infiltration in myocardium and digestive system (Fig. 5-I, Fig. 6-K). Whereas in Crayfish infected with 3×10^{4} and 3×10^{6} CFU ml^{-1} of the bacteria, heart and

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digestive system show no any changes. No differences observed in heart and digestive system in control group (Fig. 5-J, Fig. 6-L).

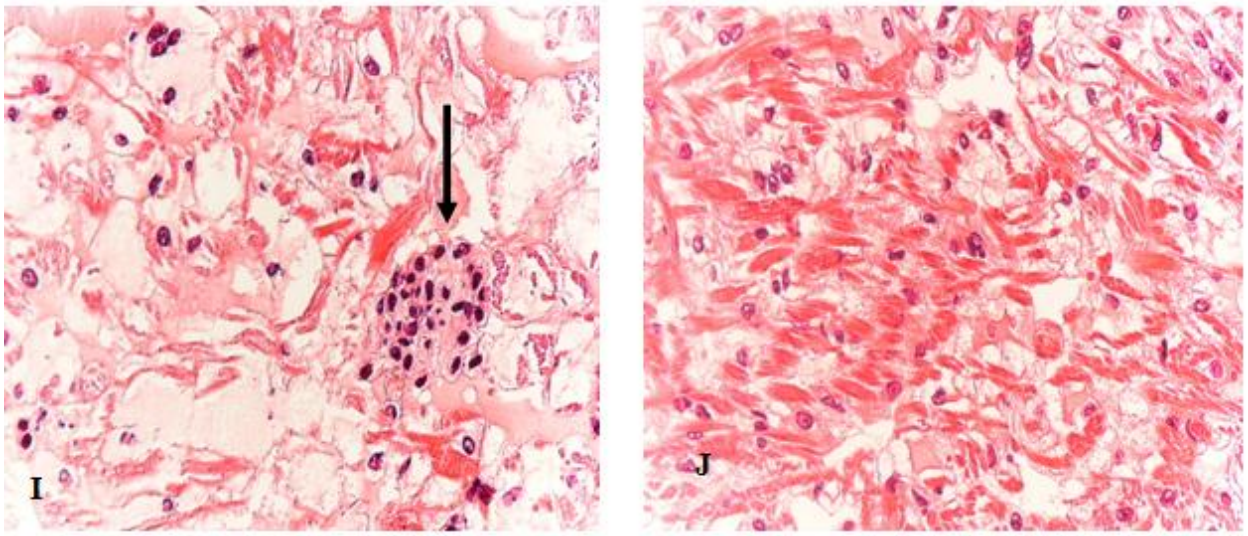


Figure 5: Myocardium changes owed to exposing to the bacteria

I; Infiltrated and aggregated hemocytes in the myocardium in Crayfish exposed to 3×10^{8} CFU ml^{-1} *A.hydrophil* (H&E ×880).

J; Normal heart tissue in control group (without of bacteria *A.hydrophila*) (H&E ×880).

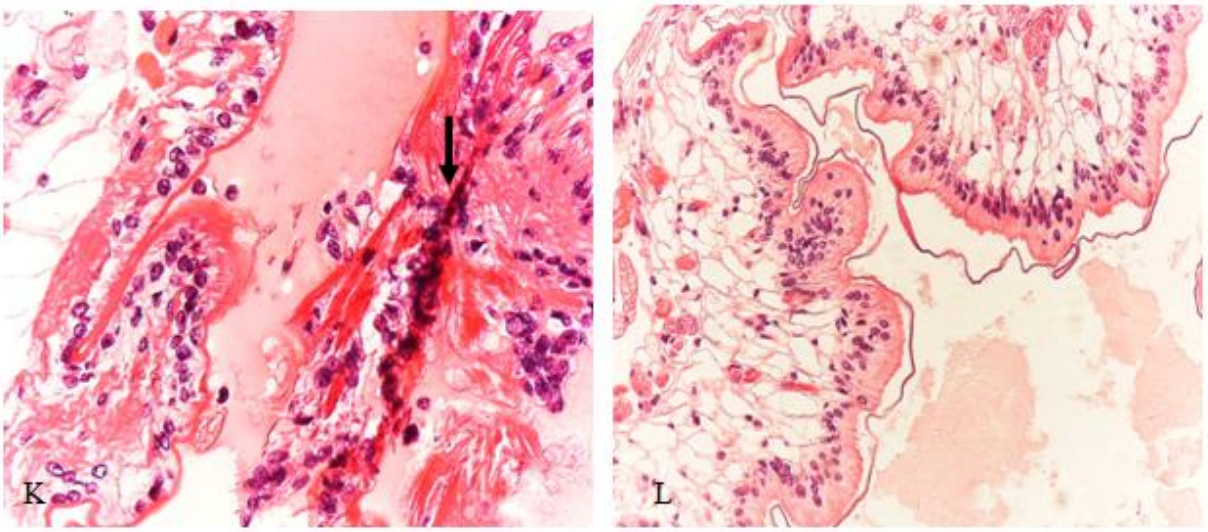


Figure 6: digestive tract changes owed to exposing to the bacteria

K; Hemocyte infiltration in digestive system in treatment 3×10^{8} CFU ml^{-1} *A.hydrophila* (H&E ×880). **L**; Normal digestive tract tissue in control group (without of bacteria *A.hydrophila*) (H&E ×440).

Discussion

In this study, the effect of different level of bacteria on fluctuations of TPP and THC was examined to determine the amount of resistance, when Cray fish encounters *A. hydrophyla*. In our research, the value of THC in Crayfish increased notability at (2h) after exposing to 3×10^{8} CFU ml^{-1} *A. hydrophila* in comparison to the control

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group, treatments 3×10^6 and 3×10^4 CFU ml^{-1}. It could be due to stress, which owes to high concentration of bacteria *A. hydrophila*. Our findings are similar to another study mentioned by Liu (2008) and jiravanichpiasal et al. (2006). Their results showed that the rate of PL crustin1 as one of the AMPS increase in occurrence of gram negative bacteria *A. hydrophila* observing in hematopoetic and hemolymph tisue (jiravanichpaisal et al., 2007).

In remaining hours, no significant differences in case of THC were observed in concentration of 3×10^8 CFU ml^{-1} in comparison to control group and treatments 3×10^6 and 3×10^4 CFU ml^{-1}. This reactions result in decreasing of stressor factors and adapted of Crayfish to environment condition.

In order to observe Crayfish treatments, It can cause adaptation of Crayfish to environment condition and probably for decreasing to concentrate of bacteria in result of environment temperature. But there was no significant differences in THC at 2, 6 and 12 h after challenged time to 3×10^6 and 3×10^4 CFU ml^{-1} . It probably indicated that because of low concentration of bacteria and not changed serological and environmental parameters in these times, the bacteria weren't able enough in disease.

The value of THC in 48 and 24 h after challenging time significantly decreased in treatments 3×10^6 and 3×10^4 CFU ml^{-1}. That can cause decrease of immune system in xrayfish by *A. Hydrophila.* Studies showed that the decreased number of hemocytes in Crayfish due to destruction by *A. hydrophilo* toxins will lead to subversion of immunological function in host and finally disease occurred and the host died (Jiravanichpaisal et al ., 2009).

Unlike THC, There were no significant differences in TPP in differences times after exposure time to bacteria between treatments with control group and treatments together. It probably indicated that in these times, the value of TPP could not be changed in exposure to *A. hydrophila* either it can cause of low concentration of bacteria or short period of these study.

In histopathological finding of this research due to *A.hydroohila* in Crayfish, in high concentration of bacteria, it had seen massive hemocyte aggregated in haepathopancreas and the lower rate in gill, heart and digestive system. That may be shown that high concentration of bacteria to *A.hydrophila* caused stresore condition and hemocyte aggregation in these organs. High hemocyte aggregated in haepatopancreas indicated that haepatopancreas probably is target organ for hemocyte aggregated and pathogenesis of bacteria. Also in high concentration of bacteria in gill sign of necrosis and the many of pyknotic nuclei were seen that can be due to pathogenesis of bacteria. Also, hemocyte aggregated in haepathopancreas,gill and necrosis of gill in other concentration of bacteria had been seen,but it was lowest in comparing 3×10^8 CFU ml^{-1}that low concentration of bacteria can be the reason for it. In this research the temperature of aquarium water in all treatments and control group was 15 ± 1^{0C} and the rate of mortality induced for *A.hydrophila* was not significant. Because of the *A. hydrophila* is an opportunistic bacterium, Then it may be shown that the

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rate of temperature aren't enough for high mortality or severity pathogenesis but with a high percentage, it can be inferred that it can induce for the high resistance of Crayfish *A. leptodactylus* to *A. hydrophila* in these temperature and these concentration.

Histopathological study performed on other Crayfish collected from the same farm showed high prevalence of bacteremic lesions (Edgerton et al ., 1995). Histopathological studies on Australian freshwater Crayfish with bacterial septicemia indicated perivascular cuffing of haepatopancreatic hemolymph vessels and granulocytic hemocyte aggregation in the heart, gills, haepatopancreas, antennal gland, abdominal muscle and connective tissue (Evans et al . , 1992 ; Edgerton et al., 1995).

References

Asha, A., Nayak, D.K., Shanker, K.M. and Mohan, C.V., 2004. Antigen expression in biofilm cells of aeromonas hydrophila employed in oral vaccination of fish. Fish and shelfish Immunology, 6, 429-436.

Austin, B., Autin, D. A., 1993. Bacterial Fish pathogens , Second ed., Disease in Farmed Fish and Wild Fish Ellis Horwood , Chichester, united Kingdom .

Bell, T. A., Lightner, D. V., 1988. A hand book of normal penaeid shrimp histology, world aquaculture society, Baton Rouge, LA.

Blair, I. S., Mcmahon, M. A. S. and Mcdowell, D. A., 1999. Food studies Research unit , university of ulsterat Jordanstom, co. Antrim, Northern Ireland . Aeromonas / introduction 25.

Cipriano, C. Rocco., 2001. Aeromonas hydrophila and motile Aeromonas septicemias of fish. Fish disease leaflet, 68.

Ebanks, R. O., Dacancy, A., Goguen, M., Pinto, D. M. and Ross, N. W., 2004. Differential proteomic analysis of aeromonas hydrophila outer membrane proteins in response to low iron and inviro growth conditions. *Proteomics*, 4, 1047-1083.

Edgerton , B. F. , Evans , L. H., Stephens , F. J., Overstreet , R. M., 2002 . Synopsis of freshwater Crayfish and Commensal Organisms . *Aquaculture*, 206, 57-135.

Edgerton, B., Owens, L., Harris, L., Thomas, A., wing field, M., 1995. Ahealth survey of farmed red claw Crayfish, cherax quadriccarinatus (Vonmartenz) , in tropical Australia. *Freshwater Crayfish*, 10,322-338.

Eissa, I. A. M., Badrani, A. F., Moustafa, M. and Fetaih, H., 1994. Contribution to motile Aeromonas Septicemia in some cultured and wild fresh water Crayfish.*Veterinary Medical Journal Giza*, 42, 63-69.

Evans , L. H., Fan , A. and Finn, S., 1992 . Health Survey of Western Australian Freshwater Crayfish. Curtin university of Technology . Perth. 136 p.

Fang, H.-M., Ge, R. and Sin, Y. M., 2004. Cloning, characterisation and expression of aeromonas hydrophila major adhesin. *Fish & Shellfish Immunology*, 16 (**5**), 645-658.

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[DOR: 20.1001.1.15622916.2012.11.3.15.5]

Groff, J. M. and S. R. Lapatra., 2000. Infactious disease impacting the commerical Salmonids. *Journal of Applied Aquaculture*, 10(**4**), 17-90.

Gross, P. S., Bartle, T. C., Browdy, C. L., Chapman, R. W. and Warr, G. W., 2001. Immunegene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the pacific white shrimp, Litopenaeus vannamei, and the Atlantic white shrimp, L. setiferus. *Developmental & Comparative Immunology*, 25,565-577.

Hazen, T. C., Filiermans, C. B., Hirsch, R. P. and Esch, G. W., 1987. Prevalence and distribution of Aeromonas hydrophila in the USA. *Journal of. Applied and Environmental Microbiology,* 36, 731-738.

Holdich, D. M., Harlioglu, M. M. and Firkins, I., 1997. Salinity adaptations of Crayfish in British waters with particular refrence to Austropotamobius pallipes, Astacus leptodactylus and Pasifastacus leniusculus, *Estuarine, Coastal and Shelf science*, 44,147-154.

Janda, J. M., Abboh, S. L., Khashe, S., Kellogg, G. H. and Shimada, T., 1996. Biochemical characteristics and serological properties of the genus Aeromonas. *Journal of clinical microbiology*, 34,1930-1983.

Jiang, G., Yu, R. and zhou, M., 2004. Modulatory effects of ammonia–NOn the immune system of penaeus Japonicus to Virulence of white spot syndrome Virus. *Aquaculture*, 241, 61-75.

Jiravanichpaisal ,p. , Roos , S., Edsman , L. ,Liu H. , Soderhall , K., 2009. Ahighly Virulent Pathogen , Aeromonas hydrophila , from the freshwater Crayfish pacifastacus Leniusculus : *Journal of Invertebrate Pathology*, 101, 56-66.

Jiravanichpaisal, P., Lee, S. Y., Kim, Y. A., Andren, T., Soderhall, I., 2007. Antibacterial peptides in hemocytes and hematopoietic tissue from freshwater Crayfish *Pacifastacus leniusculus*: Characterization and Expression pattern. *Developmental & Comparative Immunology*, 31,441-455.

Jiravanichpaisal, P., Luel lee, B. and soderhall, k., 2006. Review cell – mediated immunity in arthropods: Hematopoiesis, coagulation, melanization and opsonization. *Immunobiology*, 211, 213-236.

Kakoolaki, S., Sharifpour, I., Soltani, M., Mousavi, H. A. E., Mirzargar, S. and Rostami, M., 2010. Selected morpho-chemical features of hemocytes in farmed shrimp. *Fenneropenaeus indicus* in iran. *Iranian journal of fisheries sciences*, 9(**2**), 219-232.

Kakoolaki, S., Soltani, M., Ebrahimzadeh Mousavi, H. A., Sharifpour, I., Mirzargar, S., Afsharnasab, M. and Motalebi, A. A., 2011. The effect of different salinities on mortality and histopathological changes of spf imported litopenaeus vannamei, experimentally exposed to white spot virus and a new differential hemocyte staining method. *Iranian*

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Journal of Fisheries Sciences, 10 (**3**), 447-460.

Karunasagar, I., I. karunasagar and. Otta, S. k., 2003. Disease problems effectiny fish in tropical environment J.Applied Aquaculture, 13(3/4), 231-249.

Khardori, N., Fainstein , V., 1988. Aeromonas and plesiomonas as etiological agents. *Annual Review of Microbiology*, 42, 395-419.

Liu, H., 2008. Functional studies of some immune relevant genes in a crustacean. Acta universitatis upsaliensis. Digital comprehensive summaries of Uppsala Dissertations from the faculty of science and technology, 435. 61p.

Mathewson, J. J. and Dupont , H. I., 1992. Aeromonas Species : roleas human pathogens . in Remington .

Mc Mahon , B. R. 1976. The adaptable Crayfish. *Freshwater Crayfish*, 6, 59-74.

Matinfar, A., 2007. Prancipal program of Freshwater Crayfish *Astacus leptodactylus*. Jahad agriculture organization. Iranian Fisheries research Organization, 10.118p.

Mohajeri, J., Afsharnasab, M., Jalali, B., Kakoolaki, S., Sharifrohani, M. and Haghighi, A., 2011. Immunological and histopathological changes in penaeus semisulcatus challenged with vibrio harveyi. *Iranian Journal of Fisheries Sciences*, 10 (**2**)**,** 254-265.

Post, G. W., 1989. Textbook of fish health J.F.H population, Inc Ltd. 211west syyania Avenue Neptune City NJ00753.

Quaglio**, R., Manfrin, A., Nobile, L., Delgado, M. L., Maxia, M., Morolli, C. G. and Fioravanti, M. L., 2002.** Bacteriological investigation on allochthonous Crayfish inNorthern Italy.*Freshwater Crayfish*, 13, 280-286.

Rodríguez, J. and Le Moullac, G., 2000. State of the art of immunological tools and health control of penaeid shrimp. *Aquaculture*, 191 **(1–3)**, 109-119.

Saulnier, D., Haffner, P., Goarant, C., Levy, P. and Ansquer, D., 2000. Experimental infection models for shrimp vibriosis studies: are view. *Aquaculture*, 191, 133-144.

Silverman, L. M. and Christenson, R. H., 1996. Aminoacids and Proteins. In Burtis CA, Ashwood ER(eds): Tietz, Fundamentals of clinical chemistry, 4th ed, Saunders W.B. Philadelphia, 240-282.

Soderhall , K. and Smith , V. J. , 1983. Separation of the haemocyte populations of Carcinus meanas and other marine decapods , and prophenoloxidase distribution . *Developmental & Comparative Immunology*, 7, 229-239.

Vey, A., 1977. Studies on the pathology of Crayfish under rearing condition. *Fresh water Crayfish*, 6, 212-222.

Zorrilla, M., Chabrillon, A. S., Rosales, P. D, Manzanares, E. M, Balebona, M. C. and Morinigo, M. A., 2003. Bacterial recovered from diseased cultured gilt head sea bream (sparus aurataL.) in southeastern spain. *Aquaculture*. 218, 11-20.

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[DOR: 20.1001.1.15622916.2012.11.3.15.5]