Short communication

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

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A significant proportion of the diseases affecting farmed Nile tilapia, *Oreochromis 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 franciselllosis 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 × 10⁷, 1 × 10⁶ and 1 × 10⁵ CFU mL⁻¹, respectively) and the antibody response 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.

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Immersion vaccination, two groups of fry (n = 30) were removed from the holding tank with a net and placed in the container of vaccine without adjuvant (n = 6 fish per group). Serum from Nile tilapia, Oreochromis niloticus, vaccinated orally (gavage) or by immersion, with or without adjuvant (n = 6 fish per group).

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific IgM (mean absorbance at 450 nm ± SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 dpv</td>
</tr>
<tr>
<td>Pre-vaccination</td>
<td>Oral vaccination (dose)</td>
</tr>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Serum</td>
<td>0.18 ± 0.08</td>
</tr>
<tr>
<td>Mucus</td>
<td>0.17 ± 0.07</td>
</tr>
</tbody>
</table>

* 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 (þ-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.

Western blot of Francisella noatunensis subsp. orientalis incubated with serum from Nile tilapia, Oreochromis niloticus. Lanes: (1) control; (2) immersion (þ-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.

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 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.

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References


[5] W.A.S. Djainal, K. Shahin, M. Metselaar, A. Adams, A.P. Desbois, Larva of greater wax moth *Galleria mellonella* is a suitable alternative host for the fish pathogen 
