Fish and Shellfish Immunology 113 (2021) 86–88

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Oral vaccination of Nile tilapia (*Oreochromis niloticus*) against francisellosis elevates specific antibody titres in serum and mucus

R. Hoare [a,*,1], W. Leigh [a,1], T. Limakom [c], R. Wongwaradechkul [c], M. Metselaar [b], A.P. Shinn [c], T. P.H. Ngo [d], K.D. Thompson [e], A. Adams [a]

[a] Institute of Aquaculture, University of Stirling, Stirling, UK
[b] Benchmark Animal Health, Bush House, Edinburgh Technopole, Edinburgh, UK
[c] Benchmark R&D (Thailand) Ltd., Saensook, Chonburi, 20000, Thailand
[d] Aquacultural Biotechnology Division, Biotechnology Centre of Ho Chi Minh City, Viet Nam
[e] Moredun Research Institute, Pentland Science Park, Penicuik, UK

A B S T R A C T

Although Nile tilapia (*Oreochromis niloticus*) is a well-established aquaculture species globally, there are a limited number of commercial vaccines available or are used for this species. The majority of diseases affecting farmed tilapia are bacterial, with antibiotics frequently used to treat fish. The current study was performed to optimise the use of mucosal vaccines for tilapia by adapting an existing bacterin vaccine against *Francisella noatunensis* subsp. *orientalis* (*Fno*) as a proof of concept. This vaccine has previously provided excellent protection by injection, however, the preference for tilapia farmers would be to vaccinate fish by immersion or orally, due to the lower cost and ease of application. These vaccination routes, however, are often less efficacious probably due to the lack of adjuvants in immersion and oral vaccines. The aims of this study, therefore, were to optimise the formulation and dose of the *Fno* vaccine with mucosal adjuvants for oral and immersion delivery. Tilapia fry (av. 6 g) were given three concentrations (high, medium, low; i.e. 1×10^9, 1×10^8 and 1×10^7 CFU mL-1) of antigen combined with the oral adjuvant by oral gavage, to optimise the dose needed to induce an immune response to *Fno*, and the immune response obtained compared with fish vaccinated by immersion (with and without an immersion adjuvant). Fry were boosted by the same route at 420 degree days (DD), and samples (serum, mucus) taken at 840 DD for specific antibody responses measured by ELISA and western blotting. Specific IgM titres were significantly elevated in serum and mucus of fish given the high dose adjuvanted vaccine by gavage. In addition, by western blotting with serum, a significant immunogenic reaction was evident between 20 and 37 kDa in the fish given the high dose oral vaccine by gavage. As protection against *Fno* provided by the injection vaccine was correlated with specific antibody responses these findings suggest the oral vaccine also has potential to provide protection. Further studies are needed to optimise delivery of the vaccine via feed.

A significant proportion of the diseases affecting farmed Nile tilapia, *Oreochromics niloticus*, are bacterial, with antibiotics frequently used to treat fish. Of the few vaccines available commercially for tilapia, most tend to be administered by injection. Due to the lower cost and ease of their application, mucosal vaccines (immersion/oral) are an ideal route of vaccine delivery for this species. There is, however, a need to develop and optimise mucosal adjuvants to enhance the immunogenicity and length of protection elicited by mucosal vaccines [1–3]. This study was performed to give preliminary data on a novel oral adjuvant (Essai GR01, Seppic Courbevoie, France) by adapting a protective injectable vaccine [4] against a common bacterial pathogen of tilapia, *Francisella noatunensis* subsp. *orientalis* (*Fno*).

Nile tilapia fry were maintained in a recirculation system within the research aquarium facility of Benchmark R&D Ltd., in Thailand. An isolate of *Fno* obtained from a francisellosis outbreak in the UK was used to formulate the vaccine as previously described [4]. Tilapia (5.97 ± 0.19 g) were given an *Fno* antigen/Seppic oral adjuvant (30% antigen:70% adjuvant) by oral gavage. Three concentrations of *Fno* antigen were tested in the vaccine (high, medium, low, i.e., 1×10^9, 1×10^8 and 1×10^7 CFU mL^{-1}, respectively) and the antibody response compared with fish vaccinated by immersion (1×10^8 CFU mL^{-1} inactivated *Fno* with and without an immersion adjuvant; IMS 1312 VG PR, Seppic). Groups of tilapia (n = 30/dose) were sedated using benzocaine (100 mg mL^{-1}, 10%, Sigma-Aldrich Company Ltd., UK) and a 0.05 mL dose of oral vaccine applied into the stomach of the fish using a 20 gauge implantation tubing attached to a 2 mL syringe. The immersion adjuvant (IMS 1312 VG PR) was mixed with the formalin-inactivated vaccine concentrated to 2×10^9 CFU mL-1 (1:1) before diluting with tank water to give a final adjuvant concentration of 5%. Non-adjuvanted vaccine was prepared in same way, using sterile PBS in place of adjuvant. For

* Corresponding author. Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK.
E-mail address: rowena.hoare1@stir.ac.uk (R. Hoare).
[1] these authors contributed equally to this work.

https://doi.org/10.1016/j.fsi.2021.03.019
Received 10 November 2020; Received in revised form 22 March 2021; Accepted 29 March 2021
Available online 4 April 2021

Table 1
Levels of specific anti-*Fno* IgM in serum (diluted 1/500) and mucus (diluted 1/5) of Nile tilapia, *Oreochromis niloticus*, vaccinated orally (gavage) or by immersion, with or without adjuvant (n = 6 fish per group).

Sample	Specific IgM (mean absorbance at 450 nm ± SD)					
	0 dpv	30 dpv				
	Pre-vaccination	Oral vaccination (dose)			Immersion vaccination	
		High	**Medium**	**Low**	**- adjuvant**	**+ adjuvant**
Serum	0.18 ± 0.08	[a]**1.37 ± 0.35**	***0.48 ± 0.26**	0.34 ± 0.13	0.16 ± 0.08	0.16 ± 0.06
Mucus	0.17 ± 0.07	[a]**0.43 ± 0.24**	0.17 ± 0.07	0.18 ± 0.08	0.15 ± 0.06	0.15 ± 0.07

[a] Denotes significant differences between groups at $p < 0.0001$ or at * $p < 0.009$, (n = 6), days post vaccination (dpv).

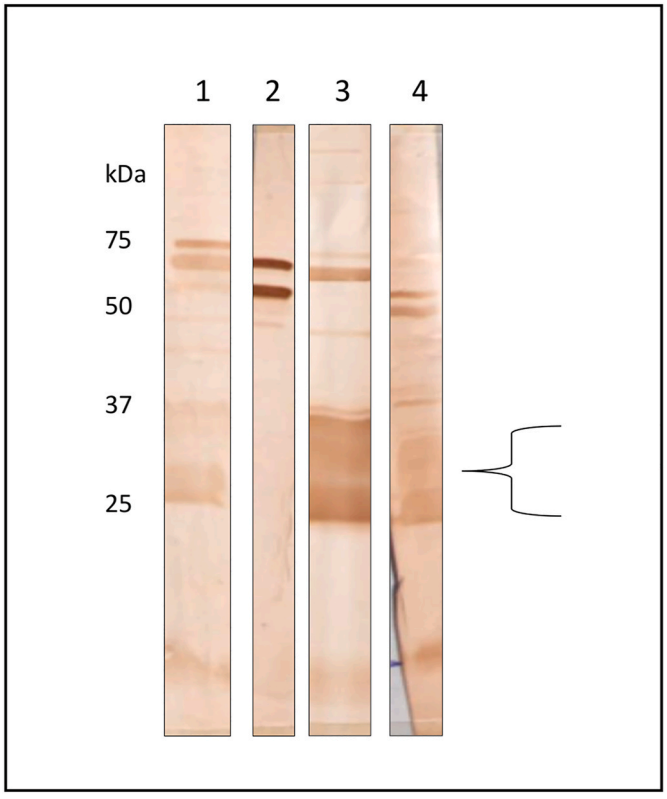

Fig. 1. Western blot of *Francisella noatunensis* subsp. *orientalis* (*Fno*) incubated with serum from Nile tilapia, *Oreochromis niloticus*. Lanes: (1) control; (2) immersion (+adjuvant) vaccinated; (3) oral gavage vaccinated (high dose); (4) positive control (intraperitoneally vaccinated). Brace indicates the strongly immunogenic area 20–37 kDa associated with the pathogenicity island of *Fno*. kDa: Kilo Dalton molecular weight.

immersion vaccination, two groups of fry (n = 30) were removed from the holding tank with a net and placed in the container of vaccine-±adjuvant for 30 s at 28 °C ±1. Fry were maintained at 28 °C ±1 throughout the vaccination period. Tissue samples were taken at day 1 and 2 post-vaccination (pv) for antigen uptake by immunohistochemistry. Fry were booster vaccinated by the respective methods (oral or immersion) as described above at 420 degree DD. Fish were sampled (n = 6) for skin mucus and blood at 0 and 30-days post-vaccination (dpv) (840 DD). An indirect ELISA was used to measure the specific antibody response in serum and mucus as previously described [4]; mucus samples were applied to the ELISA plate at a 1/5 dilution. Western blotting was also performed as described in Ref. [4]. Tilapia given the adjuvanted oral vaccine by gavage containing the highest dose of antigen (1×10^9, CFU mL^{-1}), had significantly higher levels of anti-*Fno* IgM in both serum and mucus than pre-vaccinated control fish at 0 dpv, fish vaccinated orally with the medium or low dose of antigen, and the immersion vaccinated fish (Table 1).

Tissue samples (0, 1, 2 dpv; n = 3) were analysed by immunohistochemistry (IHC) for uptake of the vaccine antigen (*Fno*) according to Ref. [5]. The presence of vaccine antigen was observed in immersion vaccinated fish at 1 dpv in gills, skin, gut and spleen and in orally vaccinated fish it was observed at 2 days post-oral vaccination in the gut and spleen, with the antigen in the spleen only seen in fish given the high dose of oral vaccine (data not shown).

By western blotting with serum, a significant immunogenic reaction was evident between 20 and 37 kDa in the fish given the high dose oral vaccine by gavage (Fig. 1, lane 3). This region was also strongly stained with serum from fish given intraperitoneal (i.p.) vaccination with an oil adjuvanted vaccine against *Fno* (obtained from the study of [4]) and is associated with the pathogenicity island of *Fno* [6] (Fig. 1, Lane 4). Previously, protection observed with the injectable *Fno* vaccine (relative percentage survival of 82%) was linked to specific serum antibodies [4]. Further studies are needed to optimise delivery of the oral vaccine by feeding and to the determine efficacy of the adjuvanted oral vaccine presented here.

CRediT authorship contribution statement

R. Hoare: Funding acquisition, conceived and designed the experiments, immune response investigations, Formal analysis, Writing – original draft, Writing – review & editing. **W. Leigh:** performed all fish trials, Data curation, immune response investigations. **T. Limakom:** performed all fish trials, Data curation. **R. Wongwaradechkul:** performed all fish trials, Data curation. **M. Metselaar:** protocol design, Project administration, Resources. **A.P. Shinn:** protocol design, Project administration, Resources. **T.P.H. Ngo:** Writing – review & editing. **K.D. Thompson:** Writing – review & editing. **A. Adams:** Funding acquisition, conceived and designed the experiments, All authors read and approved the final manuscript.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the Bacterial Vaccines (BactiVac) Network funded by the GCRF Networks in Vaccines Research and Development which was co-funded by the MRC and BBSRC with additional support from The Department of Health and Social Care's Global AMR Innovation Fund (GAMRIF). The authors would also like to acknowledge the support of the BactiVac Network in managing this funding, Benchmark Animal Health for providing the facilities in Thailand and carrying out the fish trials, and Seppic for providing the adjuvants.

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