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Efficacy of a Whole Cell *Lactococcus garvieae* Vaccine in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: In this research, a whole cell bacterin vaccine was prepared from *Lactococcus garvieae* isolated from rainbow trout farms in Turkey after an epizootic outbreak. The efficacy of vaccine on lactococcosis was tested in rainbow trout (*Oncorhynchus myciss*). In 25, 76 and 125 days following i.p. injection vaccination, the groups of vaccinates and non vaccinates were challenged by i.p. injection with 0.1 mL of the *Lactococcus garvieae* strain at concentration of approximately 10^6 cells mL^{-1} and monitored daily for clinical signs and mortality for 21 days. Dead fish were examined to confirm the reisolation of the inoculated strain from the internal organs. Calculated RPS values for day 25, 76 and 125 were found 83.3, 75 and 68%, respectively.

Key words: *Lactococcus garvieae*, lactococcosis, rainbow trout, vaccination, reisolation, Turkey

INTRODUCTION

Lactococcosis is a kind of Streptococcosis caused by *Lactococcus garvieae* (*L. garvieae*) which has been particulary devastating the freshwater culture of salmonid. The causative agent, *L. garvieae* was first described from an investigation of bovine mastitis in Great Britain. *L. garvieae* was reported to cause osteomyelitis and possible secondary endocarditis in middle aged women (Collins *et al*., 1983; Vendrell *et al*., 2006). Outbreaks affecting rainbow trout have been reported in several countries such as Australia, South Africa, Japan, Taiwan, England and countries of the Mediterranean area. Losses can exceed approximately 50-80% of the total production (Ghittino and Prearo, 1992).

The clinical manifestations of this agent in trout are characterized by uni or bilateral exophthalmia with haemorrhage in the periocular area, in the opercula, in the buccal area, at the base of the fins on the surface, darkening of the skin and distended abdomen. Internally, the peritoneal cavity can also present haemorrhage and purulent exudates (Cagirgan and Tanrikul, 1995; Austin and Austin, 2007; Avci *et al*., 2010).

In recent years lactococcosis caused by *L. garvieae* has been the principal bacterial disease affecting rainbow trout (*Oncorhynchus mykiss*) in Turkey especially during the fattening phases. *L. garvieae* is particularly virulent and can lead to mortality rates of 30-50% at the end of the productive cycle thus causing serious economic damage. Since, antibiotic treatment has become less and less efficacious (Khorvash *et al*., 2008; Najiah *et al*., 2010; Raissy and Ansary, 2011), vaccination seems the best approach to control the disease now. Vaccines to protect rainbow trout from lactococcosis have been developed and good protections levels are achieved only with intraperitoneal (i.p.) vaccines (Bercovier *et al*., 1997; Romalde *et al*., 2005; Vendrell *et al*., 2007). The objective of the research was to prepare and test a killed *L. garvieae* vaccine for its efficacy in rainbow trout (*Oncorhynchus mykiss*) against lactococcosis.

MATERIALS AND METHODS

Bacterial strain: *L. garvieae* strain was isolated from a naturally outbreak that occurred in a rainbow trout farm in the Southwest of Turkey. They were identified as *L. garvieae* by API 20 Strep (BioMerieux S.A., France) (Cagirgan, 2004; Tanrikul and Gultepe, 2011). Slide agglutination test was performed on all isolated strains using raised rabbit serum against *L. garvieae* NCDO (ATCC 43921). The strain was routinely grown onto blood agar plates at 25°C for 24-48 h. Stock cultures were maintained frozen at 20°C in Tryptone Soy Broth (TSB) (Merc) with 15% glycerol. The bacterial strain was cultured without shaking it in TSB at 25°C during 24 h for experimental infection. Serial dilutions were prepared in Phosphate Buffered Saline (PBS) and viable counts determined by plating it on Tryptic Soy Agar (TSA; Merc) medium.

Experimental animals: Rainbow trout, average weight 40±5 g were obtained from a commercial fish farm of the Bagci Balik Company, Turkey. Its health status was examined immediately using conventional microbiological

techniques upon arrival in the tank. The trout were distributed in 2000 L tanks in fresh water at 16°C with a 25% water exchange every day and continuous aeration. They were stocked at a density of 50 fish per tank. Fish fed on a commercial pelled diet (2% body weight day^{-1}).

Vaccine preparation: The *L. garvieae* antigen consisted of a 24 h culture of *L. garvieae* grown initially on 5% sheep's Blood Agar (BA) and then inoculated into Tripticase Soy Broth (TSB; Difco) for 24 h on a shaker at 25°C. Bacteria were grown to a density of approximately 10^{10} cells mL^{-1}. The cells were killed by addition of formalin to achieve a final concentration of 0.7% and preserved at 4°C overnight. Sterility of bacterin was tested by inoculating it to blood agar and observing the presence or absence of growth.

Challenge tests: The vaccine was administrated by i.p. injection (0.1 mL^{-1} fish; N = 50/vaccine). About 4 weeks after the vaccination, fish were infected by i.p. injection with 0.1 mL of the *L. garvieae* strain at concentration of approximately 10^6 cells mL^{-1} 25, 76 and 125 days after vaccination. Control fish (N = 50) were injected with sterile PBS. Challenged fish were kept at 16±1°C for 21 days. Mortalities were monitored daily and dead fish examined to confirm the reisolation of the inoculated strain from the internal organs. In addition, cumulative mortality was recorded and the vaccine efficacy calculated on the day of the trial by Relative Percent Survival (RPS) (Amend, 1981).

$$RPS = \left(\frac{\text{1-Percentage mortality in vaccinated fish}}{\text{Percentage mortality in control group}}\right)\times 100$$

Statistical analysis: In all the experiments, significance of differences in the mortalities observed among fish groups were assessed by χ^2-test (Sumbuloglu and Sumbuloglu, 1993)

RESULTS AND DISCUSSION

Some control fish died 1 day after challenge without behavioral or pathological signs. The remaining non vaccinates showed behavioral and pathological signs of *L. garvieae* infection appearing on day 4-5 post challenge. Fish presented the typical signs of disease with a rapid anorexia, uni or bilateral exophthalmia, melanosis, abdominal distension, anal prolapses and haemorrhaging at the base of fish. Non vaccinates mortality continued to 15 days post challenge. In the vaccinates, death occurred only between 3 and 12 days after challenge.

These behavioral and pathological signs were lethargy, melanosis and uni or bilateral exophthalmia. Vaccinated fish did not showed haemorrhaging sings. Kidneys from dead fish of the groups of vaccinates and non vaccinates were cultured to find out whether *L. garvieae* existed or not. Bacteria was isolated from infected fish identified (Table 1). The initial weight were 40±5 g for the vaccinated group and control group and the weights at the end of the experiment were 250 and 220 g, respectively. The cumulative mortality in a 25 days challenge of the vaccinated and control fish was 10 and 60%, respectively (Table 2). The RPS was 83.3% with an RPS value >60% being considered acceptable (Amend, 1981). Mortality was 14% in the second challenge vaccinated group and 16% in the third vaccinated group (RPS of 75 and 68%, respectively) (Table 3). In all the cases the difference in the mortalities among vaccinated groups and unvaccinated fish were significant ($p<0.001$).

Table 1: The API Strep profile for *L. garvieae* isolated from fish farm

Properties	Results
VP	+
Hippurate	-
Aesculin	+
Pyrrolidonylarylamidase	+
α-Galactosidase	-
β-Glukoronidase	-
β-Galactosidase	-
Alkaline phosphatase	-
Leucine arylamidase	+
ADH	+
Ribose	+
Arabinose	+
Mannitol	+
Sorbitol	-
Lactose	-
Trehalose	+
Inulin	-
Raffinose	-
Amygdalin	-
Glycogen	-
β-Hemolysis	-

+ = Positive; - = Negative

Table 2: Results of experimental infections in control and vaccinated fish after immunization with vaccine

Challenge	Control (died fish/N)	Mortality (%)	Vaccinated fish (died fish/N)	Mortality (%)
1st (25 days)	30/50	60	5/50	10
2nd (76 days)	28/50	56	7/50	14
3rd (125 days)	25/50	50	8/50	16

Table 3: Duration of protection and effectiveness of vaccine

Days of challenge after vaccination	Challenge dose (cfu mL^{-1})	Mortality (%)	RPS
1st challenge (25 days)			
Vaccinated fish	2.3×10^6	10	-
Control	2.3×10^6	60	83.30
2nd challenge (76 days)			
Vaccinated fish	2.1×10^6	14	-
Control	2.1×10^6	56	75.00
3rd challenge (125 days)			
Vaccinated fish	2.5×10^6	16	-
Control	2.5×10^6	50	68.00

Lactococcosis is one of the most important bacterial fish diseases in terms of economics of trout industry in Turkey. *L. garvieae* causes mortality of 60% in aquaculture farms in Summer months (Cagirgan and Tanrikul, 1995; Cagirgan, 2004). Becoming resistant to active chemotherapeutics very quickly, it can not be under control via treatment. The best procedure is vaccination to protect fish from the disease as is across all Mediterranean countries. To prevent rainbow trout lactococcosis caused by *L. garvieae* different vaccine formulations have been administered by intraperitoneal injection showing good initial levels of protection but very short duration of immunity. About 3 months after vaccination the protection level fell to RPS values of 40% (Bercovier *et al*., 1997). Therefore, a variety of adjuvants are added to vaccinations or booster vaccine preferred in order to increase duration of immunity.

Romalde *et al*. (2004) studied the efficacy of oral immunization using alginate-microparticles for a booster vaccination in rainbow trout to prevent lactococcosis. In fish that received an initial i.p. vaccination with equeous bacterin and an oral alginate-encapsulated booster vaccine 90 days later, protection reached 87% RPS 30 days after revaccination. Kubilay *et al*. (2008) reported that they acquired 100% RPS in 30th day, 100% RPS in 75th day and 84.84% RPS in 125th day following the i.p. vaccination made by bacterin+Freund's incomplete adjuvant vaccine.

They also found that i.p. vaccination performed with non-adjuvant vaccine showed 88.89, 76.67 and 54.55% RPS in 30th, 75th and 125th days, respectively. Bercovier *et al*. (1997) observed in the study with non-adjuvant vaccine that a better level of immunity of 54.55% RPS was obtained 4 months after the vaccination. Ravelo *et al*. (2006) found that application of non-adjuvant vaccine resulted in immunity degrees of 94 and 40% RPS in the 1st and 2nd months, respectively.

The study concluded a better level of immunity with a 70% RPS (125 days) following the i.p. vaccination with toxoid whole cell vaccine. In studies with *L. garvieae* vaccinations various adjuvants were added to vaccine formulations to lengthen level of immunity. Kubilay *et al*. (2008) added β-glukan to vaccine as an adjuvant finding that although, bacterin+β-glukan formulations acquired levels of immunity of 88.89 and 90% RPS 30 and 75 days after i.p. vaccination, level of RPS decreased to 54.55% in 125th day. RPS values of the vaccine used as an adjuvant were found to be 92 and 83.3% in 3 and 8 months after the vaccination (Ravelo *et al*., 2006) with an increased duration of immunity of 8 months.

CONCLUSION

This study showed that RPS values of 68% in 4 months (125 days) following the vaccination with toxoid whole cell vaccine did not fall below 60%, the least value in the level of immunity. Because hot Summer months can lengthen into early Autumn (3-4 months) in Turkey, vaccination made by non-adjuvant toxoid vaccine can protect fish from lactococcosis during risky periods of time when the disease affect them due to Summer heat. On the other hand, resources and aids should be provided for research into increasing level and duration of immunity by trying a variety of fish vaccine formulations in Turkey.

REFERENCES

Amend, D.F., 1981. Potency testing of fish vaccines. Dev. Biol. Stand., 49: 447-454.

Austin, B. and D.A. Austin, 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. 4th Edn., Praxis Publishing, Chicherster, UK., pp: 15-46.

Avci, H., A. Aydogan, T.T. Tanrikul and S.S. Birincioglu, 2010. Pathological and microbiological investigation in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) naturally infected with *Lactococcus garvieae*. Kafkas Univ. Vet. Fak. Derg., 16: 313-318.

Bercovier, H., C. Ghittino and A. Eldar, 1997. Immunization with Bacterial Antigens: Infections with Streptococci and Related Organism. In: Fish Vaccination, Gudding, R., A. Lillehaug, P.J. Midtlyng and F. Brown (Eds.). Kargel, Basel, Switzerland, pp: 153-160.

Cagirgan, H. and T.T. Tanrikul, 1995. A new problem Enterococcus-like infection in rainbow trout trout (*Oncorhynchus mykiss*) farm in Turkey. J. Bornova Vet. Cont. Res. Inst., 19: 9-19.

Cagirgan, H., 2004. Biotyping of *Lactococcus garvieae* isolated from Turkey. EU J. Fish Aquat. Sci., 21: 267-269.

Collins, M.D., J.A. Farrow, B.A. Phillips and O. Kandler, 1983. *Streptococcus garvieae* sp. nov. and *Streptococcus plantarum* sp. nov. J. Gen. Microbiol., 129: 3427-3431.

Ghittino, C. and M. Prearo, 1992. Report of streptococcosis in rainbow trout (*Oncorhynchus mykiss*) in Italy: Preliminary note. Bull. Italian Soc. Fish Pathol., 8: 4-11.

Khorvash, F., K. Mostafavizadeh, S. Mobasherizadeh, M. Behjati and A.E. Naeini *et al*., 2008. Antimicrobial susceptibility pattern of microorganisms involved in the pathogenesis of Surgical Site Infection (SSI): A 1 year of surveillance. Pak. J. Biol. Sci., 11: 1940-1944.

Kubilay, A., S. Altun, G. Ulukoy, S. Ekici and O. Diler, 2008. Immunization of rainbow trout (*Oncorhynchus mykiss*) against *Lactococcus garvieae* using vaccine mixtures. Isr. J. Aquacult., 60: 265-270.

Najiah, M., M. Nadirah, I. Sakri and F. Shaharom-Harrison, 2010. Bacteria associated with wild Mud Crab (*Scylla serrata*) from Setiu Wetland, Malaysia with emphasis on antibiotic resistances. Pak. J. Biol. Sci., 13: 293-297.

Raissy, M. and M. Ansary, 2011. Antibiotic susceptibility of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) in Iran fish farms. Afr. J. Biotechnol., 10: 1473-1476.

Ravelo, C., B. Magarinos, M.C. Herrero, L. Costa, A.E. Toranzo and J.L. Romalde, 2006. Use of adjuvanted vaccines to lengthen the protection against lactococcosis in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 251: 153-158.

Romalde, J.L., A. Luzardo-Alvares, C. Ravelo, A.E. Toranzo and J. Blanco-Mendez, 2004. Oral immunization using alginate microparticles as a useful strategy for booster vaccination against fish lactococcosis. Aquaculture, 236: 119-129.

Romalde, J.L., C. Ravelo, S. Lopez-Romalde, R. Avendano-Herrera, B. Magarinos and A.E. Toranzo, 2005. Vaccination strategies to prevent emerging diseases for Spanish aquaculture. Dev. Biol. (Basel), 121: 85-95.

Sumbuloglu, K. and V. Sumbuloglu, 1993. Bioistatistic. Hatipoglu Publishers, Ankara, Turkey, Pages: 267.

Tanrikul, T.T. and N. Gultepe, 2011. Mix infection in rainbow trout (*Oncorhynchus mykiss* Walbaum): *Lactococcus garvieae* and *Vibrio anguillarum* O1. J. Anim. Vet. Adv., 10: 1019-1023.

Vendrell, D., J.L. Balcazar, I. Ruiz Zarzuela, I. de Blas, O. Girones and J.L. Muzquiz, 2006. *Lactococcus garvieae* in fish: A review. Comp. Immunol. Microbial. Infect. Dis., 29: 177-198.

Vendrell, D., J.L. Balcazar, I. Ruiz Zarzuela, I. de Blas, O. Girones and J.L. Muzquiz, 2007. Safety and efficacy of an inactived vaccine against *Lactococcus garvieae* in rainbow trout (*Oncorhynchus mykiss*). Prev. Vet. Med., 80: 222-229.