TFR
Trends in Fisheries Research
An International Peer-Reviewed Journal
www.sciencejournal.in

PATHOGENESIS OF *AEROMONAS* SPECIES IN STINGING CATFISH SHING (*HETEROPNEUSTES FOSSILIS*) OF BANGLADESH

Md. Shirajum Monir[1*], Tanvir Ahammed[2], Shuvho Chakra Borty[2], Nazneen Bagum[3], Md. Alimul Islam[2] and Yahia Mahmud[1]
[1]Bangladesh Fisheries Research Institute, Head Quarter, Mymensingh-2201, Bangladesh
[2]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh-2201, Bangladesh
[*]**Correspondence:** E-mail: monir_bau22@yahoo.com

ABSTRACT
The present experiment was conducted to evaluate the comparative ability of producing infections and causing mortality to inject Shing (*Heteropneustes fossilis*) with *Aeromonas* species. Nine *Aeromonas* isolates (*A. hydrophila*-3, *A. sobria*-3 and *A. salmonicida*-3) were injected to 9 different groups (each group consisting of 10 fish) of healthy Shing. Experimental Shing (*H. fossilis*) were injected with *A. hydrophila*, *A. sobria* and *A. salmonicida* to groups 1-3, 4-6 and 7-9, respectively while group 10 was injected with sterile phosphate buffered saline (PBS) and served as the control. For pathogenicity test, dose of 6.7×10^6 cfu/fish with each bacterial species were injected intramuscularly and monitored upto two weeks. The highest clinical infections were developed 100% in group 3 whereas only 30% in group 9. At the end of the experiment (after two weeks), the cumulative mortality rate was also recorded highest (90%) in group 3 and lowest (30%) in group 9. However, the development of infection and mortality to the injected Shing fish was associated more severely by *Aeromonas hydrophila* than *A. sobria* and *A. salmonicida*, used in this experiment.

KEY WORDS: *Aeromonas* species, Bangladesh, pathogenesis, stinging catfish.

INTRODUCTION

Heteropneustes fossilis (Bloch) is an indigenous air-breeding catfishes, stinging catfish of South-East-Asia which is locally known as Singi or Shing in different parts of Bangladesh. Among the catfishes, Shing (*H. fossilis*) is very popular and highly valuable fish species in Bangladesh. It is not only recognized for its delicious taste and market value but is also highly regarded for medicinal and nutritional aspects. It contains very high amount of iron (226 mg per 100 g) and fairly high content of calcium compared to many other catfishes (Saha and Guha, 1939). It is a very hardy fish that can survive for quite a few hours outside the water due to presence of accessory respiratory organs (Das, 1927; Khan *et al*., 2003). But, considering its high market value as well as high consumer demand, that's why it is cultured in farms with high stocking density. Although, Shing (*H. fossilis*) culture has great potential in Bangladesh but various diseases of Shing causes serious economic losses because of their high mortality under farming conditions.

Catfish like Shing (*H. fossilis*), Magur (*Clarias batrachus*) and Pangasius (*Pangasianodon hypophthalmus*) are teleosts having entire body surfaces, fins and barbells those covered with skin composed with non-keratinized stratified squamous epithelial cells (Zhao *et al*., 2008; Esteban, 2012). Actually fish skin plays an important role with the environment to maintain homeostatic conditions including protection from external environment, sensory perception, communication, excretion, osmoregulation, thermoregulation and antimicrobial activity (Elliot and Shotts, 1980; Bordas, 1996; Esteban, 2012). It provides the first attachment site for a wide range of microorganisms in aquatic environment (Bordas, 1996; Esteban, 2012). As a result, the attachment of microorganisms in skin frequently cause lesions and rupture which make possible for the pathogens to invade and multiply into the body. Thus, skin lesions and rupture causes harmful effect on normal growth and reproduction of the fishes as well as mass mortality. Generally, *Aeromonas* spp. was frequently recorded to cause disease in different species of farmed and wild freshwater fishes of Bangladesh (Rahman and Chowdhury, 1996; Sarker *et al.*, 2000). It was seen as an infective agent of ulcer type disease

occurred in different farmed fishes (Chowdhury, 1998). Iqbal *et al.* (1998) identified *A. hydrophila, A. veronii sobria* and *A. jandaei* as pathogenic bacteria isolated from EUS affected Mrigal (*Cirrhinus mrigala*). Furthermore, Rashid *et al.* (2008) identified *A. hydrophila* from EUS affected shing (*H. fossilis)*. A very few experimental works were conducted on bacterial diseases especially on pathogenicity of *Aeromonas* spp. in Shing (*H. fossilis*). Therefore, the present experiment was conducted to evaluate the comparative pathogenicity of *Aeromonas hydrophila, A. sobria* and *A. salmonicida* in stinging catfish Shing (*H. fossilis*) of Bangladesh.

MATERIALS AND METHODS

Experimental fish and set up: Apparently healthy Shing *(H. fossilis)* weighting 25-30 g, collected from a local fish farm of Mymensingh those were used in this experiment. Prior to the experiment, fish were stocked in aquariums to acclimatize in laboratory conditions from 28 to 30°C for at least 10 days providing adequate feed and better aeration by circulating water. The pathogenicity test was conducted at the Fish disease and health management laboratory of Bangladesh Fisheries Research Institute, Mymensingh.

Bacterial cultures and preparation of suspension: Stock cultures of *A. hydrophila*, *A. sobria* and *A. salmonicida* were used in this study those were isolated from different organs of naturally-infected Shing (*H. fossilis*) of different fish farms from Mymensingh district of Bangladesh. The isolates were cultured on a Typticase Soya Agar (TSA) at 32°C for 24 hours and identity confirmed using different biochemical characteristics prior to the experiment. They were then suspended in sterile phosphate buffered saline (PBS).

Pathogenicity experiment: Intramuscular injection method was used for the pathogenicity challenge test to know the efficacy of the pathogens in initiating the infection as well as observe mortality. After acclimatization for 10 days, 25-30 g, juvenile Shing (*H. fossilis*) were randomly assigned to 10 groups as 10 fish per group. For the intramuscular (IM) injection, one ml insulin syringes (sterile and disposable) were used to inject intramuscularly with 0.1 ml of pre-selected (Ahmed, 2009) bacterial dose (6.7×10^6 cfu/fish) as follows: groups 1 to 3 - *A. hydrophila*, groups 4 to 6 - *A. sobria*, while groups 7 to 9 were infected with *A. salmonicida*. A negative control group-10 of 10 fish were injected with sterile PBS as above. Each group was then released in separate aquaria and no feed was given to the experimental fishes to ensure that the water was not further contaminated (Omprakasam and Manohar, 1991; Iqbal, 1998). The temperature, pH and dissolved oxygen, ammonia concentration of the water was kept at acceptable limit during the experiment (Sofiq *et al*., 2013). The injected fishes were then observed clinical signs, symptoms and mortalities daily up to two weeks.

Re-isolation of challenged pathogens: Re-isolation of inoculated bacteria were done by collecting samples from skin lesions, kidney, liver of moribund and freshly dead or sacrificed experimental infected Shing fish and streaked on TSA plates to check the presence and absence of bacterium. Positive bacterial culture was confirmed by the morphological and biochemical characteristics of the re-isolated bacteria were identical with those of the isolates used in the experimental infection.

Statistical analysis: The data collected for rates of developing infection and mortality were subjected to descriptive statistics and expressed in percentages.

RESULTS

Clinical and gross pathology of experimental fish: After one day post-infection of intramuscularly injected all Shing groups expressed abnormal movement, loss of balance and constant rubbing of body with the aquaria glass. By day 3 post-infection, some of the fishes were observed to develop grayish-white spots or lesions on body surface. After dissection of the freshly dead fish, the liver and kidney were observed to be swollen, unsmooth, enlarged and turned blackish. Severally diffused hemorrhage was found on fin bases, edge of head and skin at day 5 post-infection in groups 1, 2, 3 (Fig. 1). In some fishes, hyperaemic patches of the fins were also observed in these groups. After day 6 post-

infection, hemorrhagic ulcerative lesions and, body and tail erosions were developed in groups 4, 5, 6, 7, 8, 9 (Fig. 2). But corrosion of the barbells and severe grayish white lesion on the caudal area were also observed in some fishes especially in groups 7, 8, 9 (Fig. 3). However, bacteria showed clinical signs in natural infection were found to be more or less similar in the injected experimental fish. But, no clinical signs and mortality were observed in the control group 10.

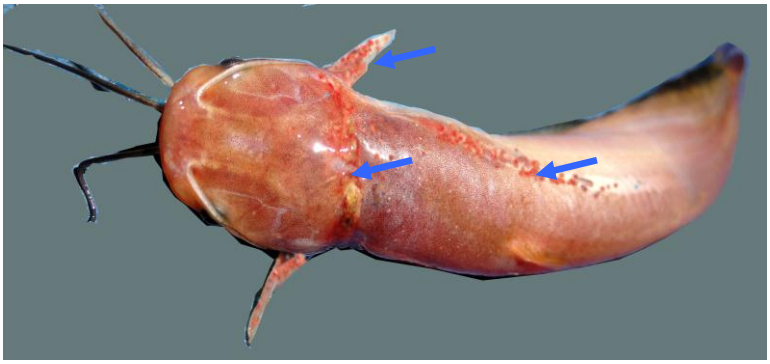

Figure 1. Hemorrhages in fins bases, edge of head and skin lesions in Shing (H. fossilis) experimentally-infected with *Aeromonas* species

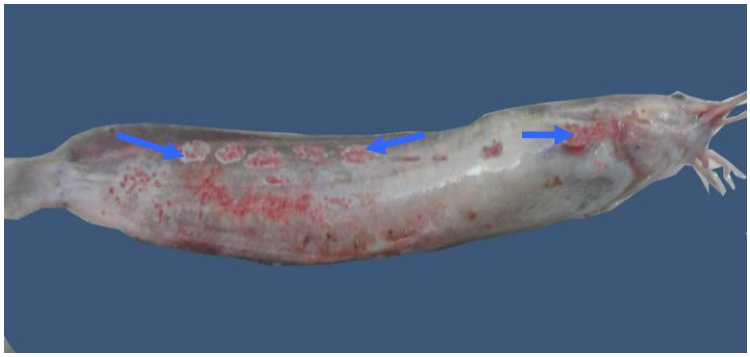

Figure 2. Hemorrhagic ulcerative lesions in Shing (*H. fossilis*) experimentally-infected with *Aeromonas* species

Figure 3. Corrosion skin lesions and barbells in Shing (*H. fossilis*) experimentally-infected with *Aeromonas* species

TFR
Trends in Fisheries Research
An International Peer-Reviewed Journal
www.sciencejournal.in

Effect of the selected *Aeromonas* spp. to develop infections in experimental Shing: At the end of the experiment (after 14 days), the highest clinical infection in the experimental fishes were found up to 100% in groups 3 whereas 50% in group 6. Sixty percent of fish in group 4 had developed infection and about 50% in groups 5 and 6. The lowest development of infection was found 30% in group 9. However, the highest average of infection was found about 86% among the groups 1, 2 and 3 during the experimental period (Table 1).

Table 1. Cumulative progression of infection in experimental Shing (*H. fossilis*) infected with *Aeromonas* spp

Group	Infected bacteria	Number of healthy Shing used per group	Mortality rate at days after injection														Percentage
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	*A. hydrophila*	10	0	0	0	2	2	3	3	3	4	4	4	4	4	4	40
2		10	0	0	0	3	4	4	4	4	4	4	4	4	4	4	40
3		10	0	0	5	8	9	9	9	9	9	9	9	9	9	9	90
Average			0	0	1	4	5	5	5	5	5	5	5	5	5	5	56
4	*A. sobria*	10	0	0	0	2	3	4	4	4	4	4	4	4	4	4	40
5		10	0	0	0	1	2	2	2	5	5	5	5	5	5	5	50
6		10	0	0	0	1	1	3	4	4	4	4	4	4	4	4	40
Average			0	0	0	1	2	3	3	4	4	4	4	4	4	4	43
7	*A. salmonicida*	10	0	0	0	1	1	2	2	2	2	2	3	3	3	3	40
8		10	0	0	0	1	1	1	2	2	2	2	2	2	2	2	50
9		10	0	0	0	1	1	2	2	2	2	3	3	3	3	3	30
Average			0	0	0	1	1	1	2	2	2	2	2	2	2	2	40
10	Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Effect of the selected *Aeromonas* spp. on mortality of experimental Shing: After 3 days of post infection, 5 severely infected fish were died in group 3 whereas no fish was died in other groups. At the end of the experiment (after 14 days), the cumulative mortality rate was recorded highest (90%) in group 3 and lowest (30%) in group 9. However, the highest average of the occurrence of mortality was recorded about 85% among the groups 1, 2 and 3 (Table 2).

Table 2. Cumulative progression of mortality rate in experimental Shing (*H. fossilis*) infected with *Aeromonas* spp.

Group	Infected bacteria	Number of healthy Shing used per group	Development of infections at days after injection														Percentage
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	*A. hydrophila*	10	0	0	4	5	5	7	8	8	8	8	8	8	8	8	80
2		10	0	0	2	4	4	5	8	8	8	8	8	8	8	8	80
3		10	0	0	7	9	9	9	10	10	10	10	10	10	10	10	100
Average			0	0	4	6	6	7	8	8	8	8	8	8	8	8	86
4	*A. sobria*	10	0	0	0	2	3	5	5	6	7	7	7	7	7	7	70
5		10	0	0	0	2	3	3	3	5	5	5	5	7	7	7	70
6		10	0	0	0	3	3	4	4	4	5	5	5	5	5	5	50
Average			0	0	0	2	2	4	4	5	5	5	5	6	6	6	63
7	*A. salmonicida*	10	0	0	0	1	1	2	3	3	3	3	4	4	4	4	40
8		10	0	0	0	1	1	1	2	2	2	3	5	5	5	5	50
9		10	0	0	0	1	1	2	2	2	2	3	3	3	3	3	30
Average			0	0	0	1	1	1	2	2	2	3	4	4	4	4	40
10	Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

DISCUSSION

In moribund condition of each group of intramuscularly injected fish were observed, abnormal movement, loss of balance and rubbing of body with the aquaria glass. The most commonly clinical signs were expressed as grayish-white spots or lesions on body surface, hemorrhages on fin bases, edge of head by the test fish during the experiment. Yarmidici and Aydin (2011) also observed body rubbing against tank walls in Nile tilapia infected with *A. hydrophila* isolate after 8 hours of infection. Rashid *et al*., (2008) observed pale body colour and fin loss in EUS affected stinging catfish (*H. fossilis*). In an experimental pathogenesis of *A. hydrophila* in shing Mostafa *et al*., (2008) experienced haemorrhagic lesions at the injection site, hyperemic anal region and, fin bases and grayish white lesion on the caudal area of the test fish. Rashid *et al*., (2008) observed that artificially infected fish with *Aeromonas* spp showed head-up-tail-down movement, some of the fish became very pale (discolored from normal grayish to pinkish) with hyperaemic spots at the base and tips of the fins. *Aeromonas* strains have been reported to produce cytotoxins and enterotoxins which destroy mucosal lining of the skin and gastrointestinal tracts resulting in excessive release of mucus and finally could be responsible for the induction (Janda and Abbott, 2010). However, more or less similar clinical signs and symptoms were also reported by Mostafa *et al*., 2008; Rashid *et al*., 2008; Ajayi, 2012.

Discoloration of skin in Rainbow trout of artificially-infected with *A. sobria* and *A. caviae* was observed by Rehulka, 2002. Change in skin colour was also found in Asian stinging catfish artificially-infected with *A. hydrophila* (Angka, 1990). However, the skin discoloration was found in experimental fish that indicates infection was established with all the injected *Aeromonas* spp. In this experiment, the hyperaemic patches were observed at the base of fins in some of the infected fishes that also reported by Yarmidici and Aydin (2011) and Thomas (2013) in experimentally-infected Nile tilapia and Indian catfish with *A. hydrophila*, respectively. The corrosive and heamorrhagic lesions were found in this experiment that has also been observed by (Angka, 1990 and Islam, 2008) in catfish species. Furthermore, more or less similar infections were reported by Ibrahem (2010) and Ajayi (2012) in naturally-diseased catfish species.

In this study, the highest percentage (100%) of infection in test fish were observed in group 3 indicates that *A. hydrophila* species used to infect the group might be more pathogenic than the other used *Aeromonas* species in this experiment. Paniagua (1990), Janda (2010); Daood (2012) were observed that *A. hydrophila* is more pathogenic and the most frequently isolated species from naturally-infected fish than other *Aeromonas* species. The average percentage of infection in *A sobria* groups (43%) was higher than the *A. salmonicida* groups (40%) that suggests *A sobria* was more pathogenic compared to *A. salmonicida*. However, this finding is more or less similar with the observation of Daood (2012). Pathogenicity of *A. hydrophila* to Shing (*H. fossilis*) by intramuscular injection was ranged from 40-90% mortality among the groups 1, 2 and 3. This mortality rate was higher than that recorded in the *A. sobria*-injected (40-50%) and *A. salmonicida*-injected (30-50%) groups. This results indicates that the *A. hydrophila* strains used in this experiment were more pathogenic than the other species. Mostafa *et al.* (2008) conducted an experimental infection of Shing (*H. fossilis*) with *A. hydrophila* by two different methods viz. intraperitoneal and intramuscular injection at a dose of 9.6×10^7 cfu/fish that caused in 100% mortality of the tested fish within 1-9 days. Sarkar and Rashid (2002) also observed 100% and 60-80% mortality in Shing (*H. fossilis)* and Magur (*C. batrachus*), Carps and Thai koi (*Anabas testudineus*) injected with $6.7x10^7$ and $6.7x10^6$ cfu/ml of *A. hydrophila*, successively. Furthermore, Hossain *et al*. (2011) found 100% and 40% mortality by 4-9 days of injection when Thai koi (*A. testudineus*) was challenged with $9.2x10^7$ and $9.2x10^6$ cfu/fish of *A. hydrophila* isolate, successively. However, the variation of the mortality rates might be due to different species of fish used in experiment, immunity of the fish, various strains of *Aeromonas* species, environmental factors, used different doses of the infective pathogens, route of administration as well as experimental duration.

CONCLUSION

It was confirmed that *Aeromonas* species used in this experiment those were able to develop infections as well as cause mortality in test stinging catfish Shing. Further researches are necessary to prepare antibody against these bacteria to prepare vaccines and to try vaccination in susceptible to save catfishes against these pathogenic bacteria.

REFERENCES

Ahmed M.B. (2009). Isolation and identification of *Aeromonas hydrophila* from carps, perch and catfishes. M.S. Thesis. Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh. 37 pp.

Ajayi A.O. (2012). Bacteriological study of catfish, *Clarias gariepinus*, from fish pond sources in Akungba-Akoko community, Nigeria. *British Microbiol. Res. J.*. 2:1-9.

Angka S.L. (1990). The pathology of the walking catfish, *Clarias batrachus* (L) infected intraperitoneally with *Aeromonas hydrophila*. *Asian Fisheries Sci.* 3:343-351.

Bordas M.A., Balebona M.C., Zorrilla I., Borrego J.J. and Morinio M.A. (1996). Kinetics of adhesion of selected fish-pathogenic *Vibrio* strains to skin mucus of gilt-head sea bream (*Sparus aurata* L.). *Applied Environ. Microbiol.* 62:3650-3654.

Chowdhury M. B. R. (1998). Involvement of aeromonads and pseudomonas in disease of farmed fish in Bangladesh. *Fish Pathol.* 33: 247-254.

Daood N. (2012). Isolation and antibiotic susceptibility of *Aeromonas* species from freshwater fish farm and farmed carp (Dam of 16 Tishreen, Lattakia). *Damascus Univ. J. Basic Sci.* 28:27-39.

Das B.K. (1927). The bionomics of certain air-breathing fishes of India, together with an account of the development of their air-breading organs. *Phil Trans*. 216:1-41.

Elliot G. and Shotts Jr.E.B. (1980). Aetiology of an ulcerative disease in goldfish *Carassius auratus* (L.): microbiological examination of diseased fish from seven locations. *J. Fish Dis.* 3:133–143.

Esteban M.A. (2012). An overview of the immunological defenses in fish skin. *International Scholarly Research Network*. 1-29.

Hossain M.F. Rashid M.M. and Sayed M.A. (2011). Experimental infection of indigenous climbing perch *Anabas testudineus* with *Aeromonas hydrophila* bacteria. *Progressive Agriculture*. 22:105-114.

Ibrahem M.D. and Mesalhy S. (2010). Determining the safety and suitability of fluorescein 281 dye for characterization of skin ulcerations in cultured Nile tilapia (*Oreochromis niloticus*) and African sharptooth catfish (*Clarias gariepinus*). *Journal of Advanced Research.* 1:361366.

Iqbal M.M., Tajima K., Sawabe Y., Nakano K. and Ezura Y. (1998). Phenotypic and genotypic identification of motile aeromonads isolated from fish with epizootic ulcerative syndrome in Southest Asian countries. *Fish Pathol*. 33(4): 255-263.

Islam M.T., Mostafa K. and Rashid M.M. (2008). Histopathological studies of experimentally-infected shing, *Heteropneustes fossilis* with *Aeromonas hydrophila* bacteria. *Progressive Agri.* 19:89-96.

Janda J.M. and Abbott S.L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity and infection. *Clinical Microbiol. Rev.* 23:35-73.

Khan M.N., Saiful Islam A.K.M. and Hossain M.G. (2003). Marginal analysis of culture of stinging catfish (*Heteropneustes fossilis*, Bloch): Effect of different stocking densities in earthen ponds. *Pak J Biol Sci*. 6(7):666-670.

Mostafa K., Islam M.T. and Rashid M.M. (2008). Experimental pathogenesis of *Aeromonas hydrophila* bacteria in stinging catfish *Heteropneustes fossilis*. *Bangladesh J. Fish. Res.* 12(1): 27-33.

Omprakasam M. and Manohar L. (1991). Experimental infection of some bacterial fish pathogens in the cichlid fish, *Oreochromis mossambicus*. *Indian J. Fisheries*. 38:106-110.

Paniagua C., Rivero O., Anguita J. and Naharro G. (1990). Pathogenicity factors and virulence for rainbow trout (*Salmo gairdneri*) of motile *Aeromonas* species isolated from a river. *Journal of Clinical Microbiology.* 28:350-355

Rahman M.M. and Chowdhury M.B.R. (1996). Isolation of bacterial pathogen causing an ulcer disease in farmed carp fishes of Mymensingh. *Bangladesh J. Fish.*, 19(1-2): 103-110.

Rahman S. and Monir M.S. (2013). Effect of stocking density on survival, growth and production of Thai *Anabas testudineus* (bloch) fingerlings under nursery ponds management in northern regions of Bangladesh. *J. Experimental Biol. Agri. Sci.* 1(6):465-472.

Rashid M.M., Hasan M.A., Mostafa K. and Islam M.A. (2008). Isolation of *Aeromonas hydrophila* from EUS affected shing *Heteropneustes fossilis* from a fish farm of Mymensingh. *Progress. Agric.*19(1): 117-124.

Rehulka J. (2002). *Aeromonas* causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology and biochemistry. *Acta Veterina Brno.* 71:351-360.
Saha K.C. and Guha B.C (1939). Nutritional investigation on Bengal fish. *Indian J. Medical Res.* 26: 921-927.
Sarkar M.J.A. and Rashid M.M. (2002). Pathogenicity of the bacterial isolate *Aeromonas hydrophila* to catfishes, carps and perch. *J.Bangladesh Agri.Univ.* 10:57-161.
Sarker M.G.A. Chowdhury M.B.R. Faruk M.A.R. Uddin M.N. and Islam M.J. (2000). Effect of water temperature on the infectivity of *Aeromonas hydrophila* isolates. *Bangladesh J. Fish.* 23(2): 99-105.
Thomas J., Madan N., Nambi K.S.S., Majeed S.A., Basha A.N. and Hameed A.S.S. (2013). Studies on ulcerative disease caused by *Aeromonas caviae*-like bacterium in Indian catfish, *Clarias batrachus* (Linn). *Aquaculture* 376379:146-150.
Yardimic B. and Aydin Y. (2011). Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *Ankara Universitesi Veteriner Fakultesi Dergisi*. 58:47-54.
Zhao X., Findly R.C. and Dickerson H.W. (2008). Cutaneous antibodysecreting cells and B cells in a teleost fish. *Developmental Comparative Immunol.* 32(5):500-508.

