

養殖アユのビブリオ病に対するスプレー法ワクチンの有効性

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著者	伊丹, 利明 楠田, 理一
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Short Paper

Efficacy of a Vaccination by Spray Administration against Vibriosis in Cultured Ayu*¹

Attention has been devoted to the oral immunization studies of cultured fishes in Japan.^{1,2)} Immunizations by oral vaccination and hyperosmotic infiltration have been established and commercialized already in America. The staffs at Dr. FRYER's laboratory are investigating a new delivery system called spray method.*³ In this study, vaccinations by spray method and modified hyperosmotic infiltration (dip method) were applied to protect vibriosis of ayu, *Plecoglossus altivelis*.

The mean body weight and length of adult ayu used in this study were 62.1 g and 180 mm. The strain used was *Vibrio anguillarum* V-36. The bacteria were incubated at 25°C for 96 hours in nutrient broth with shaking. The purity of this culture was checked with BHI agar streak plates and with slide agglutination tests. The bacteria were inactivated by overnight exposure to 0.5% formalin to prepare a bacterine. Bentonite was added to the bacterine at 0.15% and the pH was adjusted to 3.0 by concentrated hydrochloride. The prepared vaccine was administered by a commercial sand-blasting gun for 5–10 seconds to 35-fish in a net (33 by 33 by 30 cm) in such a manner that each fish received a direct application of vaccine on the body surface. The second group of 35-fish was vaccinated by being dipped into the same vaccine for 30 seconds with aeration. The third group of 35-fish was used as a control. Each group was kept at 18°C in a holding tank. Blood from fish was harvested by severing the caudal artery two weeks after vaccination and a pooled serum of 2-fish was collected from 10-fish in each group. Agglutinating antibody titer was determined by micro-titer method. Challenge test was started two weeks after vaccination. Fifteen fish in each group was immersed in a challenge tank at 23°C containing virulent strain of *V. anguillarum* (3.8×10^6 cells/ml) in 0.85% saline. After 20 minutes, these fish were retransferred to the holding tank and observation was maintained for ten days after challenge.

The diet was accepted from the second day after vaccination in dip vaccinated fish and from the first day in

spray vaccinated fish. The geometric mean of agglutinating antibody titer determined two weeks after vaccination in spray vaccinated fish was 1:32, that in dip vaccinated fish was 1:20 and that in control fish was 1:10. The percent mortality caused by vibriosis was shown in Fig. 1. All died fish showed the typical

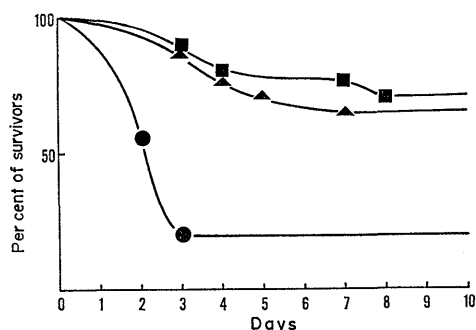


Fig. 1. Changes of survival ratio after challenge to spray vaccinated fish (■—■), dip vaccinated fish (▲—▲) and control fish (●—●).

symptoms of vibriosis. According to our observations on feeding activity of vaccinated fish, dip vaccination gave more serious stress to fish than spray method. To give them less stress, 30 seconds exposure might be shortened and the vaccine might be diluted in dip vaccination. As shown in Fig. 1, the difference in mortality between vaccinated fish and control fish is definite, reflecting the differences in agglutinating antibody titer, but that between two groups of fish vaccinated by the two different methods is not clear. This study suggests the efficacy of other methods than oral vaccination to immunize ayu against vibriosis. Detailed studies on spray vaccination will be reported elsewhere.

Toshiaki ITAMI*² and Riichi KUSUDA*²

Department of Cultural Fisheries, Faculty of Agriculture, Kochi University, Nankoku, Kochi Pref., Japan

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*² 伊丹利明・楠田理一：高知大学農学部.

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