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Investigation the Role of Physical and Chemical Parameters on Yersiniosis Outbreak in Rainbow Trout, *Oncorhynchus mykiss,* (Walbaum) Farms in Iran

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Abstract: Yersiniosis or Enteric red mouth disease (ERM) is an infectious bacterial disease that causes high mortality and economic losses in aquaculture industry. Yersiniosis has been spread in most of the Iran's provinces in last year's. Mazandaran province has the second grade of rainbow trout production in Iran and in Mazandaran province, the most rainbow trout produce in Haraz zone. Therefore, environmental parameters responsible for Yersiniosis were studied in Haraz zone (Mazandaran, Iran). Some Physical and chemical parameters of rainbow trout farms including: Nitrite, Nitrate, Ammonia, Total dissolved solid (TDS), Temperature, Dissolved oxygen (DO) and pH were measured monthly in 10 farms, one year duration. Also, liver and kidney of 1200 rainbow trout with clinical signs (410 samples) and without clinical signs (790 samples) were cultured to ensure for Yersiniosis outbreak. Effective parameters in Yersiniosis were temperature, pH increase, fish size and season. In addition, *Yersinia ruckeri* were isolated from 20% of fishes with clinical signs and 10% of fishes without clinical signs.

Key words: Yersiniosis · Rainbow Trout · Outbreak · Haraz Zone

INTRODUCTION

Fish and other aquatic animals are extremely important as a richest source of protein in human diets and most of the developed countries supply their needs to protein from fish and shellfish [1]. Recently, Rainbow trout has become the main cultured species in many of cold water fish farms in the world because of adaptation with intensive culture conditions and artificial foods and proper growth rate [2]. Bacterial diseases such as Yersiniosis are as greatest problem of aquaculture industry lead to economic losses. Yersiniosis is an infectious systemic disease that reported initially among rainbow trout in Idaho and then in other part of world such as Iran [3] Mazandaran province (Iran) has the second degree in rainbow trout production in Iran. In addition, the highest rainbow trout production in Mazandaran belongs to Haraz zone. Farms located in Haraz use from a common water source (Fig. 1), therefore, development of diseases from one farm to another is

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Fig 1: Satellite image of part of Haraz River

unavoidable. In present research, effective physical and chemical parameters in Yersiniosis outbreak in rainbow trout farms of Harz zone were investigated.

MATERIAL AND METHODS

During present study, 120 water samples and 1200 fish were sampled from 10 farms (Table 1) from August 2012 to July 2013.

Fish Sampling: Every month, 10 fish, with weight 50-500g or 10-50g and 10 samples of water were randomly been taken from selected farms. Sampled fish had either clinical signs such as darkening, exophthalmia, lethargy and redness in mouth, or no clinical signs. Fish were necropsy and microbial culture was prepared from liver and anterior kidney in Trypticase soy agar (TSA, Merck® Co., Germany). Media incubated in 20-25°C for 48 h [4-6]. Then bacteria were purified and Gram stained [7, 8]. Biochemical tests such as oxidase, catalas, KOH_3%, OF, SIM, MR, VP, growth in MacConkey agar, Lysine, arginine dihydrolase and ornithine decarboxylase production, urea, nitrate, simmon's citrate agar and Glucose, sorbitol, mannitol, manose, salicin, lactose, inositol, gsilos and trehalose were used to differential identification of isolated bacteria [9-11].

Water Sampling: One water sample was prepared in a 250cc bottle and a sterile 70 cc bottle, from each farm, monthly. Then parameters such as Nitrite, Nitrate, Ammonia and Dissolved Oxygen (DO) were measured according to the methods described by Pritzlaff and Liao [12-14]. Also pH, Total Dissolved Salts (TDS) and temperature were measured using HQC (HACH model). Water sample of sterile bottle was used to bacteria colony count (CFU/mL) [9, 15].

RESULTS

Fish: External clinical symptoms such as erratic swimming, lose of equilibrium, lethargy, darkening, exophthalmia, corneal opacity, bleeding around eyes, gills, ventral and anal fin base, mouth redness, abdominal distention and also internal signs such as Splenomegaly, liver enlargement, renal hyperemia, bleeding on the heart and liver surface and end of intestine were observed in fish.Some of symptoms were not common in all diseased fish.

Results of present study showed that 41 out of 175 fries (10-50 g) with clinical signs (23.4%) and 39 out of 307 fries without clinical signs (12.7%) and also 48 out of 235 growing fish (50-500 g) with clinical signs (20.4%) and 42 out of 483 growing fish without clinical signs (8/7%) were suffered from Yersiniosis. Generally, 20% of fish with clinical signs and 10% of fish without clinical signs were suffered from Yersiniosis.

In fact *Yersinia ruckeri* had not been isolated from 76.6% of fries with clinical signs.

Water: Results of measured Physical and chemical parameters and Bacteria total count are given in Tables 2 and 3.

Table 1: The selected farms and their geographical conditions.

The Farm	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Altitude (m)	1868	1868	1703	1703	1608	1686	1686	1657	1598	1457
Longitude	52°12 ´35 ´ ´	52°13 ´57 ´ ´	52°18 ´61 ´ ´	52°17 ´57 ´ ´	52°21´ 27 ´ ´	52°16 ´58 ´ ´	52°18 ´33 ´ ´	52°11 ´45 ´ ´	52°21 ´36 ´ ´	52°21 ´58 ´ ´
Latitude	35°56 ´22 ´ ´	35°46 ´07 ´ ´	35°37 ´14 ´ ´	35°47 ´44 ´ ´	35°49 ´54 ´ ´	35°57 ´49 ´ ´	35°57 ´62 ´ ´	35°53 ´55 ´ ´	35°50 ´18 ´ ´	35°53 ´35 ´ ´

F1, Vasar; F2, GhezelSarab; F3, GhezKaj; F4, RanginVana; F5, NeginHaraz; F6, Chand manzure Niyak; F7, Ghez Niyak; F8, Harazyek; F9, NelGhezel; F10, Ghezelalayeh Hraz.

Table 2: Physical and chemical parameters of water from 10 farms during one year

Farm	Nitrite (mg/l)	Nitrate (mg/l)	NH_4^+(mg/l)	TDS (g/l)	DO (mg/l)	Temperature (°C)	pH
1	0.0073±0.0093	0.6808±0.2501	0.1179±0.2140	0.1925±0.0501	9.03±0.76	10.63±2.36	8.30±0.30
2	0.0077±0.0080	0.7174±0.2859	0.0556±0.0374	0.2017±0.0498	8.92±0.56	10.85±1.93	8.43±0.20
3	0.0115±0.0218	0.8343±0.3551	0.0938±0.0654	0.2150±0.0638	9.32±0.51	11.08±2.71	8.42±0.10
4	0.0112±0.0198	0.7976±0.1958	0.1090±0.0969	0.2133±0.0475	9.47±0.60	10.22±3.08	8.19±0.62
5	0.0111±0.0109	0.7818±0.3433	0.1126±0.0874	0.2208±0.0337	9.24±0.54	10.94±2.84	8.33±0.45
6	0.0109±0.0136	0.7240±0.2506	0.0829±0.0442	0.2083±0.0320	9.30±0.55	10.38±3.24	8.37±0.47
7	0.0109±0.0267	0.7240±0.3853	0.0829±0.1040	0.2083±0.0294	9.30±0.62	10.38±3.16	8.37±0.34
8	0.0165±0.0199	0.8022±0.2839	0.0913±0.0510	0.2217±0.0378	9.33±0.74	10.63±3.24	8.54±0.61
9	0.0170±0.0290	0.9265±0.5420	0.2131±0.2605	0.2250±0.0437	9.29±0.64	10.51±3.05	8.54±0.20
10	0.0198±0.0260	0.9155±0.4655	0.1220±0.1050	0.2367±0.0326	9.47±0.89	10.26±3.29	8.49±0.62

Table 3: Bacteria total count of farm's water samples (Total count(CFU/ml) ±StdvTotal count(CFU/ml))

Season				
Farms	Spring(CFU/ml)	Summer(CFU/ml)	Autumn(CFU/ml)	Winter(CFU/ml)
F1	1753±1097	26607±31378	10590±13966	720±14
F2	6680±5098	28100±31301	60473±85963	433±267
F3	1880±1698	25200±28237	6500±3668	5667±2853
F4	797±262	19993±23173	5887±3905	897±75
F5	3033±671	33500±40730	50600±57602	3033±671
F6	2900±1744	20633±24872	36783±41018	1500±220
F7	900±381	15500±15160	70307±100467	1037±261
F8	7167±9230	39533±50736	46433±60158	1040±619
F9	8810±10944	33533±42804	53763±76405	1500±220
F10	1157±396	23600±27029	36700±45615	2600±724

Results showed that the amount of nitrite, nitrate, NH_4^+, TDS and DO (Except farm 2) were in limit range for rainbow trout farm water but pH and temperature were more than and less than limit range respectively, therefore pH increase and temperature decrease had significant relationship with Yersiniosis outbreak ($p<0.05$) (Table 2).

Results showed that there are significant relationship between Yersiniosis increase and maximum of bacteria total count in summer (Farms 1-4) and autumn (Farms 5- 10) ($P<0.05$) (Table 3).

DISCUSSION

Fries with clinical signs which are not infected with bacteria show that there are other reasons for clinical signs (such as improper feeding, Imbalanced diet, vitamin and mineral deficiency, or inappropriate environmental condition). In addition fish apparently healthy (Without clinical signs) were carrier and therefore *Y. ruckeri* were isolated from them.

Yersiniosis outbreak is more common in rainbow trout with length of 7.5 cm and it's less sever in larger one (12.5 cm) and often is chronic [4, 15]. In the present study, *Y. ruckeri* were isolated from fries and also growing fish but in fries were more than growing fish. Our finding is in agreement with Austin and Austin (1993) statements. They believed smaller fish are more sensitive to pathogens and mortality is more in them. Some of the fries carry the pathogen and chronically suffer from Yersiniosis, Hence they are apparently healthy but bacteria are isolated from them [4]. Behroozi and Soltani (2003) demonstrated that some fish which survive after being infected with Yersiniosis will be carrier of Yersiniosis without clinical signs and can be transport disease to other parts [17]. Meier (1986) reported outbreak of Yersiniosis in 8-12 cm rainbow trout, he also stated Yersiniosis affected larger fish (24-30 cm) after 5-7 days [18]. Her finding was in agreement with our finding. Results of present study showed that the amount of nitrite, nitrate, NH_4^+, TDS and DO (Except farm 2) were in limit range for rainbow trout farm water but pH and temperature were more than and less than limit range respectively, therefore pH increase and temperature decrease had the highest effect on Yersiniosis outbreak. Also at higher pH, Ammonia is in un-ionized form (NH_3).

NH_3 has greater toxicity, so, percent of NH_3 can be another effective reasons for outbreak of Yersiniosis. Zorriehzahra *et al*. (2012) showed that environmental parameters that affected on Yesiniosis in rainbow trout are included: low water quality i.e.; pH=5, high temperature (18-20°C), excess ammonia (NH_3=0.05 mg/L), dissolved oxygen (O_2=4-5 mg/L), marginal carbon dioxide

(CO_2=8 mg/L) and H_2S gas bubbling [19]. The fish were also subjected to low quality feed, overcrowding, handling stress and high aquatic plant growth. Although they reported lesser pH and higher temperature, generally their finding was in agreement with our findings. Also the reason of the first Yersiniosis in rainbow trout in Romania was water temperature increase in spring [20]. In addition, Hunter *et al*. (1980), reported that stress due to temperature raise, was effective in outbreak of Yersiniosis in *Salmo gairdneri* [21]. Although, Austin and Austin (2003) stated the peak incidence of Yersiniosis is at temperature 15-18°C and it decrease at temperature under 10°C, also our finding showed that Yersiniosis occur in water with low temperature (10-11°C) [4], some researcher reported it on higher temperature [19]. Therefore, it seems fish in waters with no optimum temperature, is under stress and pathogen can affect on fish. In fact, not only high temperature but also low temperature can affect of disease incidence. Rodgers (2006) statements confirm our finding about temperature. He reported there is direct relationship between ERM incidence and increase and decrease of temperature, density increase and low quality of water [22]. In addition, Zorriehzahra *et al*. (2012), expressed that the highest risk of Yersiniosis incidence is in 8-16°C. they also stated that it can be occur at 4°C but in higher temperature the rate of mortality is higher [19]. According to above evidence, peak of Yersiniosis is in temperature 15-18°C [4] but it can be also occur in lower temperature. Therefore, it seems that if the water in the farm has higher temperature, occurrence in samples is more by *Yersinia ruckeri* because the bacteria's growth conditions are suitable in higher temperature. Through, EC and follow by it TDS and also pH and DO varies with temperature change, also fish is poikilotherm and its metabolism and growth is dependent on temperature [19], increase or decrease in water temperature can be stressful and cause diseases.

In high pH, NH_3 (Un-ionized form of ammonia) is more than other ammonia forms. Therefore, in present study, high pH in all farms in most of the seasons is another effective reason for Yersiniosis incidence. Stress causes disease in fish, so that, Hunter *et al*. (1980), showed that if fish carrier *Y. ruckeri* don't be in stress condition, they won't transfer disease to other fish [21]. On summery, changes in fish environment condition such as high density and low water quality will cause stress and finally Yersiniosis [15].

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