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IMMERSIVE VACCINATION OF YOUNG RAINBOW TROUT (*ONCORHYNCHUS MYKISS* - WALBAUM) WITH *YERSINIA RUCKERI* BACTERIN

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For immunoprophylaxis of rainbow trout against yersiniosis, a vaccine has been prepared on the basis of formalin inactivated whole bacterial cells of Yersinia ruckeri. Strains of Y.ruckeri were isolated from parenchymal organs of rainbow trout suffering from yersiniosis.

The method of vaccination against yersiniosis, which is simple to use without stress was described. The vaccination was conducted by immersion in the vaccinal bath for 30 seconds.

The protective effect of the vaccine was tested by artifical infection. We found that immersive vaccination protected vaccinated young rainbow trout during exposure the reference and field pathogen isolates of Y. ruckeri bacteria.

Key words: yersiniosis, vaccine, immersive vaccination, rainbow trout

INTRODUCTION

Trout yersiniosis - enteric redmouth disease is a contagious bacterial disease, which is characterised by hyperaemia and haemorrhages on the skin of the head. The disease is caused by the gram-negative motile bacterium *Yersinia ruckeri*. The first publication about the pathology and isolation of these bacteria from trout was by Ross and Rucker (1966). The bacteria were described and named by Ewing et al. (1978). The disease is of economic significance, especially at certain locations with dense populations of salmonids (McDaniel, 1971, Wobeser 1973).

Although different isolates of the bacteria are phenotypically relatively homogeneous, serological differences exist, with five serotypes currently recognisable through the use of rabbit antisera (O'Leary, 1977, Bullock et al., 1978, Stevenson and Airdrie, 1984). They are designated: type I HL 70 (Hagerman), which is the most frequent and most virulent (Busch 1981), type II BC 74(O'Leary) which is relatively avirulent, type III (Australian) which is avirulent, and serotypes IV and V (Stevenson and Airdrie, 1984, Daly et al., 1986).

The virulence of serovar IV is unknown, while that of serovar V is very low. Serological characteristics of bacteria depend on antigenic structures on the cell surface. The largest surface molecules of gram-negative bacteria are lipopolysaccharides (LPS). Flett and Stevenson (1978) analysed the electrophoretic properties of LPS from representative serotypes I, II, III and IV. Similarities exist tbetween serotypes I, III and V and between serotypes I and II, but the latter were different from strains of serotype V. The LPS serotype II strains were heterogenous, which suggests that the serological nature of this group must depend on cross reaction between other LPS of the antigen. The bacteria occur in several serotypes. Serotype I is highly virulent and vaccine based on this serotype is very protective for other serotypes as well.

Up until now, the disease has been treated with antibiotics and through improved sanitary measures in ponds. The appearance of strains of the bacteria resistant to some antibiotics and limitations in the use of new hemotherapeutics, instigated us to start investigating prophylactic immunization using *Y.ruckeri* bacterin.

Our research considered a field attempt of vaccination against yersiniosis by simple plunging fish in the vaccinal bath. The effects of vaccination were verified by exposing specimens of rainbow trout to artificial infection with pathogenic *Y. ruckeri* bacteria.

MATERIAL AND METHODS

Pathogenic strains of *Y. ruckeri* were isolated from rainbow trout during several attacks of yersiniosis. Parenchymatous organs were seeded on tryptose soya agar (TSA) and blood agar. After incubation at 20°C for 48^h, colonies of bacteria were formed. The colonies were slightly turbid, smooth and round. There was no haemolysis on blood agar. Gram-negative rods with coccoid shape were observed under the microscope.

Using biochemical and serological methods with antihyperimmune sera, prepared from reference strains of *Y. ruckeri*, we detected Hagerman (HI 70) and O'Leary (BC 74) strains.

Preparation of Yersinia ruckeri bacterin: The vaccine was prepared from *Y.ruckeri Hagerman* and *Y.ruckeri O'Leary* isolated during numerous attacks of yersiniosis in Serbia. Strains of *Y.ruckeri* were cultivated in tryptose soya bullion (TSB Torlak) and mixed on a magnetic mixer for better aeration at 20°C for 48h. The microorganisms were simultaneously seeded on tryptose soya agar (TSA) to check their purity. Prior to inactivation of the bacterial culture in formalin, we took 1 ml of mature bullion culture to determine the number of colonies (C.F.U.). The number of colonies was calculated by spreading on plates (in triplet) 1 ml of serial 10-fold dilutions of the cultures in physiological saline on TSA. After 48^h, each culture was inactivated by gradual addition of formalin (final concentration 0,4%) with further incubation at 20°C for 24^h. Sterility was tested by inoculation of TSA and blood plates with a sample of the inactivated culture. Plates seeded in this

way were incubated at 20°C. The plates were examined 24, 48 and 72^{h} after seeding. In this way, vaccine for immersive vaccination was prepared.

Procedure of the immersive vaccination: Prepared vaccine was diluted 1:10 with water from spawning ponds. The water temperature was 10°C. We used young rainbow trout, of average weight about 3 g.. The first group of 1000 young rainbow trouts was immersed in the vaccinal bath for 30 seconds, with continuous aeration. After that, the fish were transferred to the pools. The same number of fish were bathed in pool water without vaccine administration to form a control group.

Pathogenicity testing procedure - biological experiment - challenge infection: Artificial infection was carried out 30 days after vaccination. Vaccinated and sham vaccinated young rainbow trouts were immersed in the solution of *Y.ruckeri* live culture for 60 minutes with continuous aeration. Experimental infective doses of *Y.ruckeri* were prepared from 48^{h}-old cultures, grown on tryptose soya bullion at 20°C. Inoculated cultures for artifical infection were prepared from our isolates. The number of colonies C.F.U. was 30 x 10^{6}. The prepared culture was diluted 1:20 with water from spawning ponds. After infection, fish were observed for two weeks at 10°C. During this time, dead and moribund fish were removed and tested for the presence of *Y.ruckeri*.

RESULTS AND DISCUSSION

Yersiniosis is a disease which mainly attacks young salmonids in the intensive systems of aquaculture, so that vaccination against this disease is one of the most effective methods of control. Different methods of administering vaccine against yersiniosis have been tested: oral, injection, immersive, spray and anal administration.

We prepared a vaccine from formalin inactivated whole bacterial cells of *Y.ruckeri*. Amend et al. (1983) extracted LPS antigen from bacteria using butanol, and they concluded that an immersive vaccine prepared from LPS extract similarly effective as formalin inactivated whole bacterial cells.Immersive vaccination, according to Ellis (1988) is very effective, with a high level of protection for a significant period of time. Although the bacteria occur in several serotypes, serotype I (Hagerman) is highly virulent, and vaccine based on this serotype is very protective for other serotypes as well (Bullock and Anderson 1984).

Two weeks after artificial infection of young rainbow trouts, a large number of control and some of the vaccinated trouts died. After artificial infection with *Y.ruckeri*, the course of disease was very similar to the natural one. Fish started to die 4 days after infection without clinical signs, which appeared 6 days after infection. 50% mortality was achieved 10 days after infection. Mortality stopped after 14 days.

The macroscopic changes in artificially infected young rainbow trouts were characterised by hemorrhages on the mouth, palate, tongue, jugular region, thoracic and abdominal fins. A large number of fish had strong exophthalmus and

bleeding in the eyes. The abdomen was soft. During pressure with the fingers, unsavoury and purulent exudate emerged from anus. In the abdominal cavity, there was some red and serous fluid. The liver was pale, yellow and brown coloured, with bleeding under the capsule, and with shabby consistention. The stomach was enlarged, filled with transparent yellow fluid. Pyloric processes were with stappled bleedings. The spleen was enlarged with circular edges and with shabby consistention. The kidney was enlarged, grey colured with necrotic foci.

In all cases, the presence of *Y. ruckeri* was confirmed on the base of colonial morphology and agglutination with rabbit *Y.ruckeri* antisera. At the end of the two-week observation period, we determined total mortality of the fish.

Table 1. Mortality of vaccinated and control fish after the biological experiment

Treatment	Number of fish	Mortality	
		Number	%
Immersive vaccination	920	240	26.08
Control	930	680	73.12

Sham vaccinated fish had a greater mortality than vaccinated fish (Table 1). Thus the percentage mortality was 73.12% in the control group and 26.08% in the vaccinated group.

Our results show that immersive vaccinated fish had a lower mortality than unvaccinated group.

Comparing the relative level of protection (RLP) between the unvaccinated control and the immersive vaccinated group, we found that immersive vaccination gave a high level of protection (64.33%) as indicated in Table 2.

Table 2. Comparison of relative level of protection (RLP) between the unvaccinated control and the immersive vaccinated group

Treatment method	Number vaccinated	Challenge day	Mortality (%)		RLP
			Vaccinated	Control	
Immersive vaccination	1000	30	26.08	73.12	64.3

Our results agree with the results of Ellis (1988) and Bullock and Anderson (1984). These authors found that immersive vaccination is very effective with a high level of protection. Tebbit et al. (1984) announced detailed information about the efficacy of field experiments with immersive vaccination, by following more

than 22 million vaccinated trouts during a two-year period on three separate fish farms. Their experiments showed that mortality from yersiniosis was reduced by 84%. Vaccinated fish needed 77% less medicaments. Johnson and Amend (1983) compared the efficacy and duration of immunity in trouts which were vaccinated by injections, immersive and spray methods, and they concluded that immersive vaccination insured a high level of protection.

The advantage of immersive vaccination is its relatively simple use (immersion of fish in the vaccine) and minimal stress compared with other methods of vaccination.

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IMERZIONA VAKCINACIJA MLAĐI KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS - WALBAUM*) *YERSINIA RUCKERI* BAKTERINOM

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SADRŽAJ

U svrhu imunoprofilakse mlađi kalifornijske pastrmke protiv jersinioze pripremljena je vakcina od formalinom inaktivisane cele bakterijske ćelije *Yersinie ruckeri*. Sojevi *Y.ruckeri* izolovani su iz parenhimatoznih organa kalifornijske pastrmke obolele od jersinioze, koji su kultivisani na triptoza bujonu.

Opisan je lako primenljiv metod vakcinacije protiv jersinioze koji ne izaziva stres kod riba. Vakcina je aplikovana kupkama u trajanju od 30 sekudi. Posle veštački izazvane infekcije, ispitivan je protektivni efekat primenjene vakcine. Utvrdili smo da imerziona vakcinacija štiti mlađ kalifornijske pastrmke nakon izlaganja patogenom referensu i terenskom izolatu *Yersiniae ruckeri*.