

Evaluation of an *Edwardsiella ictaluri* live attenuated vaccine against edwardsiellosis in Nile Tilapia (*Oreochromis niloticus*)



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Introduction

- Edwardsiellosis is a major emerging bacterial disease impacting Nile Tilapia aquaculture (Figure 1)
- Edwardsiellosis is caused by several *Edwardsiella* sp., including *Edwardsiella ictaluri*



Figure 1. Clinical signs and gross changes associated with edwardsiellosis in Nile tilapia. A. Coelomic distention. B. Hemorrhagic enteritis. C and D. Reno- and splenomegaly with multifocal white nodules (granulomas).

Edwardsiellosis management in aquaculture
Extra-labelled use of antimicrobials
Only three options in the USA
No commercial vaccines available

The overall goal of this study is to evaluate if live attenuated mutant candidates of *Edwardsiella ictaluri* are effective at protecting Nile tilapia against edwardsiellosis

Live Attenuate Candidate

- Live attenuated vaccine candidates were developed for *E. ictaluri* by passaging in increasing concentrations of rifamycin media inducing random mutations in the bacterial genome
- Attenuation of the mutants was confirmed using *in-vitro* and *in-vivo* methods (Figure 2)

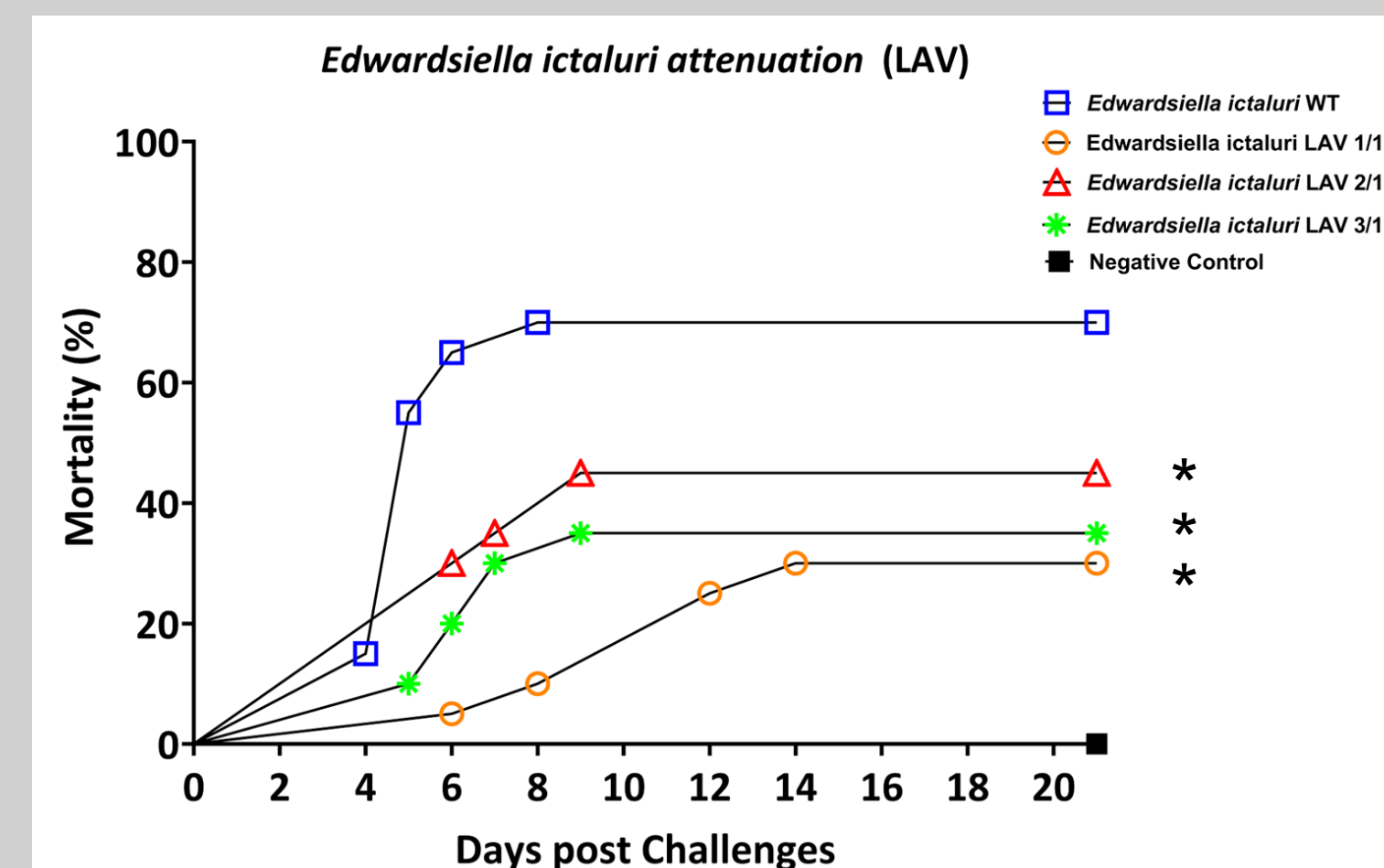


Figure 2. *In-vivo* attenuation in three live attenuated vaccine candidates (LAV) of *E. ictaluri* compared to Wild-type strain (WT). Ten Nile tilapia fingerlings were challenged via intra-coelomic injection to the different strains and mortality evaluated during 3 weeks. * indicates statistical significance ($p < 0.05$) when compared to WT strain using Logrank (Mantel-cox) test.

Protection conferred by LAV 1/1 against wild-type strain

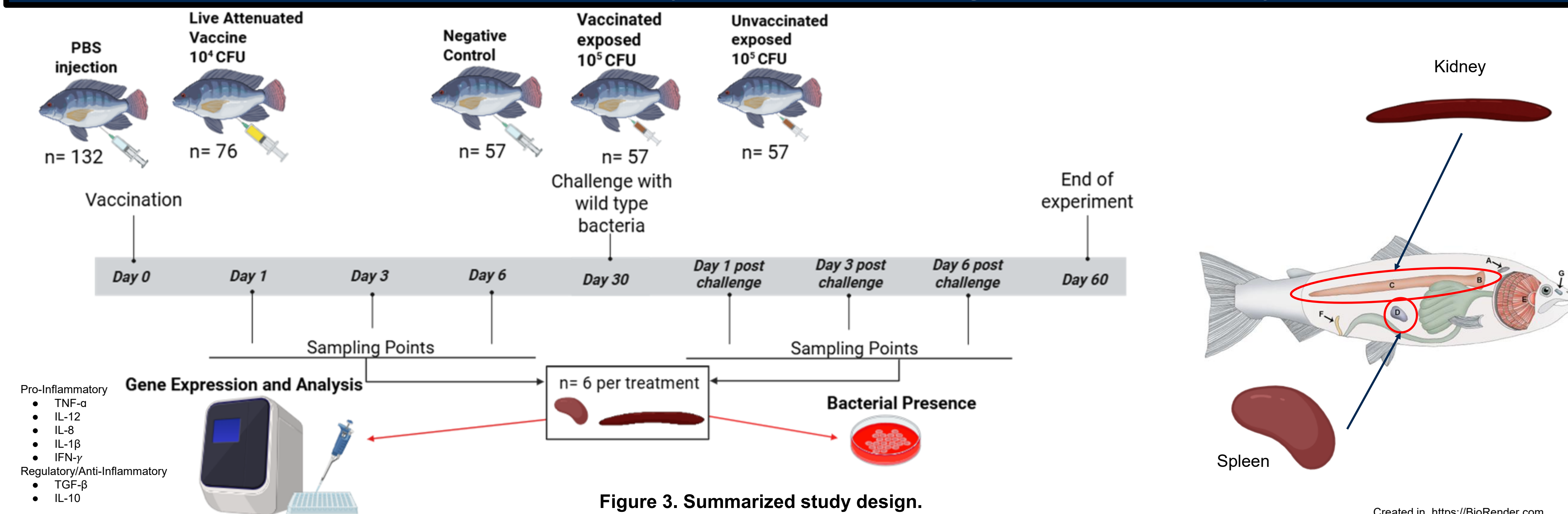


Figure 3. Summarized study design.

Gene Expression of pro-inflammatory and regulatory cytokines

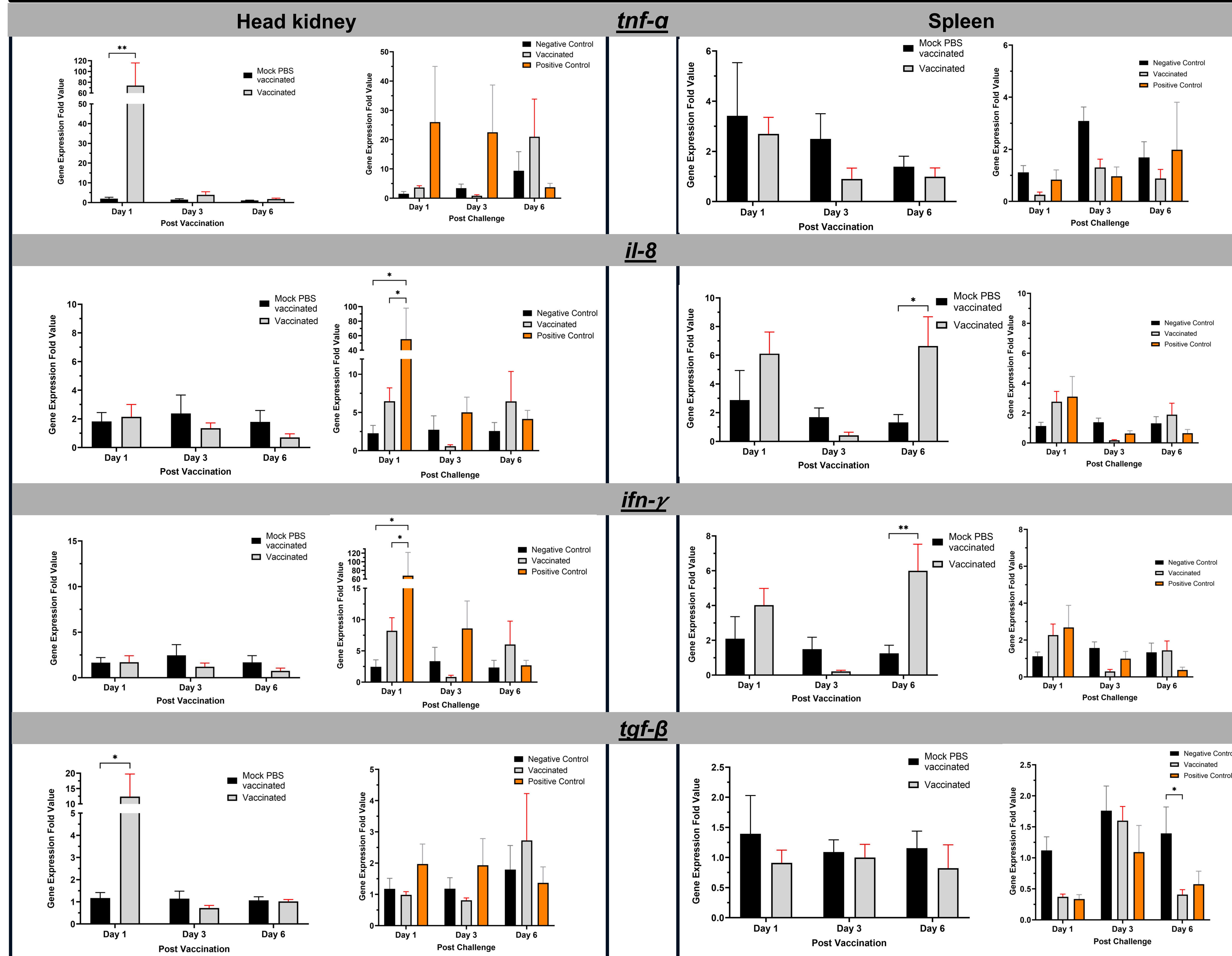


Figure 5. Relative transcript quantification of pro-inflammatory and regulatory cytokines post vaccination or post challenge in primary (head kidney) and secondary (spleen) lymphoid organs in mock vaccinated (PBS) and vaccinated group. *, ** indicate statistical significance at $p < 0.05$ or $p < 0.01$, respectively using 2-way ANOVA.

Vaccination = Reduced Morbidity and Mortality

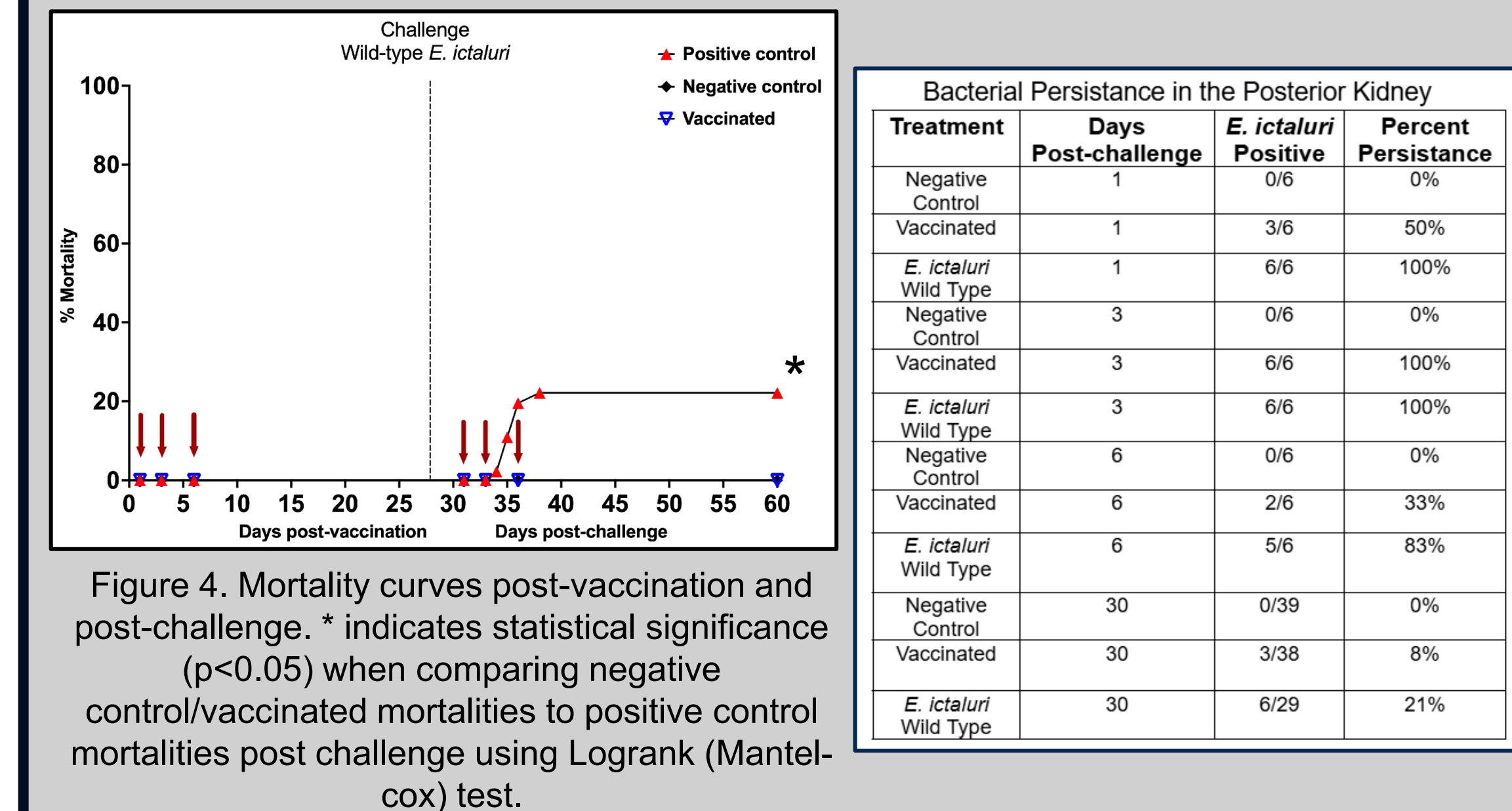


Figure 4. Mortality curves post-vaccination and post-challenge. * indicates statistical significance ($p < 0.05$) when comparing negative control/vaccinated mortalities to positive control mortalities post challenge using Logrank (Mantel-cox) test.

Conclusion

- Attenuation of *Edwardsiella ictaluri* by random mutations in rifamycin media was successful as indicated in in-vivo trials
- LAV 1/1 was successful decreasing morbidity and mortality associated with *E. ictaluri* infections in Nile tilapia.
 - Significantly different survival curve
 - Bacterial persistence in survivors
- Vaccinated fish display higher transcript abundance of pro-inflammatory cytokines following vaccination in spleen and head kidney
- After being exposed to *E. ictaluri* WT, vaccinated fish expressed lower pro-inflammatory cytokines compared to positive controls

The developed LAV candidate represents a novel prophylactic alternative for Nile tilapia producers in piscine edwardsiellosis endemic areas

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