# Immune response of Nile Tilapia (*Oreochromis niloticus*) vaccinated with novel diatom-based oral vaccines against piscine francisellosis

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## Background

- Nile tilapia (*Oreochromis niloticus)*
  - Farmed fish with an estimated market value of $9.8 billion[1]
  - Infectious diseases can result in mortalities and delayed growth, decreasing the profitability
- *Francisella orientalis*
  - Gram-negative facultative intracellular bacteria[2]
  - Highly infectious and environmentally persistent with a mortality rate up to 95% in tilapia[2]
  - Etiologic agent of piscine francisellosis
    - Systemic granulomatous disease[2]
  - No commercial vaccines available or approved antimicrobials for treatment in food fish
  - Oral vaccines are highly desirable
    - Eliminate the need to handle the thousands of individuals
- Diatoms (*Thalassiosira pseudonana)*
  - Algae used as a nutritional supplement for fish
  - Can be modified to express foreign proteins
    - Can be used as a "vaccine-vector"[3,4]
  - Transgenic diatoms conferred a protective immune response against *F. orientalis* when injected[3]

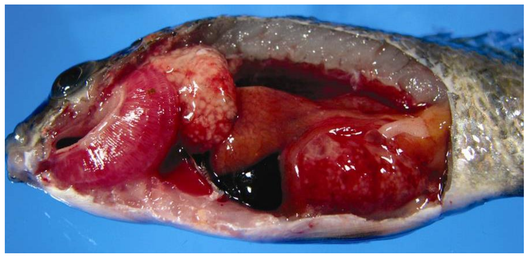

**Fig 1**: Francisellosis in Nile tilapia. Note the severe spleno and renomegaly with multifocal white nodules.

## Objectives

**Hypothesis:** Recombinant eGFP-flagellin-IglC fusion proteins expressed in diatoms confer a protective immune response against *F. orientalis* infection in Nile tilapia fingerlings when administered orally.

- eGFP is fluorescent to aid in cell selection during transformation[3]
- IgLC is an immunodominant *F. orientalis* antigen[3]
- Flagellin is a potent immunostimulant used as an adjuvant[5]

**Objective 1:** Assess the survival rates of orally immunized Nile tilapia by transgenic diatoms and respective controls after challenging with virulent *F. orientalis.*

**Objective 2:** Evaluate markers of cell immunity and bacterial load in orally immunized Nile tilapia following infectious challenges with *F. orientalis*.

## Methods

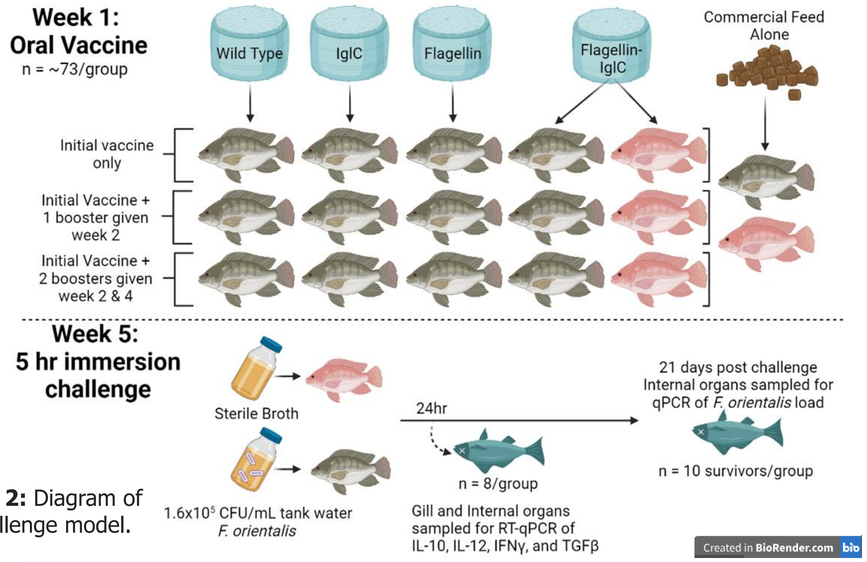


**Fig 2:** Diagram of challenge model.

## Results

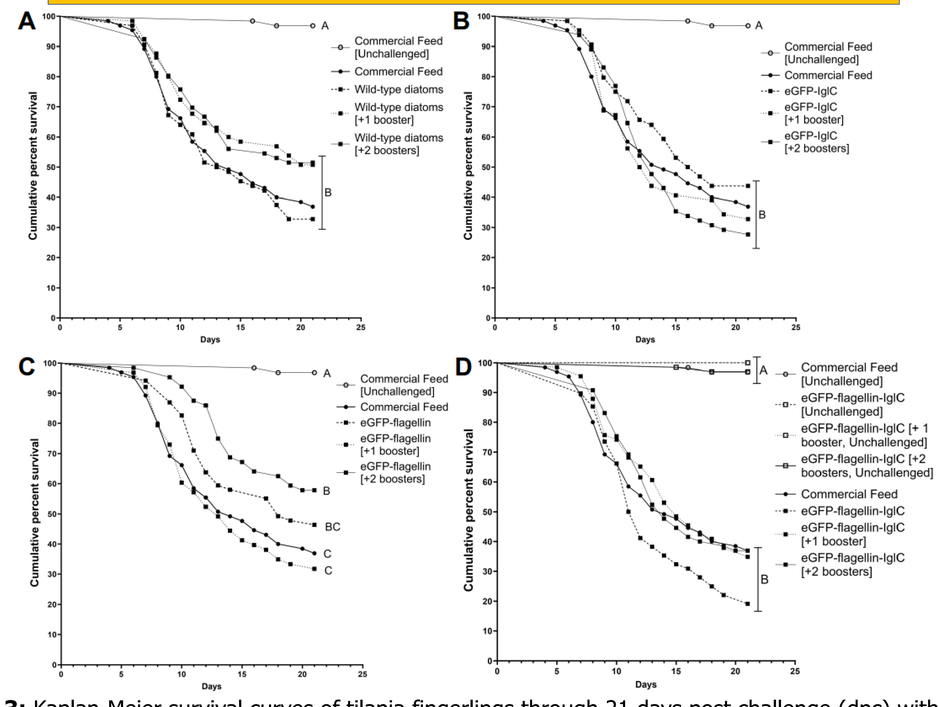


**Fig 3:** Kaplan-Meier survival curves of tilapia fingerlings through 21 days post challenge (dpc) with 1.6x10$^5$ CFU/mL tank water/5hr of *F. orientalis*. Each curve represents the average of 4 parallel tanks holding n=16 fish. Groups that do not share letters are significantly different ($p < 0.05$) as determined by Log-rank (Mantel Cox) test. (A) Wild Type (B) IglC (C) Flagellin (D) Flagellin-IglC

## Results continued

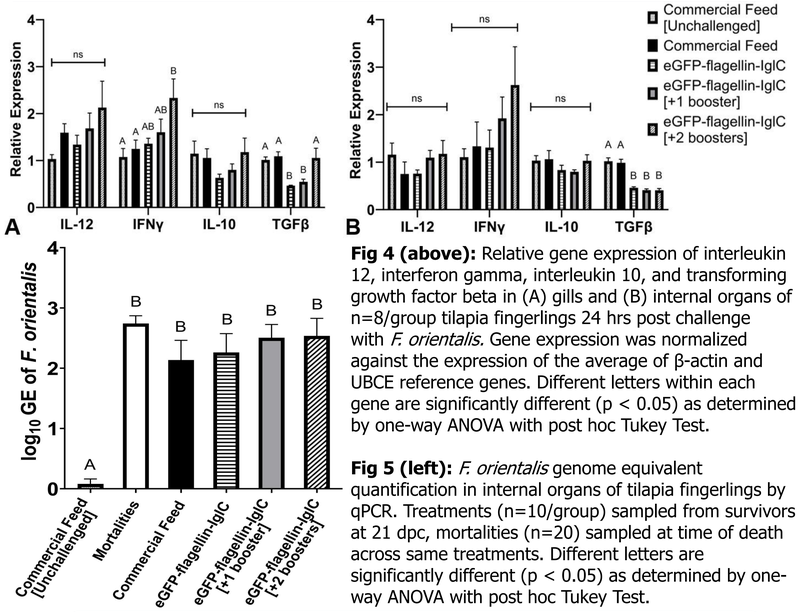


**Fig 4 (above):** Relative gene expression of interleukin 12, interferon gamma, interleukin 10, and transforming growth factor beta in (A) gills and (B) internal organs of n=8/group tilapia fingerlings 24 hrs post challenge with *F. orientalis.* Gene expression was normalized against the expression of the average of β-actin and UBCE reference genes. Different letters within each gene are significantly different ($p < 0.05$) as determined by one-way ANOVA with post hoc Tukey Test.

**Fig 5 (left):** *F. orientalis* genome equivalent quantification in internal organs of tilapia fingerlings by qPCR. Treatments (n=10/group) sampled from survivors at 21 dpc, mortalities (n=20) sampled at time of death across same treatments. Different letters are significantly different ($p < 0.05$) as determined by one-way ANOVA with post hoc Tukey Test.

## Conclusions

- The recombinant eGFP-flagellin-IglC fusion proteins expressed in diatoms are safe to consume by Nile Tilapia fingerlings
- None of the tested transgenic diatoms were effective in reducing mortality or bacterial load against *F. orientalis*
- Transgenic diatoms expressing eGFP-flagellin-IglC fusion proteins were associated with a significant decrease of TGFβ in both gills and internal organs, and increase of IFNγ in the gills

**Although the novel delivery method shows promise, further research is needed to produce an effective oral vaccine against this disease.**

## Acknowledgments

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