

Results of Field Test on Vaccination Trails against Vibriosis  
of Milkfish (*Chanos chanos*) and Edwardsiellosis of Eel  
(*Anguilla japonica*)

虱目魚紅斑病及鰻魚肝腎病疫苗之田間試驗

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Abstract

The results of field test showed that significant protection against vibriosis and edwardsiellosis was obtained in milkfish (*Chanos chanos*) and Japanese eel (*Anquilla japonica*) vaccinated by single immersion of formalin-killed bacterin. A better protection was obtained in milkfish after vaccine administration. The duration of protective immunity could last for approximately 4-5 months in experimental milkfish and eels.

Introduction

*Vibrio anguillarum* is one of the major pathogens for both marine and freshwater fish. In Taiwan this bacterium causes more than 50% loses of milkfish (*Chanos chanos*) annually in winter season (Huang, 1977; unpublished data). The immunization of *V. anguillarum* has been acknowledged in salmonid (Antipa, 1976; Croy and Amend, 1977; Hayash *et al.*, 1964; Itami *et al.*, 1978) and ayu (Aoki *et al.*, 1982; Kusuda *et al.*, 1978). Using HIVAX *V. anguillarum* bacterin milkfish fingerlings were vaccinated and a gnicant protection was obtained. (Lin, 1983).

The eel culturist in Taiwan should be familiar with the hovee that *Edwardsiella anguillimortifera* (*E. tarda*) can cause on the fish farm. In 1977 Lin reported that edwardsiellosis and red disease initiated approximately 36% of all eel diseases in Taiwan. Total losses of cultured eel from elver to adult was more than 50%. In recent years, the mortality of cultured eels caused by edwardsiellosis become more serious than before. However, due to the presence of R plasmid among *E. tarda*, most chemotherapeutic treatments for the disease become useless (Chen and kou, 1978; Chen *et al.*, 1984). It is therefore, vaccination was considered to be a better tool to prevent the occurrence of edwardsiellosis in eel. Immune reponses of eel against *E. tarda* has been investigated for years, including the potencies of vaccines prepared from *E. tarda* and the methods of vaccination and their effectiveness against *E. tarda* (Song and kou, 1981; Song *et al.*, 1982). The results showed that vaccinated eels produced significantly high antibody titers and possessed protective immunity against *E. tarda*.

Since 1983 we have attempted to make *V. anguillarum* and *E. tarda* vaccines by using formalin-inactivated method. The results on the efficacy of vaccine against edwardsiellosis and vibriosis in the field will be reported in the present paper.

## Materials and Methods

### Bacterin preparation

Biochemically identified *E. tarda* isolated from eel with typical symptom of edwardsiellosis was used for the present study. The bacteria were proliferated in 40 ml kidney infusion broth (KIB) for 24 hrs at 37°C, subcultured in 4-liter KIB and inactivated by addition of formalin to a final concentration of 0.2% formaldehyde. The solution was then kept in room temperature for 72 hours to evaporate formalin and stored in 10°C until experimental uses.

*Vibrio anguillarum* (Serotype I) isolated from diseased milk fish (*Chanos chanos*) by Chang and Kou (1983) and designed as 820108-4L was used for the production of bacterin. Formalin-treated vaccine prepared as described for *E. tarda* was also employed in the present study.

Both *V. anguillarum* and *E. tarda* bacterins were tested for the viability by re-inoculating 0.1 ml solution into 10 ml freshly prepared trypticase soy broth (TSB) and KIB respectively and observed for the occurrence of bacterial proliferation. The sterility of bacterin was demonstrated using liquid thioglycolate medium and incubated at 23°C and 28°C respectively.

### Vaccination of Fish

The field tests for the efficacy of bacterin against edwardsiellosis in Japanese eel (*Anguilla japonica*) and milkfish (*Chanos chanos*) was performed in March to July, 1983 in Ja-Yi and December, 1983 to April, 1984 in Kaohsiung respectively.

A total of 7,500 eel weighing 5-6 gms each and 5,000 milkfish weighing 2-3 gms each were used for the vaccination experiment. Prior to the experiment, ten fold dilution of bacterin containing  $1-2 \times 10^8$  cells per ml was made using pond water. Subsequently, 0.15% bentonite (Difco) was added into bacterin solution and shaken well. A group of 300 fish was then placed in 1 m<sup>3</sup> net and dipped into a plastic tank containing 40-liter bacterin for 3 minutes. The solution could be repeatedly used for ten times. Thus, 40-liter bacterin was used to vaccinate 3,000 fish. Following vaccination, the milkfish and eels were transferred immediately into over-winter pond and cultured pond respectively. During the experimental period, all the dead fish with typical symptoms of edwardsiellosis and vibriosis were recorded. Confirmation of the diseases was also made by isolating of *E. tarda* and *V. anguillarum* from randomly sampled moribund fish. For comparison, 7,000 eels and 5,000 milkfish with similar size as those employed in bacterin immersion experiment were used for control test. The control fish were immersed in pond water with 0.15% bentonite.

### Results and Discussion

The efficacy of *V. anguillarum* bacterin against vibriosis in milkfish is presented in Table I. The result showed that when the vaccinated fish were maintained in over-winter ponds for 4 months, 70% survival rate was obtained. However, the control fish gave only 30% survival. These figures should be resulted from the protective immunity caused by bacterin immersion.

Using HIVAX *V. anguillarum* bacterin, significant protection against vibriosis was also obtained (Lin *et al.*, 1983). It also revealed that the immunity of milkfish immunized with *V. anguillarum* bacterin was still solid after 5 months at 12-38°C. These studies may suggest that the duration of protective immunity conferred by immersion could last at least four months after vaccination. Although vibriosis is the most important disease in milkfish, it only occurs during December and March in Taiwan. High level of protection with four months after vaccination may reflex a potentiality of using bacterin on the prevention of vibriosis in milkfish. Similarly, immersion of ayu (Aoki *et al.*, 1984; Kawano *et al.*, 1984), salmonid (Croy and Amend, 1977; Rohvec *et al.*, 1981; in Johnson *et al.*, 1982 *a, b*) with lyophilized cells or bacterin of *V. anguillarum* also resulted in a high level of protection against vibriosis for more than 3 months' period.

The efficacy of *E. tarda* bacterin administration by immersion for the control of edwardsiellosis in Japanese eel is shown in Table II 10% mortality was observed in the vaccinated eels during the course of experiment. Whereas, the mortality of unvaccinated fish caused by edwardsiellosis was up to 20%. The result may demonstrate that a single exposure to *E. tarda* bacterin could induce an effective protection of cultured eel against edwardsiellosis. The potential of *E. tarda* bacterin was also investigated by Song *et al.*, (1982). The effective protection for eel after 3 minutes immersion was obtained. They also suggested that triple exposures of bacterin would give a better response for cultured eels. We are therefore working on the multiple vaccination including immersion and oral administrations and the results will be published elsewhere.

Table I. Field Test for the Efficacy of *Vibrio anguillarum* Bacterin against Vibriosis in Milkfish (*Chanos chanos*).

Treatment	Experimental Fish	%Survival (Survive No.)
Vaccinated	5,000	70* (3,500)
Unvaccinated	5,000	34* (1,700)

Vaccinated and unvaccinated experiments were performed in different over-winter ponds at a period between December, 1983 and April, 1984.

\*Significant difference was obtained between these two experimental fish ( $P < 0.01$ )

Table II. Field Test for the Efficacy of *Edwardsiella tarda* Bacterin against Edwardsiellosis in Eel (*Anguilla japonica*).

Treatment	Experimental No.	Mortality (%)
Vaccinated	7,500	10*
Unvaccinated	7,000	20*

Vaccinated and unvaccinated experiments were performed in different cultured ponds at a period between March and July, 1983.

\*Significant difference was obtained between these two experimental fish ( $P < 0.01$ )

### 摘 要

本研究結果顯示虱目魚及鰻魚經疫苗處理後對紅斑病及肝腎病有很顯著之保護免疫作用，兩種疫苗比較以虱目魚疫苗有較好之效果。實驗結果同時顯示虱目魚及鰻魚經1次免疫後有4-5個月之保護免疫作用。

### Acknowledgement

This study was supported by a grant from Council of Agriculture in Republic of China.

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