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Vaccination trials against 'red plague' in eels

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ABSTRACT: From killed cultures of *Vibrio anguillarum* and *Aeromonas hydrophila*, 4 vaccines were obtained: a formalin- and an ampicillin-inactivated preparation of each culture. Vaccination of eels *Anguilla anguilla* was performed by intramuscular injection and by immersion. Intramuscular vaccination with the *V. anguillarum* and *A. hydrophila* vaccines prepared by either method of inactivation induced statistically significant protection in eels experimentally challenged with the corresponding pathogen. Using immersion vaccination the *A. hydrophila* vaccines induced a statistically significant degree of protection but the *V. anguillarum* vaccines did not.

INTRODUCTION

Red plague is a disease that frequently occurs in eels farmed in brackish waters. The acute form of the disease is favoured by high water temperatures (Groberg et al. 1978, Cognetti et al. 1980, Groberg et al. 1983). The aetiological agents commonly isolated are *Vibrio anguillarum* and *Aeromonas hydrophila*. They are normally present in brackish water (Cognetti et al. 1980, Ghittino 1985) and when eels are stressed by increasing water temperatures, they can cause a high mortality rate and result in considerable economic loss (Rucker 1959, Cognetti et al. 1980, Ghittino 1985).

Many workers have attempted vaccination against red plague and other fish diseases using vaccines administered by injection, immersion or spraying (Garrison & Gould 1976, Antipa & Amend 1977, Gould 1977, Gould et al. 1978, Antipa et al. 1980, Giorgetti et al. 1981). The present authors have previously isolated both aetiological agents from a natural outbreak of this disease (Celano et al. 1985) and after some encouraging results with vaccination tests performed in the laboratory they now report on vaccination trials conducted on an eel farm (Ittica, Ugento, Lecce, Italy).

MATERIALS AND METHODS

Micro-organisms. Strains of *Vibrio anguillarum* and *Aeromonas hydrophila* used in these experiments were isolated during natural outbreaks of red plague (Celano et al. 1985). They were cultured on Nutrient agar supplemented with 2 % NaCl, even though only *A. hydrophila* subsequently proved to be halo-tolerant. The bacteria were maintained by (weekly) subculture on Nutrient agar which was supplemented with 2 % NaCl in the case of *V. anguillarum*.

Preparation of vaccines. The 2 organisms were cultured at 30 °C for 48 h in aerated Nutrient broth with added 2 % NaCl. Each broth culture was then divided into 2 portions to permit the preparation of the 2 different vaccines.

Formalin-inactivated vaccine: Formalin (final concentration 0.3 %) was added to a broth culture of each organism and then left at ambient temperature (20 °C) for 7 d. After this period, sterility was demonstrated. The broth cultures were centrifuged at 3000 × *g* for 20 min. The whole killed cells obtained were suspended in sterile distilled water and stored at 4 °C until used.

Ampicillin-inactivated vaccine: The second portion of each broth culture was used for the preparation of a vaccine inactivated with ampicillin. Ampicillin (final concentration 0.3 %) was added to each broth culture and the culture left at 30 °C for 5 d, at which time sterility was demonstrated. The cultures were centrifuged at 3000 × *g* for 20 min. The resulting sediment was suspended in sterile distilled water and stored at 4 °C until used.

Experimental animals. For the experiment, 500 eels *Anguilla anguilla* weighing 80 to 90 g each were employed. The eels were kept in 100 l tanks with circulating water, and the water temperature was

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maintained at 20 °C. The eels were placed in the tanks 7 d before the start of the experiment to accustom them to the new conditions, and were not fed during the experiment.

The eels were subdivided into 10 groups of 50 subjects each and treated as follows:

Group 1: Each eel was injected intramuscularly with 0.5 ml of formalin-inactivated vibrio vaccine.

Group 2: The eels were vaccinated by immersion for 5 min in a plastic tank containing 6 l of water and 500 ml of formalin-inactivated vibrio vaccine.

Group 3: Each eel was injected intramuscularly with 0.5 ml of ampicillin-inactivated vibrio vaccine.

Group 4: The eels were vaccinated by immersion for 5 min in a plastic tank containing 6 l of water and 500 ml of ampicillin-inactivated vibrio vaccine.

Groups 6, 7, 8 and 9 received the same treatment as Groups 1 to 4 except that the corresponding *Aeromonas hydrophila* vaccines were substituted for the vibrio vaccines.

Group 5 was used as the unvaccinated control for the groups treated with vibrio vaccines (Groups 1 to 4), and Group 10 was used as the control for the groups (6 to 9) treated with the *Aeromonas hydrophila* vaccines. The control groups (5 and 10) were not sham-treated in any way before challenge.

Twelve d after the vaccinations, all groups were challenged by intramuscular injection with 0.5 ml of the appropriate broth culture (grown for 24 h at 30 °C) as follows: Groups 1 to 5 were challenged with the *Vibrio anguillarum* culture and Groups 6 to 10 were challenged with the *Aeromonas hydrophila* culture. The eels were observed for 30 d post-challenge. Every eel that died during the experiment was examined by culture for the presence of the challenge organism.

Evaluation of results. Statistical evaluation of the results was performed by the chi square test (χ^2) (Cavalli-Sforza 1977).

RESULTS AND DISCUSSION

During the period between the start of the experiment and the challenge, 24 eels died. Necropsy and bacteriological tests on these eels demonstrated that death was not due to red plague.

The results of the experiment are shown in Table 1. Necropsy performed on all of the eels that died after challenge demonstrated typical lesions of red plague and it was possible to reisolate the injected aetiological agent in pure culture.

Intramuscular vaccination performed either with the formalin- or the ampicillin-inactivated vibrio vaccine induced statistically significant protection. The ampicillin-inactivated vaccine gave slightly better protection, but the difference was not statistically significant.

Results with eels treated intramuscularly with the formalin- and ampicillin-inactivated *Aeromonas hydrophila* vaccines also showed a significant degree of protection. Again, the ampicillin-inactivated vaccine gave a slightly (statistically insignificant) better protection.

Table 1 *Anguilla anguilla*. Results of eel vaccination trials. Eels were vaccinated with inactivated whole cell vaccines administered by dipping them for 5 min in the vaccines or by intramuscular injection. Challenge was administered by intramuscular injection. Survival in each vaccinated group was compared with survival in the appropriate unvaccinated control group; + = significant protection

Group no.	Vaccine	Vaccination method	No. of eels immunized and challenged	No. of eels that died on challenge	No. of surviving eels	χ^2	Statistical evaluation
	Vibrio anguillarum						
1.	Formalin-	Injection	48	6	42	58.52	+
2.	inactivated	Immersion	49	41	8	1.37	–
3.	Ampicillin-	Injection	50	2	48	76.28	+
4.	inactivated	Immersion	48	43	5	0.09	–
5.		Unvaccinated control group	46	43	3		
	Aeromonas hydrophila						
6.	Formalin-	Injection	49	8	41	59.83	+
7.	inactivated	Immersion	47	22	25	26.24	+
8.	Ampicillin-	Injection	49	1	48	82.68	+
9.	inactivated	Immersion	41	14	27	36.22	+
10.		Unvaccinated control group	49	47	2		

The immersion-administered vaccines obtained from *Vibrio anguillarum* did not induce significant protection. However, similar vaccines prepared from *Aeromonas hydrophila* induced protection that was statistically significant, even though the protection obtained was lower than that observed with the vaccines administered by intramuscular injection.

We are not able to explain why the immersion-administered vibrio vaccines failed to induce significant protection in the eels. More promising results might have been obtained if the vaccine had been prepared to contain the soluble extracellular products released by *Vibrio anguillarum* during its growth in broth culture. The vaccine tested contained only cell-associated immunogens.

The protection conferred by the more promising vaccines in this study must have been strong because an extremely severe challenge was used to assess vaccine efficacy. With both pathogens, the challenge dose administered was large enough to kill over 90 % of the unvaccinated controls. This level of challenge was more severe than that normally occurring in eel farms.

The results indicate that further studies are needed if immersion vaccines effective against both forms of red plague disease are to be possible. Vaccination by immersion would be preferable to vaccination by intramuscular injection because it is easier to perform, is less traumatic for the eels, and would likely be less costly to administer.

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