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Survey of Experimental Contamination to *Ichthyophthirius multifiliis* in Cultural Rainbow Trout Consequently Vaccination with *Aquavac garvetil*

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Abstract: The rainbow trout is the only cold water fish in Iran which has specific economic importance. One of the major bacterial diseases among the rainbow trout is streptococcosis which can be controlled through *Aquavac garvetil* vaccine. It has been shown that this vaccine has stimulation effects on specific and non-specific immune system in the fish. So the aim of this study was determination of Ichthyophthirius experimental infectious in rainbow trout after *Aquavac garvetil* vaccination. In this study, 2000 fish (1 g) randomly selected and ten infected fish by Ichthyophthirius added to pool after 15 days. After 60 days, fishes randomly divided in control and treatment groups. In treatment group, vaccination was done in two stages. First stage in day 67 which fish were sunk in *Aquavac garvetil* vaccine and in second stage vaccine were administrated orally in day 127. In control group, vaccine were not used. After one month, in both groups, 500 fish randomly were selected and anesthetized, after wet mount preparation the numbers of parasites were counted. Mean of number of parasites compared in control and treatment groups.

Key words: Ichthyophthirius, *Aquavac garvetil* vaccine, rainbow trout, fish, parasites, Iran

INTRODUCTION

Rainbow trout is one the best fishes in most cold water aquaculture farms in furthermore of world. Production rate of rainbow trout in Iran in years of 1997 and 2008 was approximately 3000 tons and predicts with initiation other farms in future years, achieved outstanding progress. Thus, hygienic and cultural management about this fish has great importance (Dremond, 2000; Farzanfar, 2005; Rabert and Sheferd, 1999; Ritez, 2004; Soltani, 2001). Streptococcosis is one of the chief bacterial diseases in rainbow trout that for its prevention used of *Aquavac garvetil* vaccine. Administration of this vaccine has been obligated since spring 2007. Because of high cost, using of this vaccine with exception of in high epidemic areas, isn't prevalent. Vaccination in farms especially in salmons, for prevention of infectious disease used as one of the major method for prevention. Several infectious diseases caused by gram positive and gram negative bacteria which were controlled significantly by vaccination. Positive effect of vaccination in farms is reduction of mortality and use of antibiotics (Gudding *et al*., 1997; Makhir, 2006; Noga, 1995; Rabert and Sheferd, 1999; Stoskopf, 1993). With attention to this point that vaccination causes high resistance in fishes, herd health and improving sanitary situations of fishes thus results rapidly growth, reduces fatalities, improves feed conversion ratio and reduces spoilage (Ellis, 1988; Makhir, 2006). *Aquavac garvetil* vaccine mainly includes 2 major dead bacteria (*Lactobasillus garvieae* and *Streptococcus iniae*) that used in rainbow trout. These bacteria are the most causative agent of streptococcosis in fishes and other animals (Barnham *et al*., 1987; Goh *et al*., 1998; Koh *et al*., 2004; Lau *et al*., 2003). *Aquavac garvetil* used as induction of immune response against this disease. Vaccine are used in fishes as injection, oral and overwhelming of package in water. Small fishes were vaccinated by overwhelming method because injection in these fishes is very hard. Fishes with 20 g and more, recommended that vaccination exert by injection method and about in fishes with 5 g and more were vaccinated by overwhelming method. Because immune system in these fishes weren't evolutes and created immune are short term, remembrance vaccination must be repeated after 60-90 days orally (Makhir, 2006; Rabert and Sheferd, 1999; Stoskopf, 1993). This shown that using of this vaccine causes excitation of specific and non-specific immune system. Ichthyophthirius is ciliated

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parasite in the multifiliis species has significant importance and accounted as dangerous external fish's parasite. Carp is chief host of this parasite but capable to infected all freshwater fishes. Infection rate and their potential related to primary infection severity. For treatment we can use many physical and chemical methods such as increasing water temperature, malachite green, formalin, potassium permanganate and Methylene blue. The best and common way to prevention and controlling is immunizing. Although, in current situations there aren't any efficient and practical method against ichthyophthirius in the market but there are compounds for induction of immune (Dremond, 2000; Entergaster, 1999; Rabert and Sheferd, 1999; Ritez, 2004; Noga, 1995; Buchmann *et al*., 1999; Swennes *et al*., 2007). With attention to existed problems in treatment field and more cost of drug treatment and also with attention to this fact that during treatment the reduction of growth and fatalities were existed thus prevention is beneficial and efficient than treatment. In these cases with exerting correct and sanitary managements and using of vaccine and immune excitants can be preventing from incidence of disease (Ellis, 1988; Gudding *et al*., 1997; Makhir, 2006; Stoskopf, 1993). Prevention and controlling the parasite will be successful when sanitary management of farm capable to exert of controlling polices. Vertices of polices includes: prevention of adjacent of fish and parasite together, rapidly detection of parasite in infection incidence time, treatment or immunizing of them and accuracy at purchasing new fishes (Dremond, 2000; Makhir, 2006; Rabert and Sheferd, 1999; Soltani, 2001; Stoskopf, 1993). With this introduction and attention to this fact until today there aren't any reports indicates effect of this vaccine against ichthyophthirius, the aim of this research is determination of experimental contamination rate to ichthyophthirius in rainbow trout consequently vaccination with *Aquavac garvetil*.

MATERIALS AND METHODS

In this research 2000 pieces of 1g rainbow trout fish child's were selected by chance and were cultured in round pool with 5 m^3 capacity at 12°C with 0.01 mg L^{-1} ammonia, 5 ppm CO_2 and 7-8 ppm O_2. After 15 days, 10, 1 g rainbow trout which were suffered from ichthyophthirius released to these pools and after 1 month to wit day 45 of research, fishes exited from pools. With experimental assessments of these fishes revealed that these fishes were suffered from ichthyophthirius. In continuance of research in day 60 (weight of fish was 4-5 g) fishes were divided into 2 chiliad groups and maintained in 2 round pools (each pool has 10 m^3 capacity) with same conditions. One of the pools selected as control pool or unvaccinated group and other pool selected as treatment pool or vaccinated group. In treatment group 1 week after (day 67), *Aquavac garvetil* vaccine in stage 1 by overwhelming method and according to creator company recommendation were administrated whereas control group didn't received vaccine (Ellis, 1988; Gudding *et al*., 1997). After 2 months, to wit day 127 of research in treatment group stage 2 vaccination orally administrated as remembrance according to the creator company recommendation. For best distribution of vaccine in fish diet, we used fish oil (volume of used oil was 3% of feed) (Stoskopf, 1993). After 1 month administrating of oral vaccine in treatment group (day 157 of research) of each control and treatment groups 500 samples were selected by chance and after anesthesia by wet mount, existence of ichthyophthirius were assayed by light microscope. Of each fish 3 wet mounts supplied from dorsal surface and dorsal fin, right and left sides and right and left sides of tail fin, respectively. Numbers of seen parasites in total 3 slides for each fish recorded and numbers of seen parasites in 500 fishes from each group in separate tables were collected. Finally, mean of seen parasites in each fish were compared with control and treatment groups. Data expressed as mean±standard deviation and for comparison of parasite's mean in control and treatment group used of t-test. $p<0.05$ considered as significant levels between groups.

RESULTS AND DISCUSSION

Results showed that numbers of isolated parasites from control fishes (19.59±3.89) were significantly ($p<0.05$) more than treatment group (4.83±2.37). To wit using of *Aquavac garvetil* vaccine in treatment group fishes significantly can reduce number of parasite than to other group (Fig. 1).

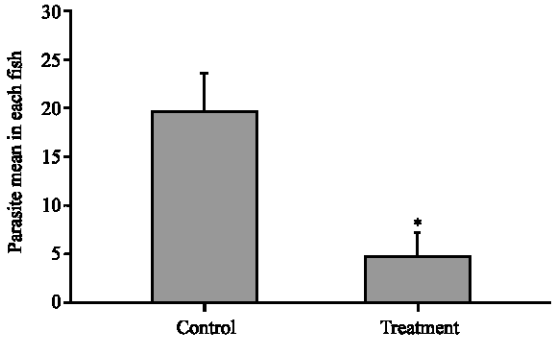

Fig. 1: Mean of isolated parasite from control and treatment groups, $p<0.05$ is in compared with testifier group

Many researches were done in association with ichthyophthirius to found a suitable way to prevention of suffering from this parasite (Makhir, 2006; Buchmann *et al*., 1999; Stoskopf, 1993; Swennes *et al*., 2007). Immune response in infected fishes to ichthyophthirius demonstrated by researchers and fishes that escape from one epidemic disease achieved relatively immune against re-infection. Immune in carp, lasts 8 months (Dremond, 2000; Makhir, 2006; Stoskopf, 1993). Many vaccines were marked against this parasite such as G5, G12 or produced vaccines with *Gyrodactylus derjhavini* that not immunizing directly but with creation of partial crossover immune prevented from suffering to ichthyophthirius (Buchmann *et al*., 1999; Swennes *et al*., 2007). Swennes made vaccines with two ichthyophthirius serotypes and examined on catfishes. They used G5 and G12 on fishes and achieved that fishes which were treated with G12 immunized perfectly against two serotypes next invasions. Whereas, fishes which were treated with G5 only relatively immunized against two serotypes (Swennes *et al*., 2007). In the other researches that were done by Buchmann *et al*. (1999) has shown that fishes which were contacted with *Gyrodactylus derjhavini*, achieves a partial crossover immune against ichthyophthirius and infection and incidence rate to ichthyophthirius reduced (Buchmann *et al*., 1999). In the other study that were done by Fagani in Iran demonstrated that use of *Aquavac garvetil* against streptococcosis causes increasing of white blood cell, average of cell volume, haemoglobin weight in the cell and haemoglobin percentage. In the other research were done by Ogut *et al*. (2005) in Turkey revealed that prevalence rate of ichthyophthirius in September, August and October is very high than other months that probably is related to Turkey weather. Results of this study were shown that use of *Aquavac garvetil* vaccine against ichthyophthirius causes reduction of suffering to this parasite (Ogut *et al*., 2005). In the other study which carried out by Ekanem *et al*. (2004) demonstrated that use of Mucuna pruriens leaves and the petroleum-ether extract of seeds of Carica papaya causes 90% reduction in numbers of *I. multifiliis* on fish after treatment in baths of each plant extract at 200 mg L^{-1} compared to untreated controls (Ekanem *et al*., 2004). *Aquavac garvetil* used especially against streptococcosis. Vaccination against streptococcosis in most cultural fishes has positive results. After vaccinating, achieved immune causes 85-95% protection. Whereas, after contamination of unvaccinated farms, fatality rate would be between 5-100% (Makhir, 2006; Stoskopf, 1993). Immune system of fishes can be divided to 3 portions: specific immune system, non-specific immune system and cell-mediated immune system. Non-specific immune system divided to two classes includes: physicochemical defense and cell defense. Physico-chemical defense is collection of agents that aquatic Wight used of them as defensive tools includes: skin, flake and mucosa (Ellis, 1988; Gudding *et al*., 1997; Makhir, 2006; Rabert and Sheferd, 1999). Mucosal discharges on external surfaces of skin, gills and gastrointestinal lumen play a physicochemical role against pathogenic agents. Physical specificity causes that because of high viscosity, pathogenic agents can't passing easily. In addition, mucosa in fish continuously produced and turnover. Thus, pathogenic agents excreted from body surfaces. From chemical aspects, mucosa has specific pH that isn't suitable for pathogenic survival. In mucosa, there are some proteins which play a role in immune system includes: Lysozyme, agglutinin, persipeptin and etc. (Ellis, 1988; Gudding *et al*., 1997; Makhir, 2006).

Aquavac garvetil vaccine cause creation of specific immune against streptococcosis and this immune wasn't decreased cause of ichthyophthirius on the fish body surface but this vaccine fulfilled as immune system excitant and consequently non-specific immune system reached in the mucosa. Lysozyme is one of the effective protein macromolecule in the skin mucosa and gastrointestinal that plays an important role in the non-specific immune system. Lysozyme with disturbing in the bacterial osmotic pressure and with perforating bacterial cell wall causes bacterial death and also causes hydrolyzing of glycochitin compounds in the cell wall of fungus, some parasitic crustaceans and protozoan. Nevertheless, the role of this vaccine against ichthyophthirius needs another more studies.

CONCLUSION

Results showed that in treatment group after vaccination the number of parasites significantly decreased ($p<0.05$) compared with control group. This effect of *Aquavac garvetil* vaccine is probably via stimulating effect of this agent on nonspecific immune system in fishes. However, study of the mechanism of this vaccine in decreasing of Ichthyophthirius infections needs to more investigation.

RECOMMENDATIONS

Prevention and controlling of parasite will be successful when sanitary management of farm capable to exert of controlling polices. Vertices of polices includes: prevention of adjacent of fish and parasite together, rapidly detection of parasite in infection incidence time, treatment or immunizing of them and accuracy at

purchasing new fishes. Also in farms that suffering from this parasite is exist must be used of *Aquavac garvetil* without fearing of sides effects.

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