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Prevalence of Fish Flavobacterium Infection at Pond Farms of Ararat Region, Armenia

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Abstract **– The goal of current work was research of prevalence and detection of causes for *Flavobacterium columnare* infection at fish farms of Ararat Region. Epidemiological, clinical and laboratory research was conducted at "Eco Fish Trade", "Bigama Fruit" and "Mary Fish" farms, as well as at the laboratory of the Department of Epidemiology and Parasitology, National Agrarian University of Armenia. Affected fish was researched by classic method for ichthyopathological examination. Laboratory diagnostics and identification of the disease was conducted by means of microscopic examination. Research demonstrated high prevalence of the disease in all species and age groups of fish grown in aquaculture of the region. The incidence of the fish *Flavobacterium columnare* infection at the fish farms of Ararat Region was rather high (49.0%). Disease incidence in young fish (1.5-6 months) was much higher (72.01%) than in adult fish (6 months to 1.5 years) (37.12%) that could be probably explained by higher susceptibility of young fish to various stressors. Presence of predisposing factors and economic relations between fish farms promote the further spreading of the disease in Armenia.**

Keywords **- *Flavobacterium Columnare* Infection, *Saprolegnia* Infection, Fish, Pond, Fish Farm, Prevalence.**

I. INTRODUCTION

It is known that fish evolution lasted a few hundred million years, and they adapted both to environmental conditions and numerous parasites during this period. All fish diseases are mainly determined by factors present at fish pond farms. Fish keeping conditions, feeding, variations of water temperature etc. Preventing the mentioned impairments and avoiding fish stress, we often prevent development of diseases, especially when causative agents of the diseases are saprophyte microorganisms. Fish *Flavobacterium* infection is one of such kind of diseases.

Flavobacterium columnare infection (columnaris infection, myxobacterial disease, cotton wool disease, saddleback, fin rot) used to be common disease of ornamental aquarium fish. Causative agent *Flavobacterium columnare* Bernardet et al, 2002 (formerly named *Flexibacter columnaris*) is a common saprophyte of sediment developed on the organic matter that is opportunistic microorganism [4], [12], [13]. Various stress factors, e.g. chemical changes, poor nutrition, mechanical injuries, and especially high water temperature, result in clinical manifestation [4], [6], [10], [19], [23]. Many species of wild and cultivated fish are susceptible to this disease worldwide [4], [8], [14], [16], [17], [22], and significant economic losses can be caused by this pathogen [4], [7], [17]. White margins and mucous film appear on the scales and fins at early stage of the disease. Progressing lysis and necrosis of deeper tissues, severe gill lesions (discoloration, inflammation and necrosis), ulcerations, tissue maceration etc. develop later [3]-[5], [16]-[17], [22].

It hasn`t been registered at fish pond farms of Armenia before, but currently it`s one of the most prevalent fish diseases, although there is absolutely no literature data about *Flavobacterium* infection in Armenia [15].

Based on the above-mentioned, the goal of current work was research of prevalence and detection of causes for *Flavobacterium columnare* infection at fish farms of Ararat Region.

II. Material and Methods

Research was conducted at "Eco Fish Trade LLC", "Bigama Fruit LLC" and "Mary Fish LLC" fish farms that are located in Ararat Region in 2019. All the farms have artificial ponds that are nourished by underground artesian water.

"Eco Fish Trade LLC" pond farm is located in village Zorak. The farm has surface equals to 1.6 hectares, and 1.2 hectares of them is pond water surface. Water source mean temperature is $+13^0$C, and it fluctuates within $+15^0$C and $+16^0$C in summer. Oxygen concentration in water equals to 9 mg per l due to presence of water aerators. Farm is specialized on growing of silver trout (*Salvelinus agassizii* Garman, 1885), rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), and river trout (*Salmo trutta morpha fario* L., 1758). The total number of fish at the farm is 80,000.

"Bigama Fruit LLC" pond farm is located in village Sayat Nova. The farm has surface equals to 13 hectares, and 11 of them are due to pond water surface. Water source mean temperature is $+14^0$C, and it fluctuates within $+16^0$C and $+17^0$C in summer. Oxygen concentration in water equals to 8 mg per l due to presence of water aerators. Farm is specialized on growing of silver trout (*Salvelinus agassizii* Garman, 1885), rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), and Californian golden trout (Oncorhynchus mykiss aguabonita Jordan, 1893). The total number of fish at the farm is 140,000.

"Mary Fish LLC" pond farm is located in village Marmarashen. The far has surface equals to 16 hectares, 13 of that is pond water surface. Water source mean temperature is $+15^0$C, and it fluctuates within $+18^0$C and $+19^0$C in summer, because of local climatic conditions. Oxygen concentration in water equals to 6 mg per l due to presence of water aerators. Farm is specialized on growing of silver trout (*Salvelinus agassizii* Garman, 1885) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). The total number of fish at the farm is 80,000.

Research was conducted in summer, from May to August 2019. 200 fish of the following species were examined: silver trout (*Salvelinus agassizii* Garman, 1885), rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), Californian golden trout (Oncorhynchus mykiss aguabonita Jordan, 1893), and river trout (*Salmo trutta morpha fario* L., 1758). Young fish of age from 2 months to one and half year was examined.

Epidemiological investigation and clinical examination was conducted at the farms prior to laboratory examination [18].

Laboratory research was conducted at the laboratory of the Department of Epidemiology and Parasitology, National Agrarian University of Armenia. Examination of the affected fish was conducted by classic method for ichthyopathological examination [1].

Laboratory diagnostics and identification of the disease was conducted by means of microscopic examination. Skin scrapings and gill smears from affected fish from all the above-mentioned pond farms were examined. Smears were stained by methylene blue and gentian violet stains. Besides, the agent was incubated in tryptone-yeast extract medium, at 25^0C for 48 hours.

Smears were also prepared from the grown colonies and stained by methylene blue and gentian violet stains [2], [9], [16], [21].

III. Results and Discussion

Results of the fish examination are demonstrated in Table 1.

Table 1. Fish Flavobacterium Columnare Incidence.

Fish Farm Name	Age Groups Examined	Number of the Fish Examined from Each Group	Number of the Infected Fish	Incidence, %
"Eco Fish Trade"	Adult Fish 6 months – 1,5 years	66	9	13.64
"Bigama Fruit"	Young Fish 2 – 6 months	34	29	85.29
	Adult Fish 6 months – 1,5 years	33	24	72.73
"Mary Fish"	Young Fish 2 – 6 months	34	20	58.82
	Adult Fish 6 months – 1,5 years	33	16	48.48
TOTAL		200	98	49.0

Thus, incidence of the fish *Flavobacterium columnare* infection at the fish farms of Ararat Region was rather high (49.0%).

Disease incidence in young fish (1.5-6 months) was much higher (72.01%) than in adult fish (6 months to 1.5 years) (37.12%) that could be probably explained by higher susceptibility of young fish to various stressors.

Affected fish with clinical signs specific for *Flavobacterium columnare* infection, was detected at all examined farms. Ulcers covered by grey mucus were detected in different parts of the fish body, mainly on dorsal and tail fins, head and around the mouth. Fin destruction and muscle denudation is observed in some fish. The described signs are specific for chronic course of disease (see Fig. 1 to 4).

Epidemiological investigation at "Eco Fish Trade LLC" pond farm shew that sanitary condition of the farm is unsatisfactory, and similar clinical signs were detected in fish during last two years. Great economic damage caused to the fish farm by mass death of the young fish during the mentioned period. Currently the disease is being registered in all age groups of silver trout, rainbow trout and river trout, however fish death cases are comparatively rare.

Epidemiological investigation and clinical examination conducted at "Bigama Fruit LLC" pond farm shew that two months old young fish was bought from the neighbor farm. In 3 days after young fish were placed into basins, death cases appeared. There were no clinical signs in young fish, however, occurrence of greyish mucous spots on fins and around mouth was observed in older fish. Silver, rainbow and golden trout was affected at the mentioned farm.

Fig. 1. Specific affects in gills.

Fig. 2. Specific affects in gills.

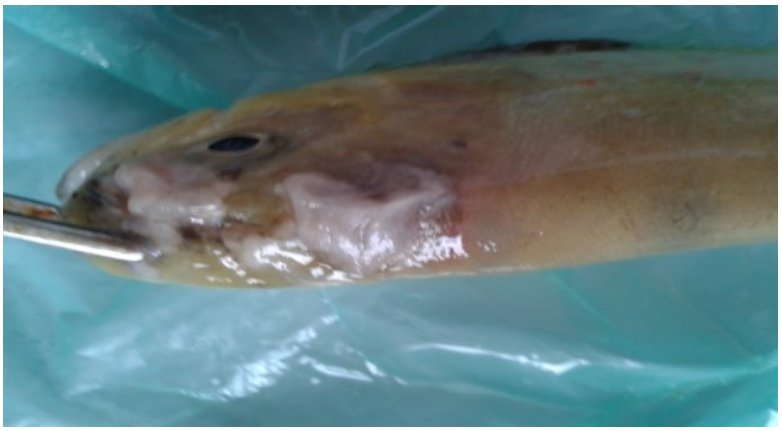

Fig. 3. Specific lesions on head.

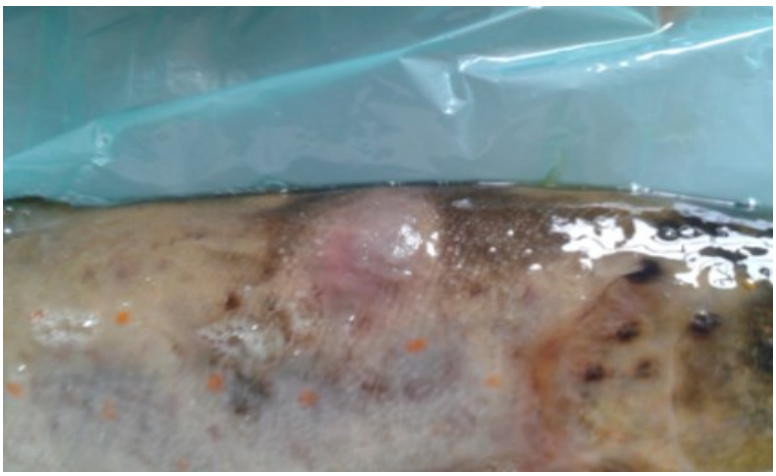

Fig. 4. Dorsal lesions due to *Flavobacterium* infection.

Following clinical signs were registered in silver and rainbow trout of "Mary Fish LLC" pond farm: anorexia, horizontal swimming on the pond surface, darkening and mucus coating, tachypnea expressing as "cough-like" movements of operculum. In several cases opercula are open, and hyperemic and mucus-covered gills are visible (see Fig. 1 and 2). Disseminating necrosis of gill petals starting from the peripheral parts was detected during the microscopic examination.

Specific growth was observed in tryptone-yeast extract medium, where yellow small rhizoid adherent colonies were formed in central part, and white ones were at periphery.

Numerous irregularly arranged narrow rods that have flattened ends with approximate size 1.2-10 x 0.6-2.0 Um were detected during microscopic examination of the stained smears prepared from both pathologic material and grown cultures (see Fig. 5).

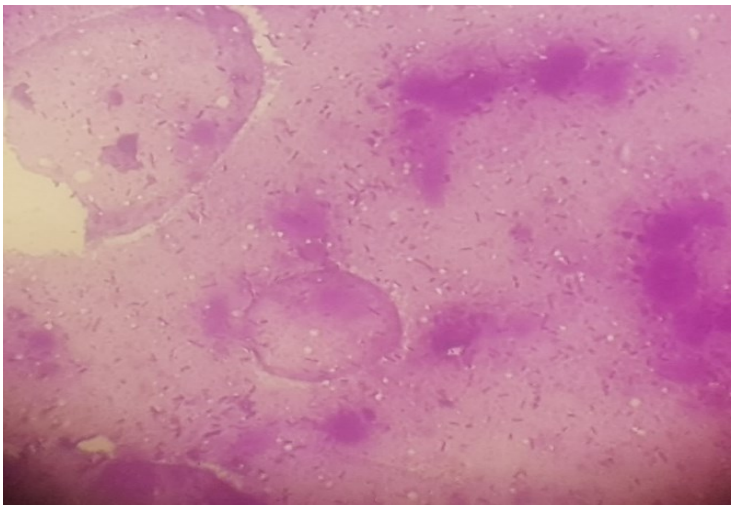

Fig. 5. *Flavobacterium columnare* in rainbow trout gill smear (630x, stained by methyl violet).

Mix-infection by *Flavobacterim columnare* bacteria and *Saprolegnia sp.* fungi was detected at "Eco Fish Trade" pond farm was detected during laboratory examination of skin and gill smears of the affected fish.

Flavobacterium columnare is a saprophyte living and reproducing in decaying organic matter of the pond sediments. Besides, clinically healthy fish can serve as asymptomatic carrier of this pathogen unless stress and exposure by unfavorable environment conditions, i.e. predisposing factors.

In each farm where the disease was detected, such factors were specific.

For example, poor sanitary conditions of ponds, and generally of farm territory (e.g. presence of carcasses of the death fish, foreign objects, and fodder remainder in the pond water), serve as predisposing factors at "Eco Fish Trade" fish farm.

For "Bigama Fruit", general stress and external injuries of the young rainbow trout bodies during their capture and transportation were predisposing factors.

According to literature data, *Flavobacterium columnare* infection is usually registered in summer period, when water temperature is $+15^0$C and higher [9], [18], [20], therefore, the main predisposing factors of high prevalence of *Flavobacterium* infection at fish farm of Ararat Region are high temperature of water and low oxygen concentration in it.

The finding of *Flavobcterium columnare* in Armenia is significant in that this is the first report of the mentioned serious fish pathogen in our country. Presence of predisposing factors and economic relations between fish farms promote the further spreading of the disease in Armenia, especially in summer period with a high water temperature, taking into consideration climate warming. Additional studies are in progress.

IV. Conclusion

Basing on results of epidemiological investigation, clinical examination and laboratory research conducted at the targeted fish farms, we can conclude with confidence that fish *Flavobacterium columnare* infection that has

not been formerly registered in our country, is currently widely distributed at fish farms of Ararat Region. Poor sanitary conditions of the farms, high temperature of pond water, low oxygen concentration, mechanical injuries of the fish skin, unsatisfactory feeding, and transportation stress promotes the disease development. Fish of all species and age groups, especially young fish, is susceptible to the disease.

Disease entrance to our country probably occurred from abroad with infected fertilized roe and fish fodder.

Close economic ties between fish farms of our country, i.e. transportation of the fertilized roe and fish from infected farms, as well as joint use of the contaminated transport means and capture implements, promoted the distribution of the disease.

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