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Side effects in sea bass (*Dicentrarchus labrax* L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis

A. Afonso[a,b,c,*], S. Gomes[a,b], J. da Silva[a,b], F. Marques[a], M. Henrique[a]

[a] *IBMC — Institute for Molecular and Cell Biology, Rua do Campo Alegre 823, 4150-180 Porto, Portugal*

[b] *CIIMAR — Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas 177, 4050-123 Porto, Portugal*

[c] *ICBAS — Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Largo do Prof. Abel Salazar 2, 4050 Porto, Portugal*

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Abstract

Sea bass (*Dicentrarchus labrax* L.) were injected intraperitoneally with monovalent (*Photobacterium damselae* subsp. *piscicida* or *Vibrio anguillarum*) and divalent (*Ph. damselae* subsp. *piscicida* and *V. anguillarum*) vaccine formulations, with or without adjuvants (mineral oil, liposome or alginate), to evaluate the short and long-term pathological effects. Eight animals from each group were sampled one, two, four and 11 months after intraperitoneal injection. The acute peritoneal response and the progression to a chronic status were evaluated by analysing peritoneal leucocytes collected during the first days post-injection. To evaluate the chronic response, the late peritoneal leucocyte response was analysed and the peritoneal cavity was examined and the intra-abdominal lesion level scored based on a pre-defined scale. Correlation between leucocyte exudative response, tissue inflammatory response and the development of granuloma were sought. The acute leucocyte response was characterized by an early (24–48 h) mobilization of neutrophils and macrophages, with phagocyte numbers dependent on the formulation, but no significant variations were observed in lymphocytes/small cells and EGCs. Later on, a steady increase occurred in lymphocytes/small cells and EGCs and a high concentration in neutrophils and macrophages was maintained up to 30-60 days in groups i.p. injected with oil adjuvanted formulations with antigen.

* Corresponding author. Rua dos Bragas 177, 4050-123 Porto, Portugal. Tel.: +351 223 401800; fax: +351 226 099157.
E-mail address: aafonso@ibmc.up.pt (A. Afonso).

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All the lesions observed were moderate, indicating that in sea bass, the pathological effects due to intraperitoneally injected vaccines are less severe than in other fish species. The divalent oil adjuvanted vaccine induced the most severe side effects, with macroscopic granulomas consistently present up to 11 months.

Keywords: Sea bass; Fish vaccination; Side effects; Inflammatory response; Peritoneal leucocytes; Cytochemistry; Immunohistochemistry; Electron microscopy

1. Introduction

Sea bass (*Dicentrarchus labrax*, L.) is one of the most used fish species in Mediterranean mariculture [1]. Two of the most pathogenic bacterial species affecting cultured sea bass are *Photobacterium damselae* subsp. *piscicida* [2] and *Vibrio anguillarum* [3], etiologic agents of pasteurellosis and vibriosis, respectively. An important method of controlling bacterial diseases in aquaculture, especially salmonids, is vaccination.

The most efficacious route of vaccination is intraperitoneal (i.p.) injection and the vaccines are usually adjuvanted [4–7]. In particular, the introduction of oil-adjuvanted vaccines has been very effective in the control of furunculosis in salmonids [8]. Although useful in the enhancement of the immune response, adjuvants such as mineral oil induce side effects in salmonids, including decreased growth rates, chronic peritonitis, adhesions, granulomas and pigmentation in the peritoneal cavity [6,7,9–14]. Since the side effects of vaccines can have an economic impact, implementation of safer vaccination strategies is, therefore, of major importance for the progress of vaccine technology [15,16].

As the side effects induced by i.p. vaccination may differ between species, this work aimed to evaluate the pathological effects induced in sea bass by i.p. injection of different vaccine formulations. The structure of vaccine-induced granulomas was also studied in detail, both by light and electron microscopy.

2. Material and methods

2.1. Experimental fish

Non-vaccinated sea bass weighing between 12 and 22 g were purchased from a commercial fish farm. Animals were maintained in a 600 L tank, with recirculating aerated seawater at 18–20 °C. Water quality was maintained with mechanical and biological filtration and fish were fed ad libitum on commercial pelleted feed. A control non-injected group of 16 fish and 12 groups of 80 fish were split into 160 L fibreglass tanks and maintained under the same conditions. The fish were acclimatized in the experimental tanks for at least 2 weeks prior to use. Only healthy fish, indicated by their activity and external appearance, were used in the assays.

2.2. Vaccination

Monovalent vaccines (vibriosis or pasteurellosis) containing 1×10^9 formalin-killed bacteria ml^{-1}, and divalent vaccines (combination of pasteurellosis and vibriosis) with the same final bacterial concentration were used. All vibriosis vaccines contained *Vibrio anguillarum* strains An112 (serotype O1) and An104 (serotype O2), and all pasteurellosis vaccines contained *Photobacterium damselae* subsp. *piscicida*, strain AL 5051. The mentioned experimental vaccines both water-based and formulated with different adjuvants, were supplied by Alpharma, S.A. (Table 1). Adjuvants consisted of mineral oil, liposome or alginate.