IMMUNE RESPONSES AND PROTECTION OF FRY NILE TILAPIA (*OREOCHROMIS NILOTICUS*) IMMUNIZED BY IMMERSION AND ORAL BIVALENT VACCINES AGAINST *STREPTOCOCCUS AGALACTIAE* AND *AEROMONAS VERONII*

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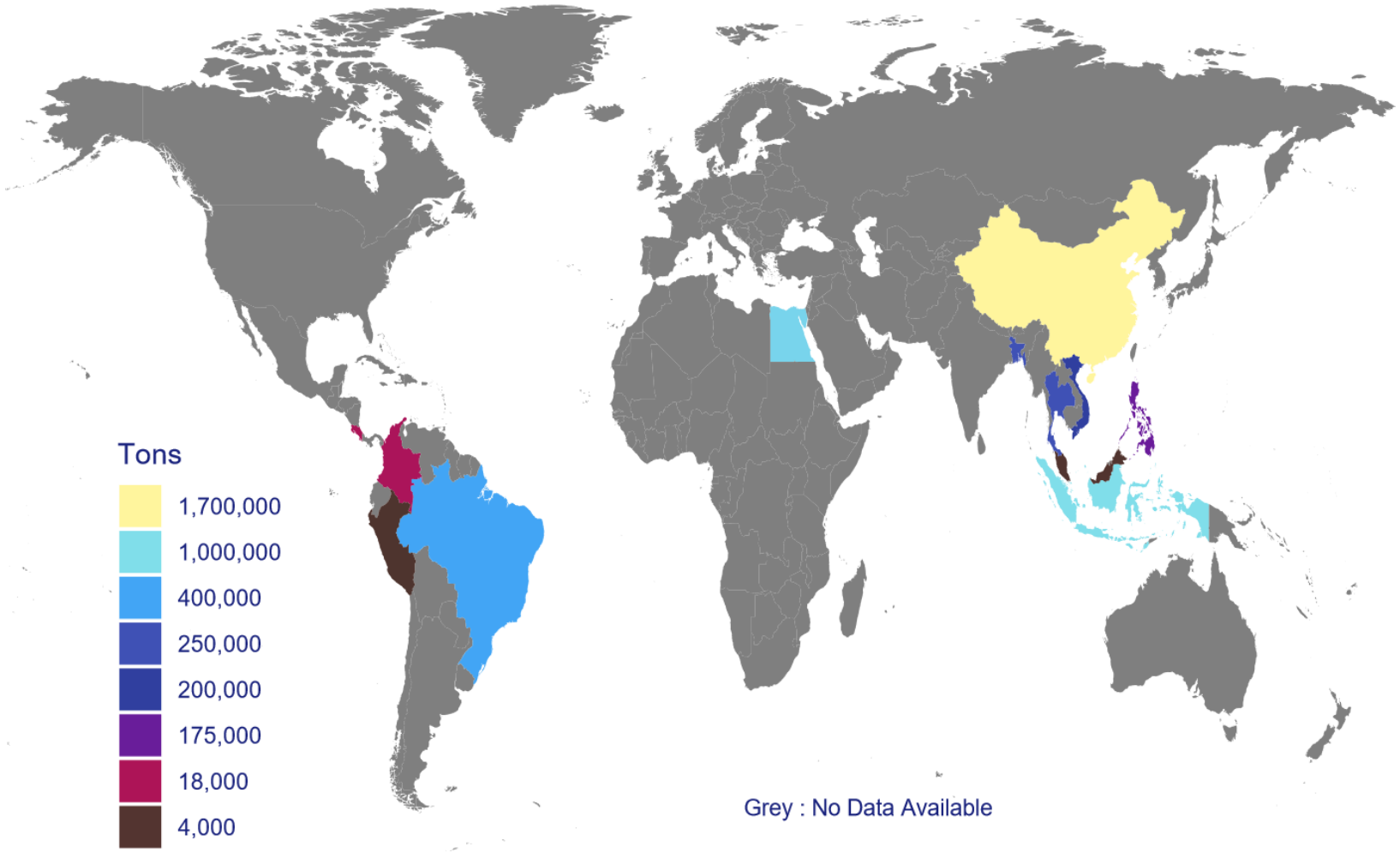
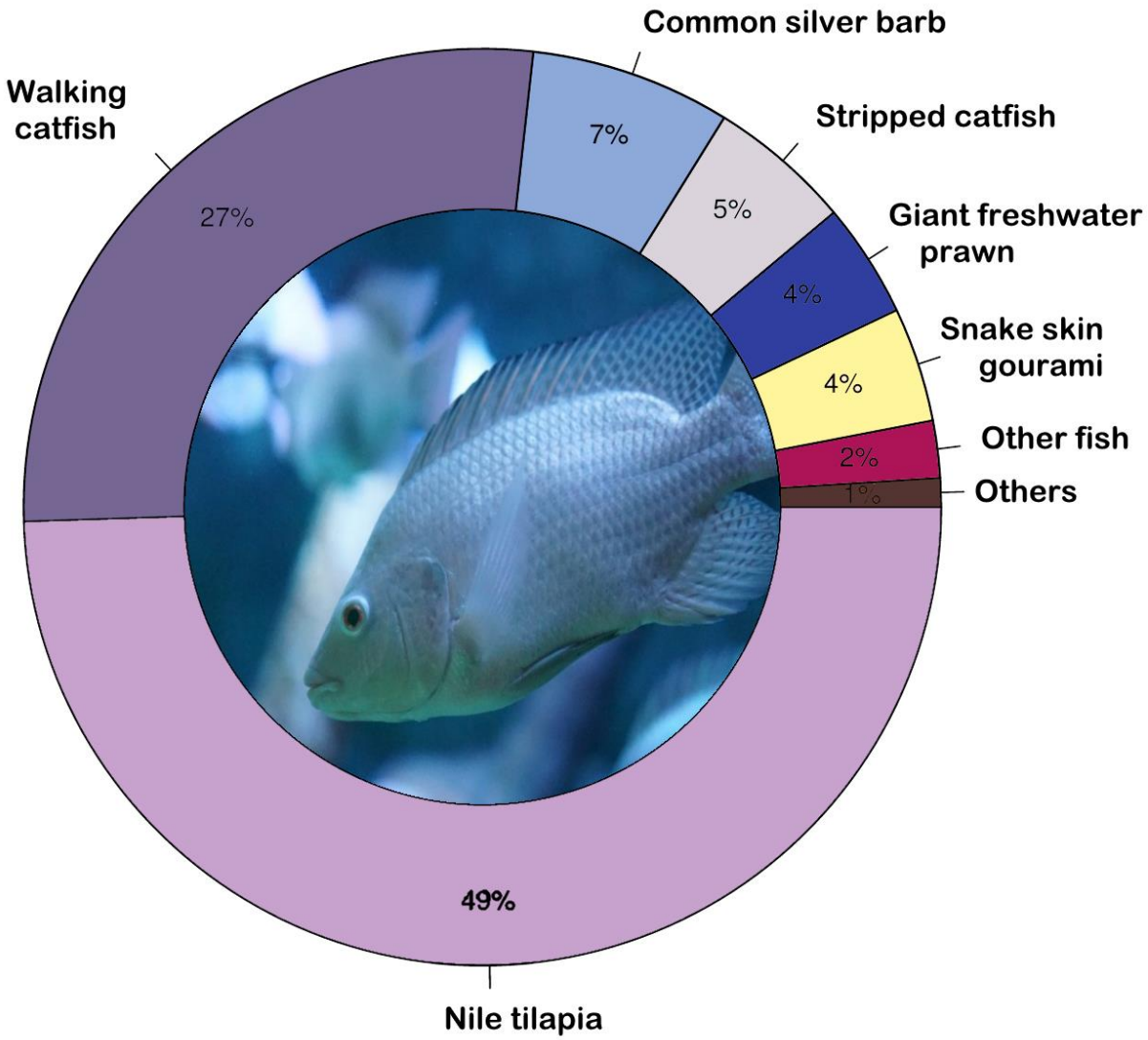
1 INTRODUCTION

Importance of tilapia and diseases, types of vaccines for aquaculture and overview of vaccination strategies.

FARMED TILAPIA PRODUCTION BY COUNTRY (2019)

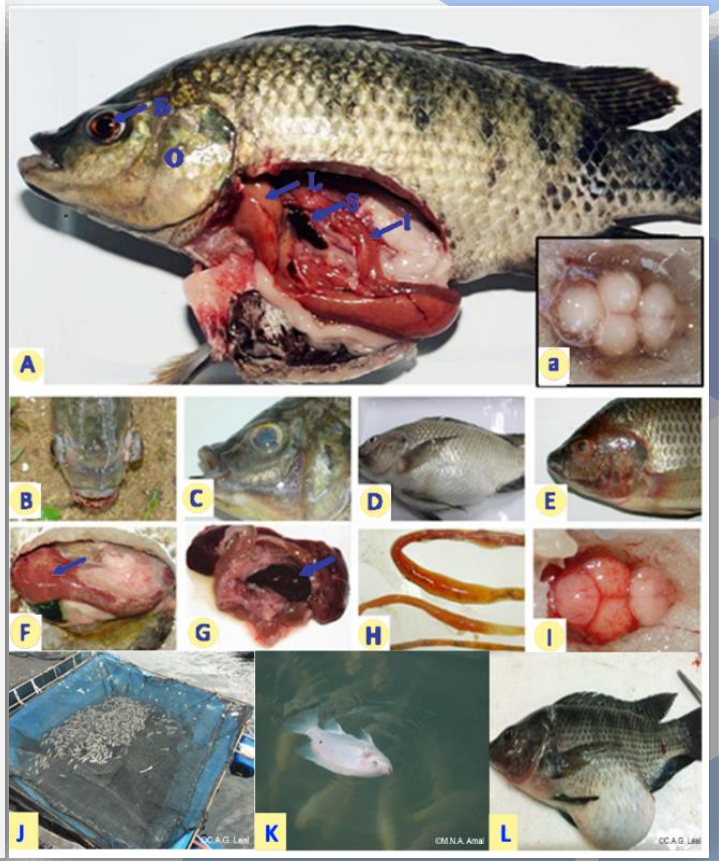


PRODUCTION OF NILE TILAPIA IN THAILAND

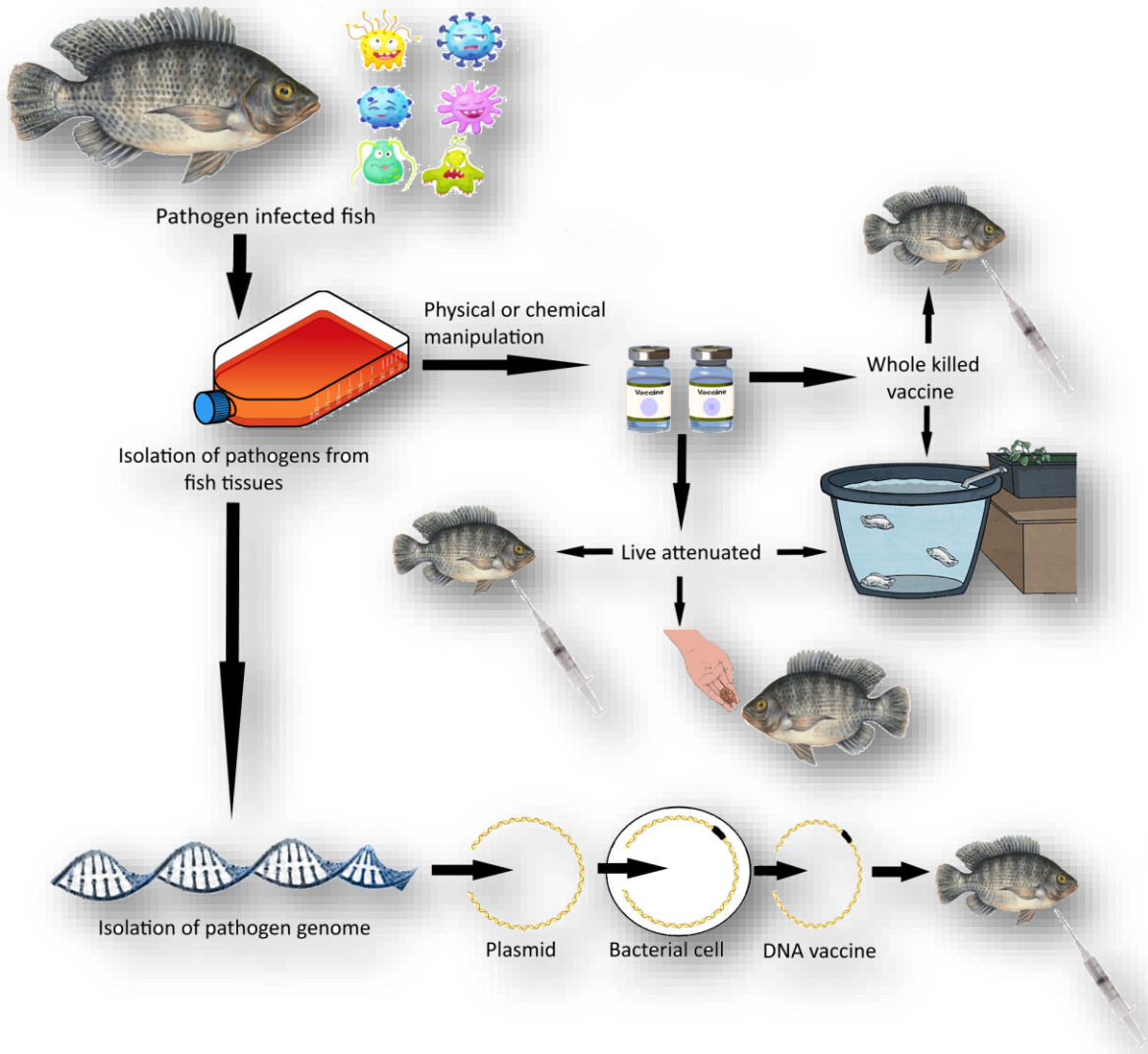


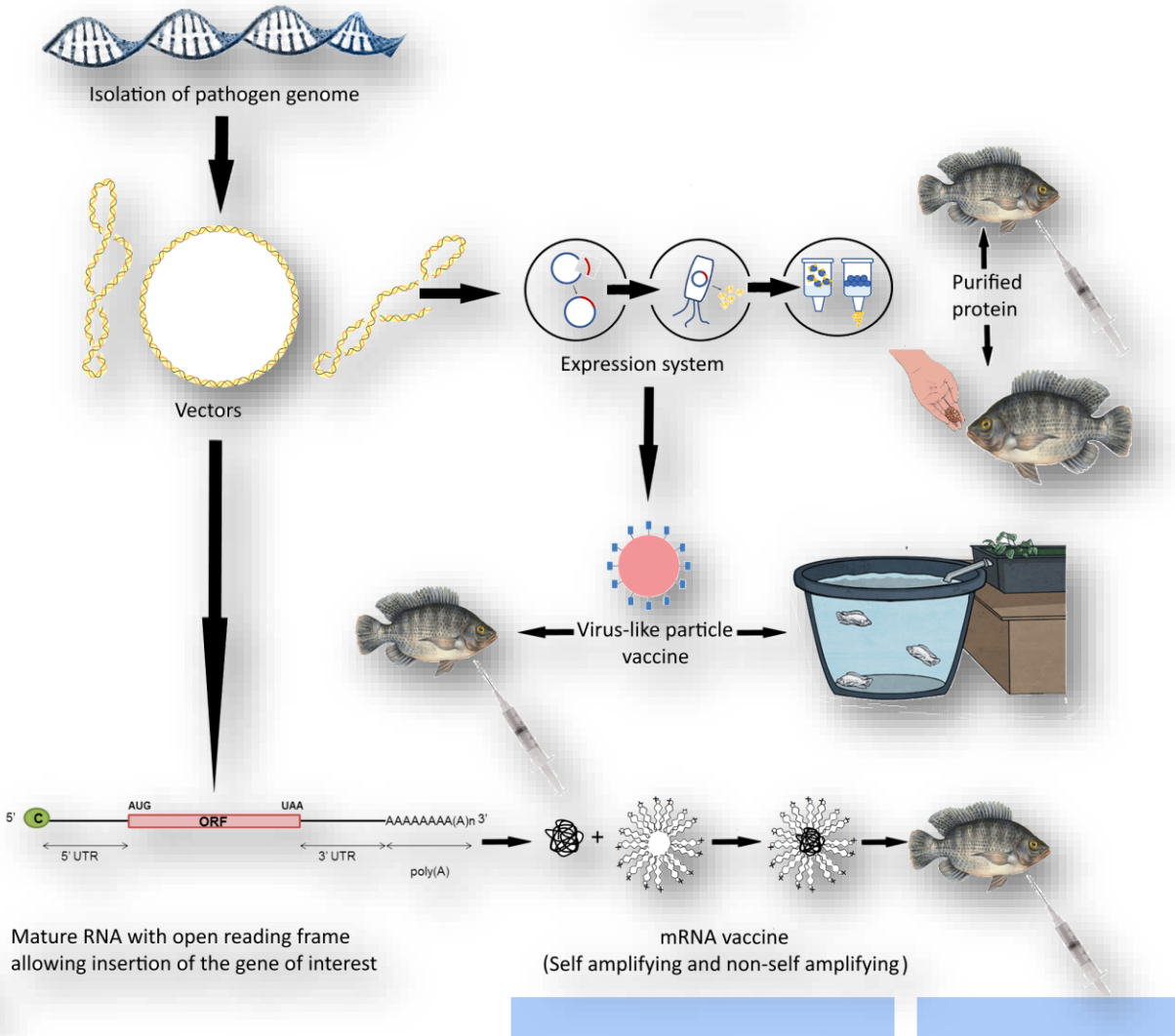
DISEASES CAUSED BY *STREPTOCOCCUS AGALACTIAE* AND *AEROMONAS VERONII*

Species	Fish species	Clinical criteria
Streptococcus agalactiae	Nile tilapia, Barcoo grunter, Golden pompano, Giant Queensland grouper, Ya-fish, Silver pomfret	Erratic swimming, appetite, lethargy, uncoordinated movements, exophthalmia (uni- or bi-lateral), intraocular hemorrhage, opaqueness of cornea, ascites
Aeromonas veronii	Asian seabass, Carassius auratus, Cyprinus carpio, Ctenopharyngodon idella, Nile tilapia, Silurus asotus	Anorexia, ascitic fluid appear yellow, distended abdomen, hemorrhage, lethargy, scale protrusion, sepsis, ulcer syndrome



TYPES OF VACCINES FOR AQUACULTURE





OVERVIEW OF COMMERCIAL VACCINES FOR *STREPTOCOCCUS AGALACTIAE* VACCINES IN NILE TILAPIA AND VACCINATION METHODS

Pathogen	Vaccine type	Antigens/targets	Delivery method	Vaccine name	Company
Streptococcus agalactiae Serotype Ia & Serotype III	Inactivated	Whole cell inactivated Streptococcus spp.	IP	Strep Sa	AQUAVAC
Aeromonas veronii	Inactivated Oil-based (Palm oil)	Whole cell inactivated Aeromonas spp.	IP	Autogenous Aeromonas veronii vaccine	PHARMAQ AS

	Immersion	Injection	Oral
Application	Easy	Delicate	Very easy
Stress	Light	Moderate	No
Job / labor	Moderate	Intensive	No
Efficiency	Good	Excellent	Passable
Duration	3-12 months	12-24 months	2-4 months

OVERVIEW OF VACCINATION STRATEGIES AND ANTIGEN DELIVERY SYSTEMS FOR *STREPTOCOCCUS AGALACTIAE* VACCINES IN NILE TILAPIA

Vaccine	Vaccination	Challenge	RPS *	Ref.
Live attenuated vaccination	Intraperitoneal	Intraperitoneal	70%	[10]
Live attenuated vaccination (YM001)	Intraperitonal	Intraperitneal	96.88%	[8]
Live attenuated vaccination (YM001)	Immersion	intracoelomic	67.22%	[8]
Live attenuated vaccination (YM001)	Oral	Intraperitoneal	71.81%	[8]
Live attenuated vaccination	Intraperitoneal	Intraperitoneal	75%–100%	[9]
DNA vaccine (Sip) *Salmonella typhimurium* vector	Oral	Intraperitoneal	70%–100%	[12]
Recombinant DNA feed based vaccine	Oral	Intraperitoneal	70%	[13]
Whole cell Inactivated vaccine—formalin killed	Intraperitneal	Intraperitneal	49%	[14]
Whole cell Inactivated vaccine—formalin killed	Intraperitneal	Intraperitneal	50%	[15]
Whole cell Inactivated vaccine—formalin killed	Intraperitneal	Intraperitneal	80%	[16]
Whole cell Inactivated vaccine—formalin killed	Bath	Intraperitneal	34%	[16,17]
Whole cell Inactivated vaccine—formalin killed	Oral	Intraperitneal	97%	[16,17]
Whole cell inactivated vaccines—heat killed	Oral	intracoelomic	38.9%	[18]
Whole cell Inactivated vaccine—formalin killed	Spray	Immersion	80%	[19]
Whole cell inactivated vaccines—heat killed	Spray	Injection	70%	[19]
Extracellular product (ECP)—formalin treated	Intraperitneal	Intraperitneal	29%	[14]

2 OBJECTIVES OF THE RESEARCH

RESEARCH QUESTIONS

1. Can a soybean oil-based oral bivalent inactivated vaccine with oral booster dose **(OR+OR)** stimulate IgM responses against both *S.agalactiae* and *A.veronii* in Nile tilapia fingerlings ?

2. Can a bath immersion inactivated bivalent vaccine with oral booster dose **(IM+OR)** stimulate IgM responses against both *S.agalactiae* and *A.veronii* in Nile tilapia fingerlings?

3. Which one of the two methods (OR+OR or IM+OR) is better at protecting fingerling Nile tilapia from *S.agalactiae* and *A.veronii* infections?

METHODS OF VACCINATION

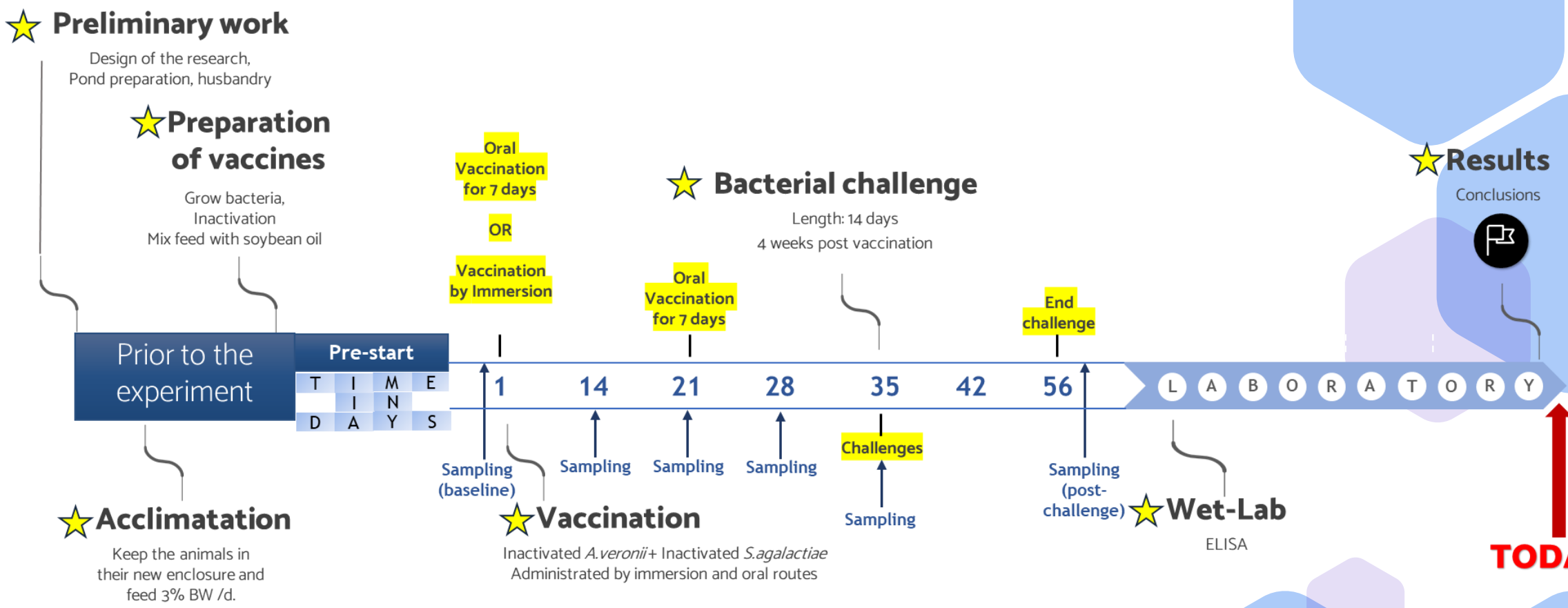
Immersion + Oral booster (IMM+OR)

Nile tilapia (1.1±0.1 g) were randomly assigned to 2 tanks (150 fish / tank) and immunized with immersion vaccine (day 1) plus oral booster vaccine fed daily (day 21 – 28)

Oral vaccine + Oral booster (OR+OR)

Nile tilapia (1.1±0.1 g) were randomly assigned to 2 tanks (150 fish / tank) and immunized with oral vaccine (day 1 – 7) fed daily plus oral booster vaccine (day 21 – 28).

TIMELINE


Preliminary work
Design of the research,
Pond preparation, husbandry
Preparation of vaccines
Grow bacteria,
Inactivation
Mix feed with soybean oil
Oral Vaccination for 7 days
OR
Vaccination by Immersion
Bacterial challenge
Length: 14 days
4 weeks post vaccination
Oral Vaccination for 7 days
End challenge
Results
Conclusions
Prior to the experiment
Pre-start
TIME IN DAYS
1
14
21
28
35
42
56
LABORATORY
Sampling (baseline)
Sampling
Sampling
Sampling
Challenges
Sampling
Sampling (post-challenge)
Acclimatation
Keep the animals in their new enclosure and feed 3% BW /d.
Vaccination
Inactivated A.veronii + Inactivated S.agalactiae
Administrated by immersion and oral routes
Wet-Lab
ELISA
TODAY

3

METHODOLOGY

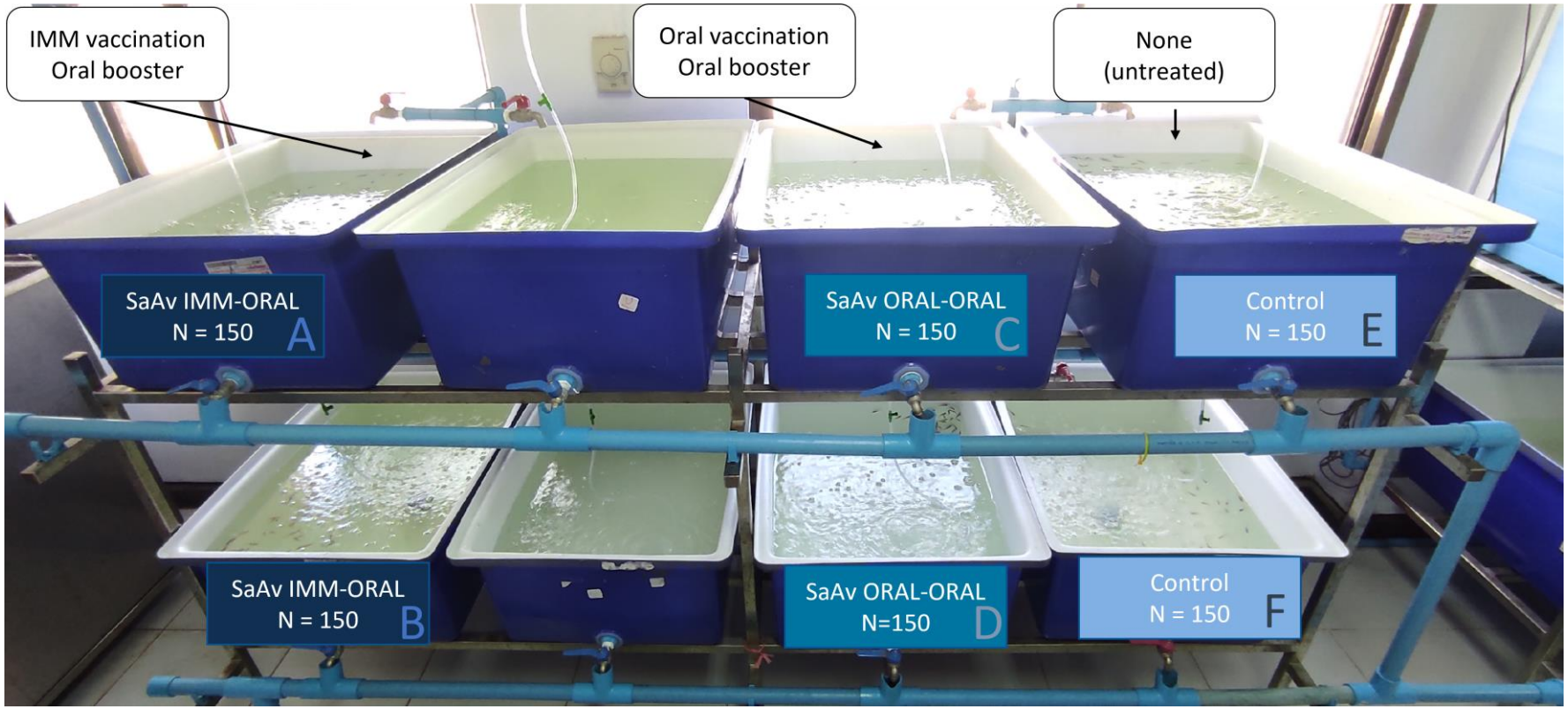
Experimental fish and husbandry, preparation of vaccines, administration of vaccines, indirect elisa, challenge trials.

KEY MEASUREMENTS

Two methods of vaccination for streptococcus infection and motile aeromonas septicemia were evaluated in *Nile tilapia (Oreochromis niloticus)* fingerlings for their effect on:

- Disease specific antibody levels (IgM)
- Survival rates upon artificial infection trials

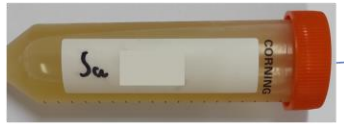
HUSBANDRY


IMM vaccination
Oral booster
Oral vaccination
Oral booster
None
(untreated)
SaAv IMM-ORAL
N = 150
A
SaAv ORAL-ORAL
N = 150
C
Control
N = 150
E
SaAv IMM-ORAL
N = 150
B
SaAv ORAL-ORAL
N=150
D
Control
N = 150
F

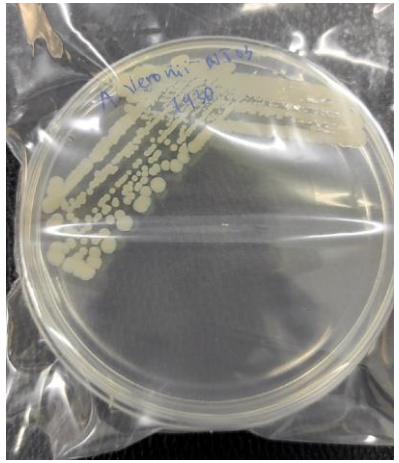
CULTURE OF BACTERIA

Streak Sa from stock onto TSA

Inoculation of a single colony of bacteria Sa in 30mL (overnight)



Inoculation of O.N 30mL Sa in 800mL TSB



Streak Av from stock onto TSA

Inoculation of a single colony of bacteria Av in 30mL (overnight)

Inoculation of O.N 30mL Av in 800mL TSB

CULTURE OF BACTERIA (2)

Harvest 5-6 hours later and read OD. Take sample for viable bacterial count. **Either use live bacteria or inactivate**

Read OD600, Using blank TSB Dilute 10 times to read OD. Sa = 0.170*10 = 1.7

Serial dilutions in TSB 8 times 1+9mL. 10^-1 to 10^8

Streak 100uL Sa onto TSA 10^-7, 10^- 8, 10^- 9

Harvest 5-6 hours later and read OD. Take sample for viable bacterial count. **Either use live bacteria or inactivate**

Read OD600, Using blank TSB Dilute 10 times to read OD. Av = 0.220*10 = 2.2

Serial dilutions in TSB 8 times 1+9mL. 10^-1 to 10^8

Streak 100uL Av onto TSA 10^-7, 10^- 8, 10^- 9

CULTURE OF BACTERIA (3)

Count colonies between 30-300

For Sa OD=1.7
CFU.mL^-1=1.18*10^9

Inactivate to produce vaccines **or** use live bacteria for challenge

Count colonies between 30-300

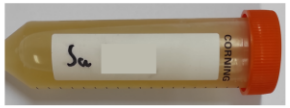
For Av OD=2.2
CFU.mL^-1=0.217*10^9

Inactivate to produce vaccines **or** use live bacteria for challenge

PRODUCTION OF HEAT-KILLED VACCINE SOLUTION (HKV)

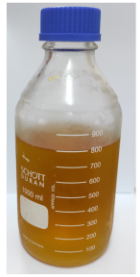
A.

Inoculation of bacteria Sa 30mL, O.N → Culture Sa in 800mL → Inactivation with heat → Prepare vaccine for the desired concentration.

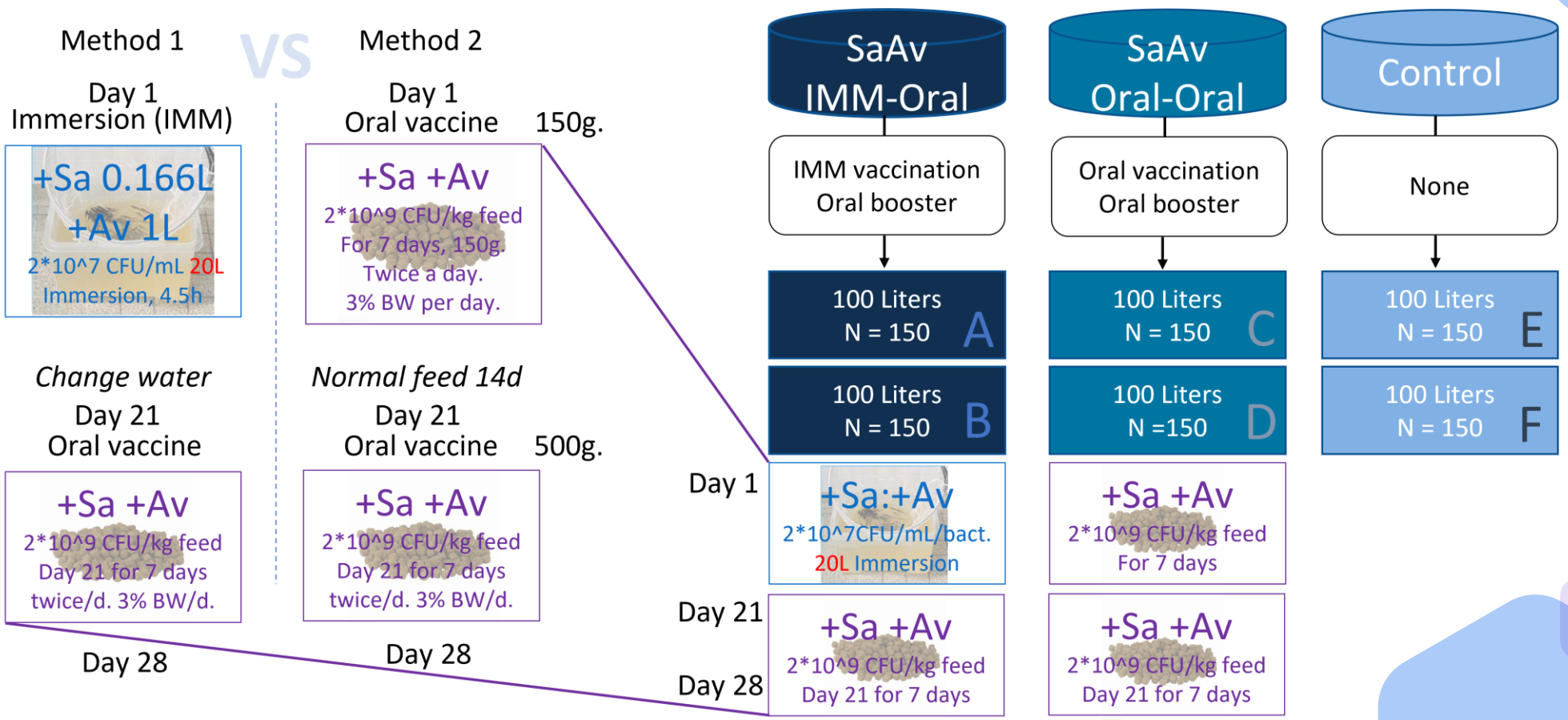


B.

Inoculation of bacteria Av 30mL, O.N → Culture Av in 800mL → Inactivation with heat → Prepare vaccine for the desired concentration.

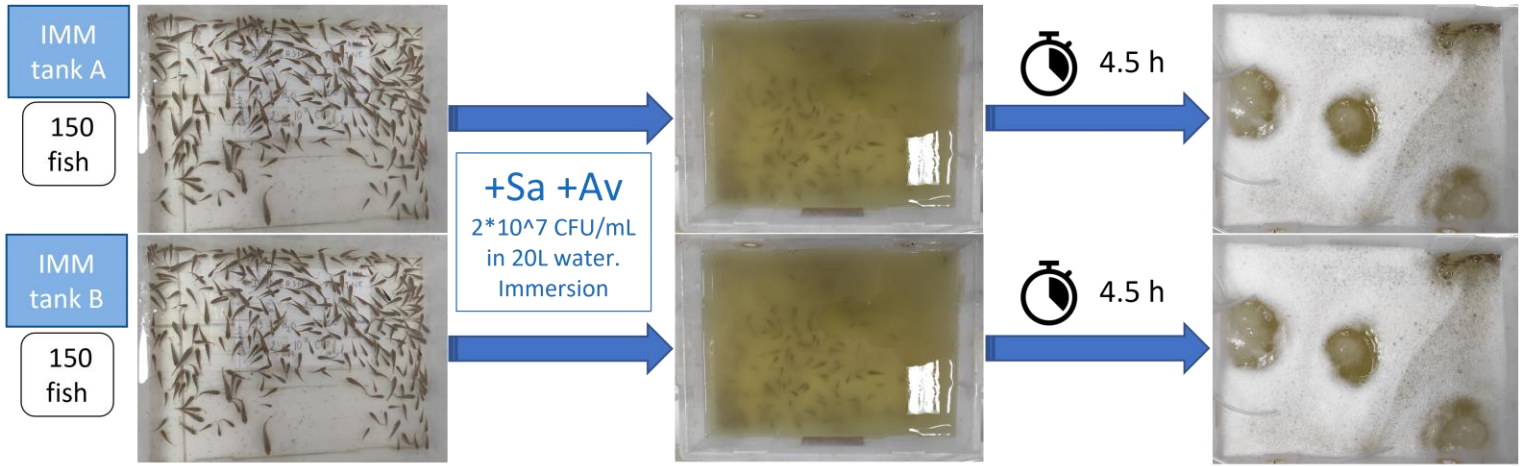


VACCINATION



VACCINATION: BATH IMMERSION

A.



B.

2*10^7 CFU/mL

4.5 h

Mortality after 4.5 hours = 3 %

VACCINATION: FOOD PELLETS

A.

Inactivated Bacteria Sa, OD 1.7 CFU/mL 1.18*10^9

+

Inactivated Bacteria Av, OD 2.2 CFU/mL 0.217*10^9

+

1 Kg of nursery feed pellets

+

100 mL soybean oil (10 % of total)

=

B.

Oral vaccines ready to use

1 billion CFU Av + 10 billion CFU Sa per Kg
Mixed with 100 mL of soybean oil (coating agent)

First vaccination day 1

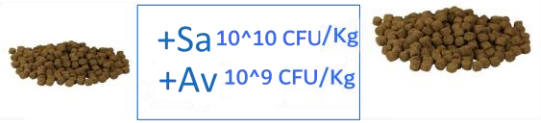
2 tanks of 100 fish (1.2g), fed 3% BW /day.

In total 150g of oral vaccine feed is prepared.

Booster vaccination Day 21

4 tanks of ~100 fish (1.5-2g), fed 3%BW /day.

In total 500g of oral vaccine feed is prepared



150g. 500g.

PREPARATION OF SAMPLES

A.

Randomly sample 6 fish per experimental condition, every week

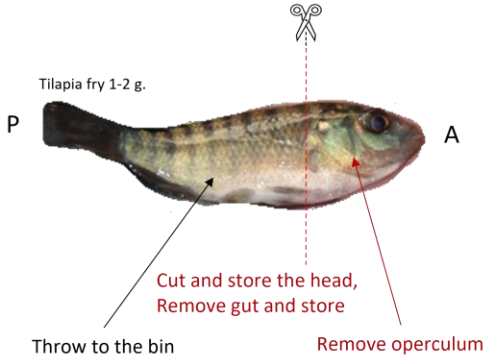
6 — 3 in tank A, 3 in tank B

6 — 3 in tank C, 3 in tank D

6 — 3 in tank E, 3 in tank F

Move to a beaker and terminate the fish using a few drops of love oil

Put the fish on LDPE bag. Remove operculum, remove the posterior body parts, separate gut from head.



B.

Add PBS-NaN3 to the eppendorf 300uL for gut and 600uL for head, crush using the device.

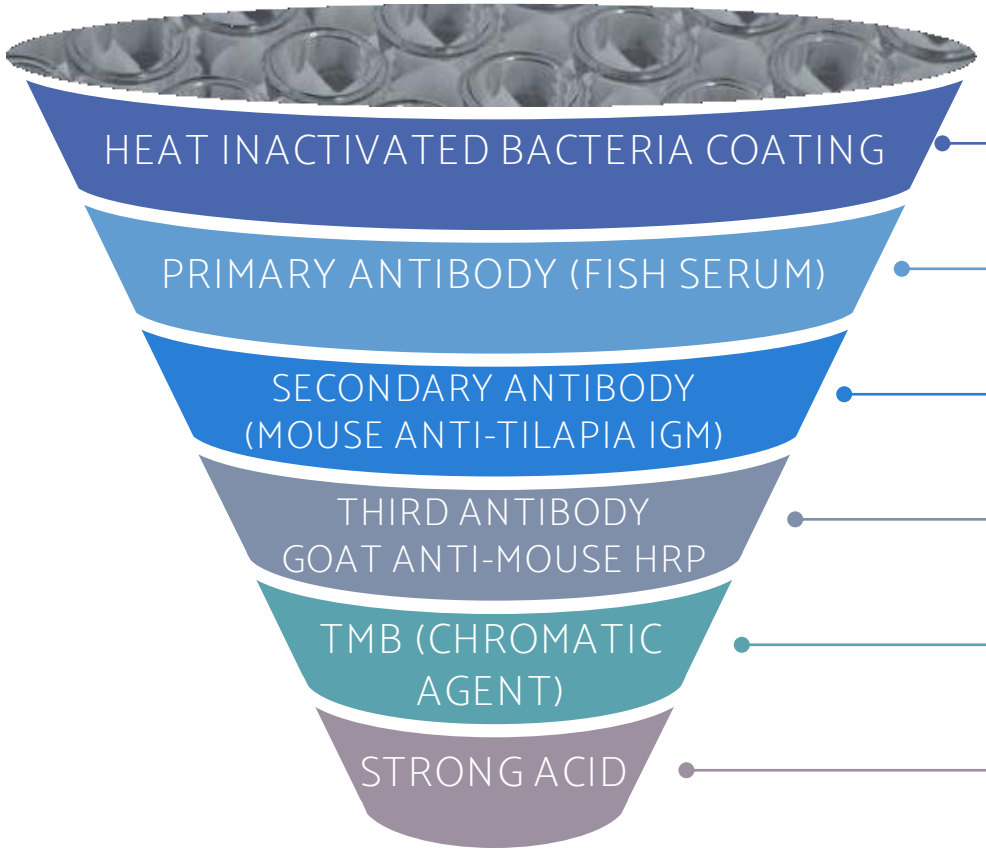
Head — 600uL — PBS pH 7.4, Sodium azide 0.02%

Gut — 300uL — PBS pH 7.4, Sodium azide 0.02%

Centrifuge 12,000 rpm, 10mn, Save supernatant, discard pellet

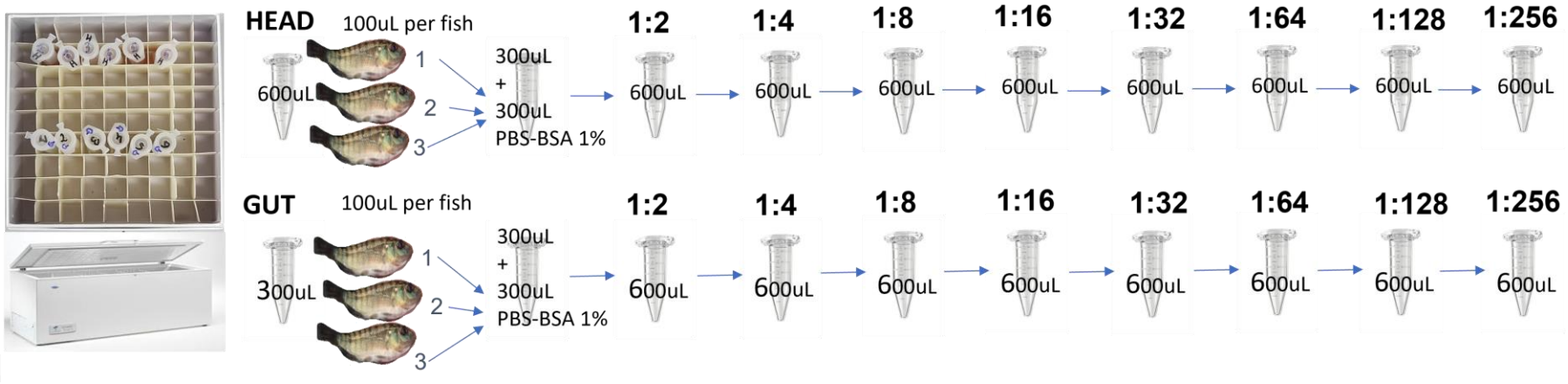
Store the samples at -20 Celsius

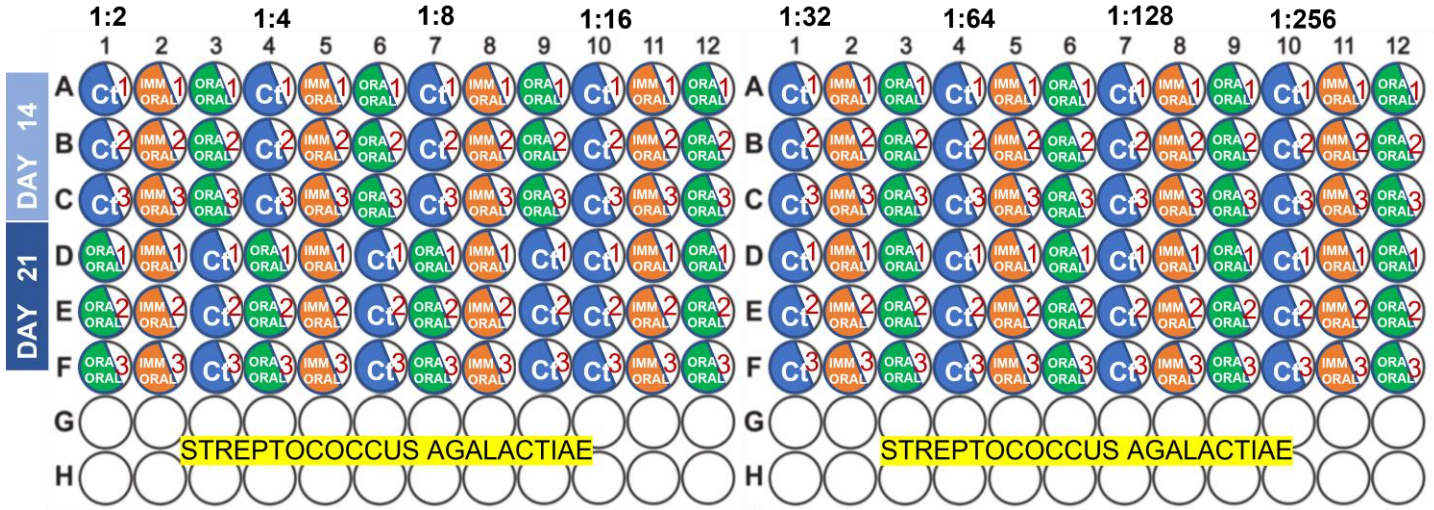
INDIRECT ELISA ASSAY FOR ANTIGEN-SPECIFIC IgM LEVELS



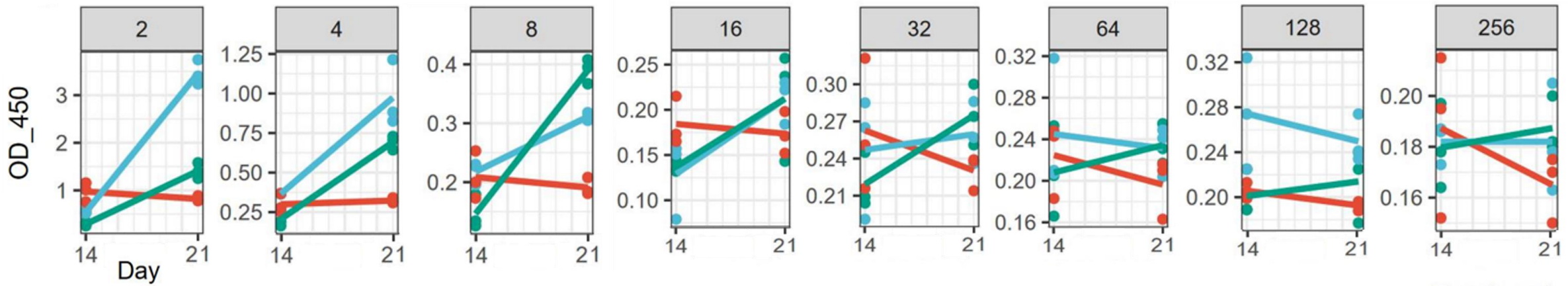
- S.Agalactiae or A.veronii killed whole cells are adsorbed to the PVC microplate wells.
- The primary antibody (IgM) is contained in the serum from the head or the gut of the fish.
- The secondary antibody is produced in a mice and is able to bind to tilapia IgM (first antibody).
- The third antibody is fused with the enzyme horseradish peroxidase (HRP) and binds to the secondary antibody.
- The TMB added to the wells and is a substrate of HRP and creates a yellow upon oxydation.
- Stop the enzymatic reaction

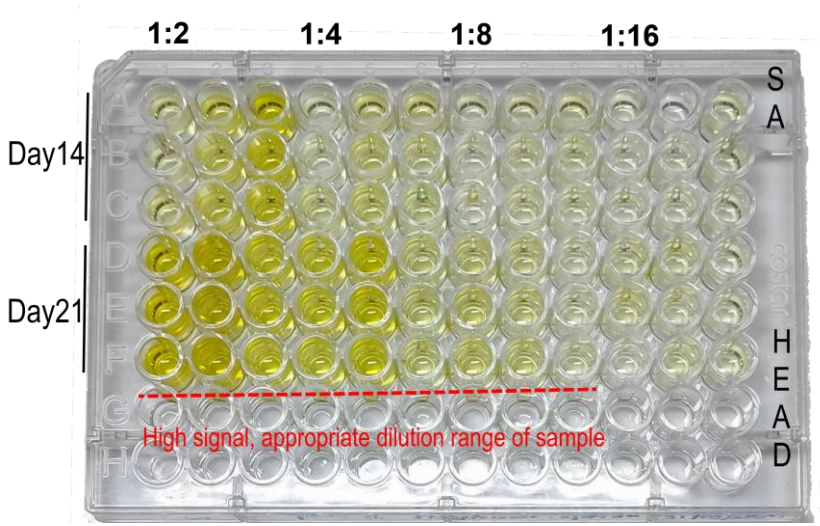
DETERMINATION OF THE OPTIMAL SAMPLE DILUTIONS

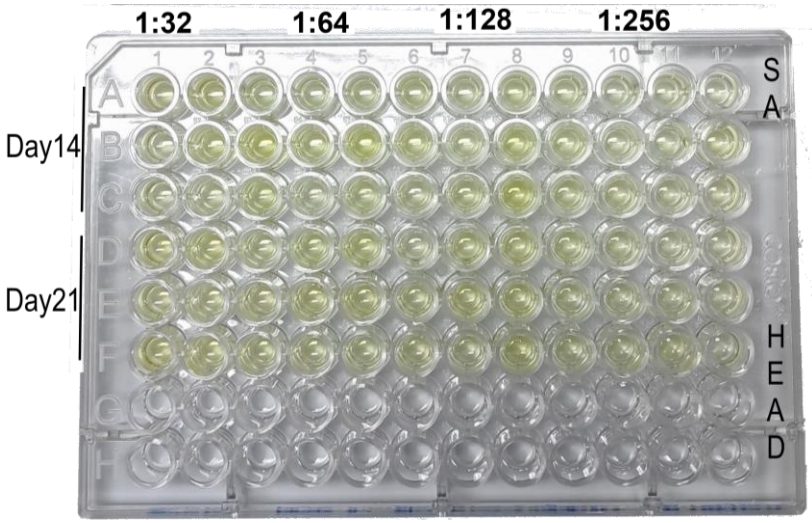




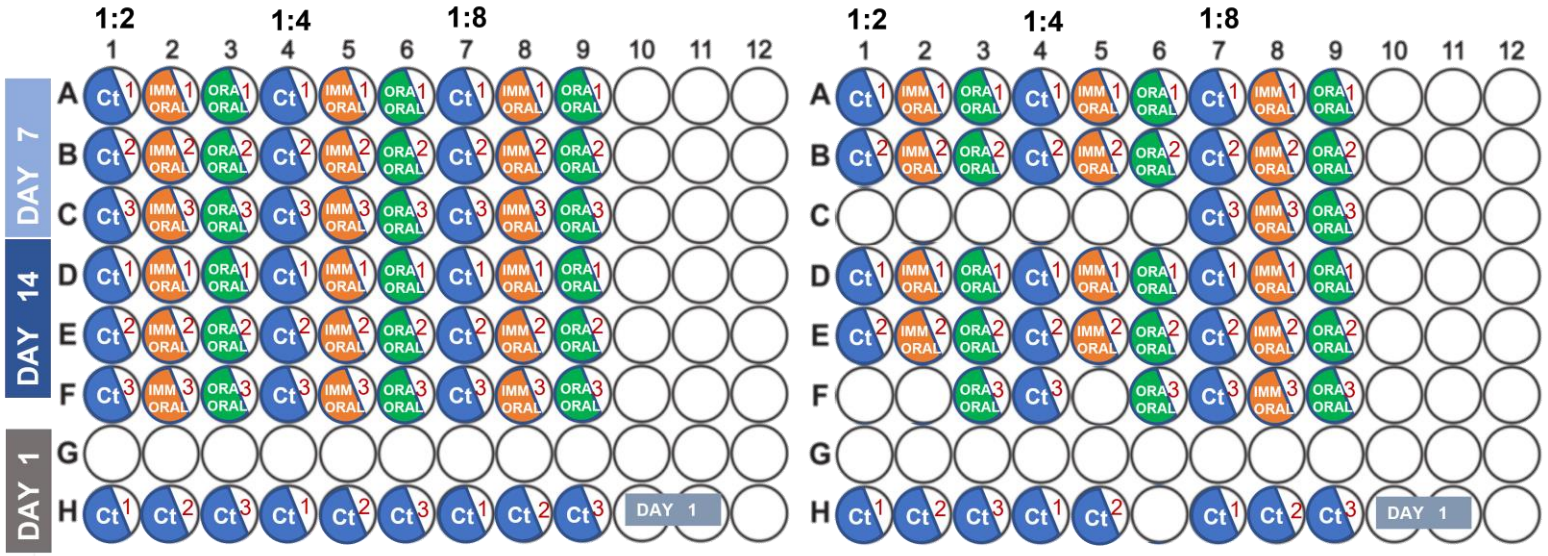
DETERMINATION OF THE OPTIMAL SAMPLE DILUTIONS (2)





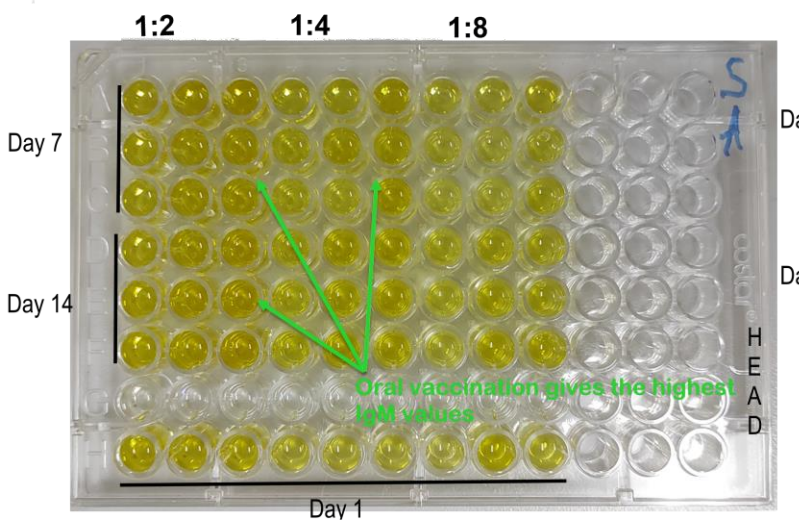


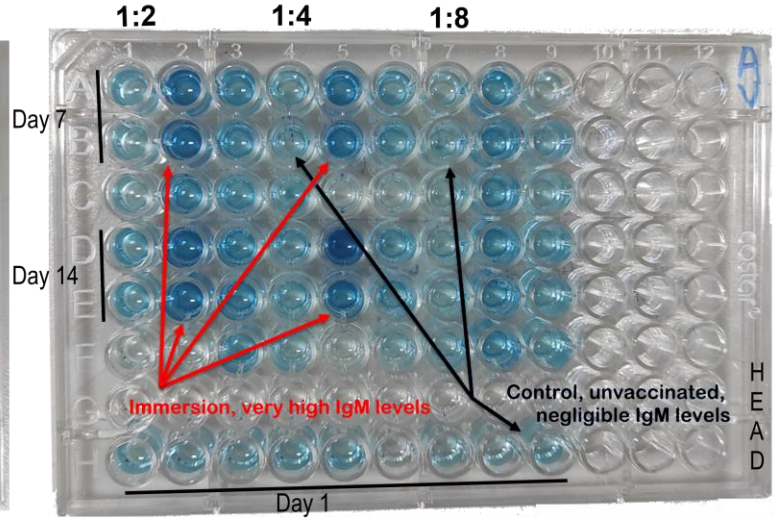
EXAMPLE OF INDIRECT ELISA



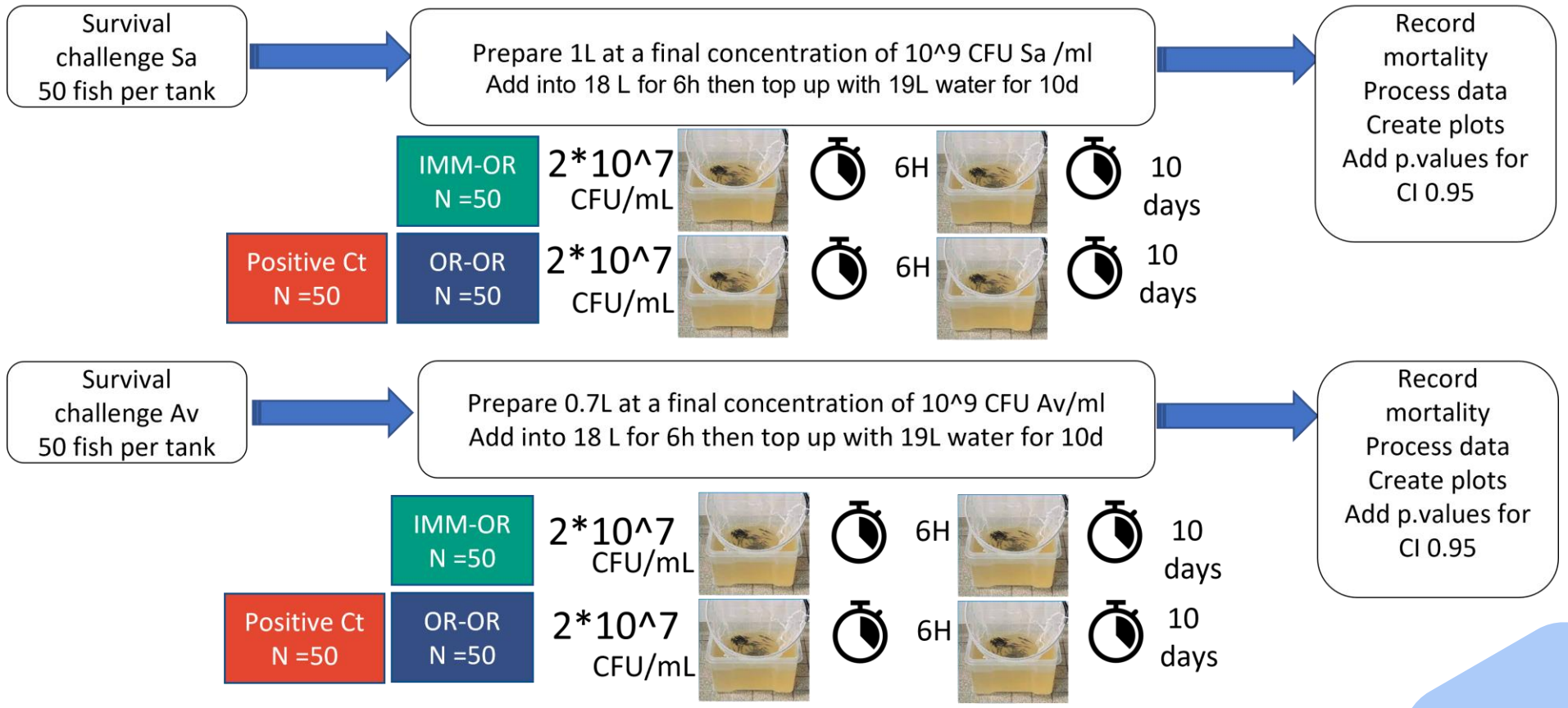
STREPTOCOCCUS AGALACTIAE (head)

AEROMONAS VERONII (head)

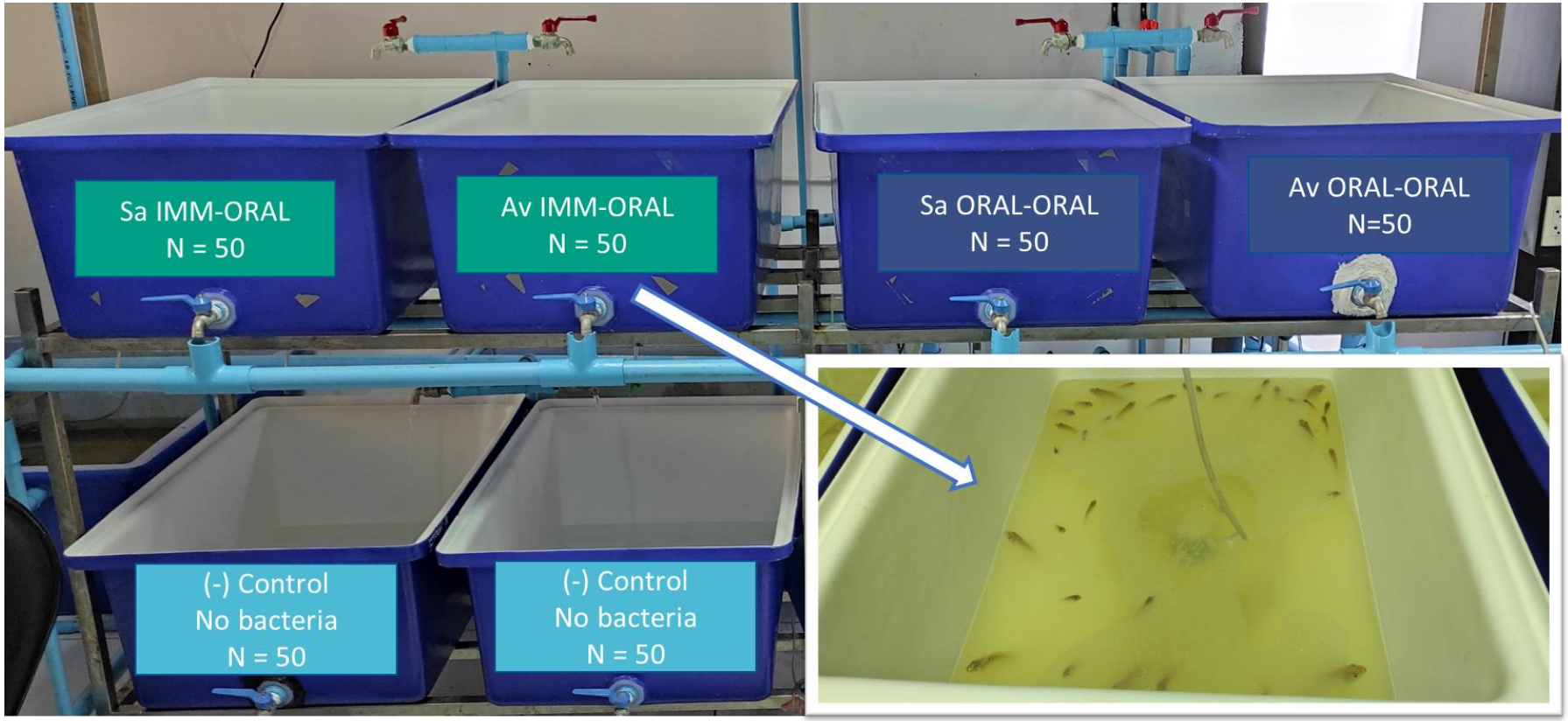




CHALLENGE TRIALS



HOUSING FOR THE CHALLENGE TRIALS

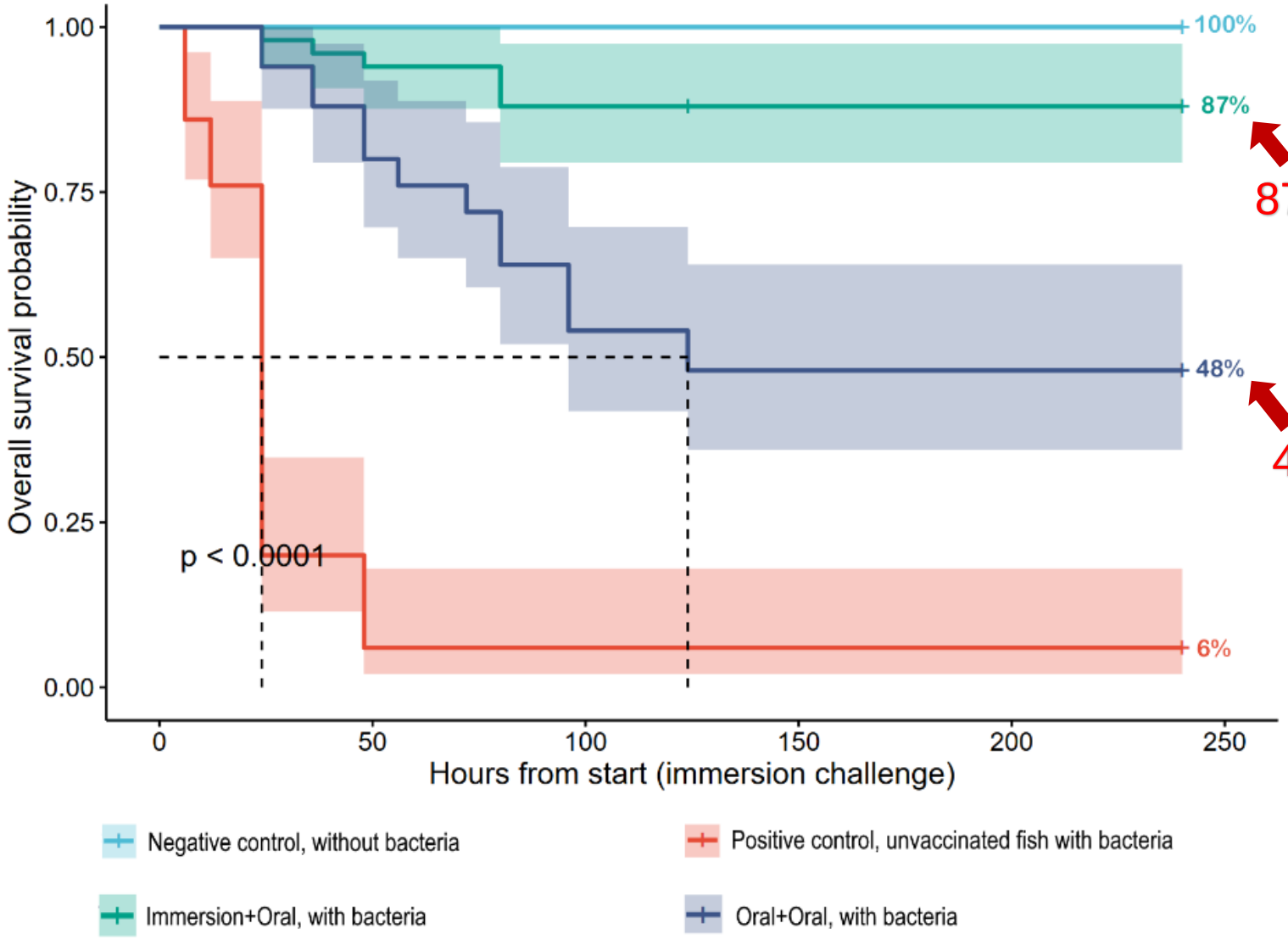


4 EXPERIMENTAL RESULTS

Results of infection challenge trials for survival,

indirect Elisa for specific antibodies.

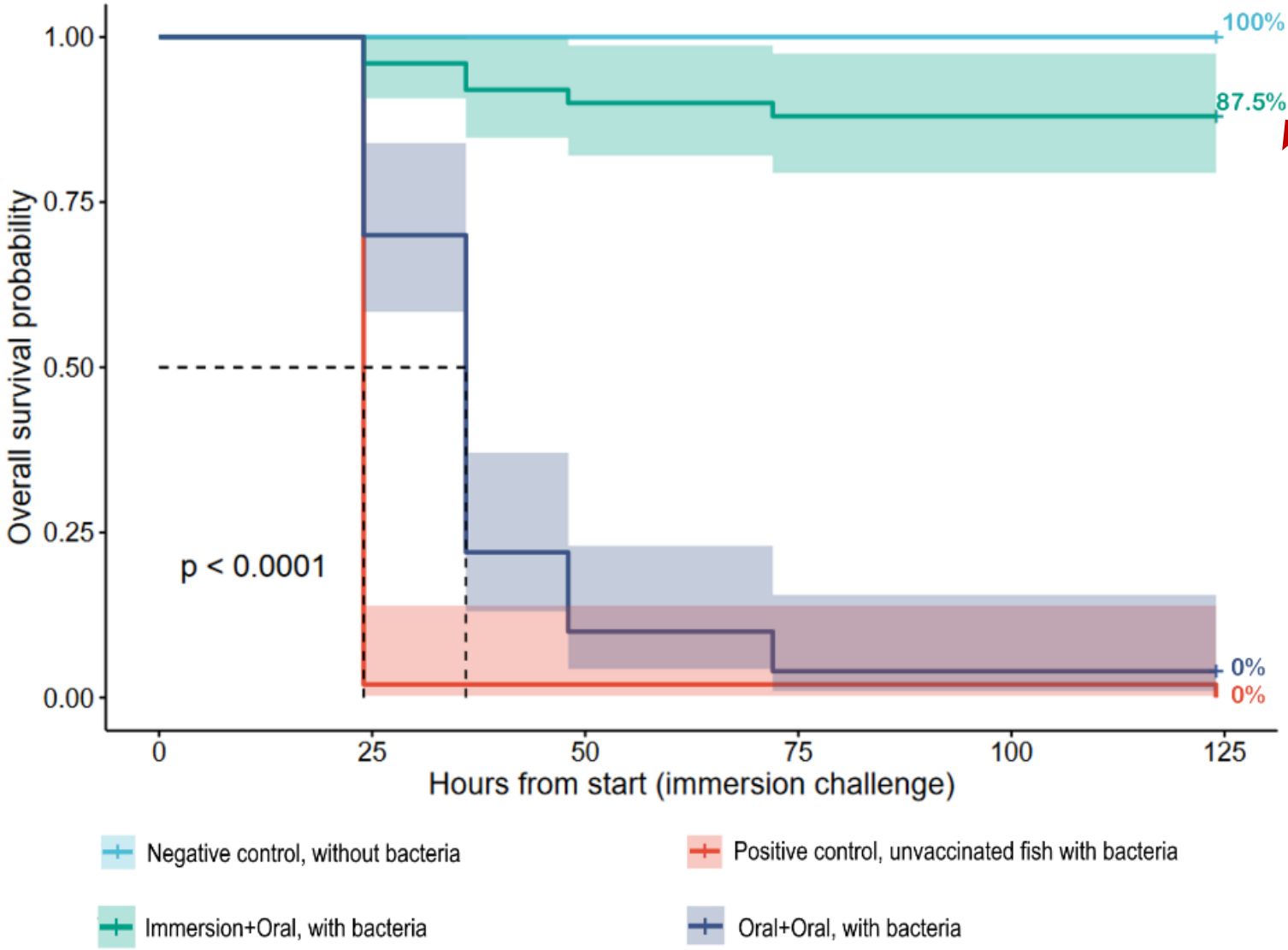
CHALLENGE TRIAL 1: IMMERSION WITH STREPTOCOCCUS AGALACTIAE



87 % survival with immersion – oral vaccination against Sa

48 % survival with oral – oral vaccination against Sa

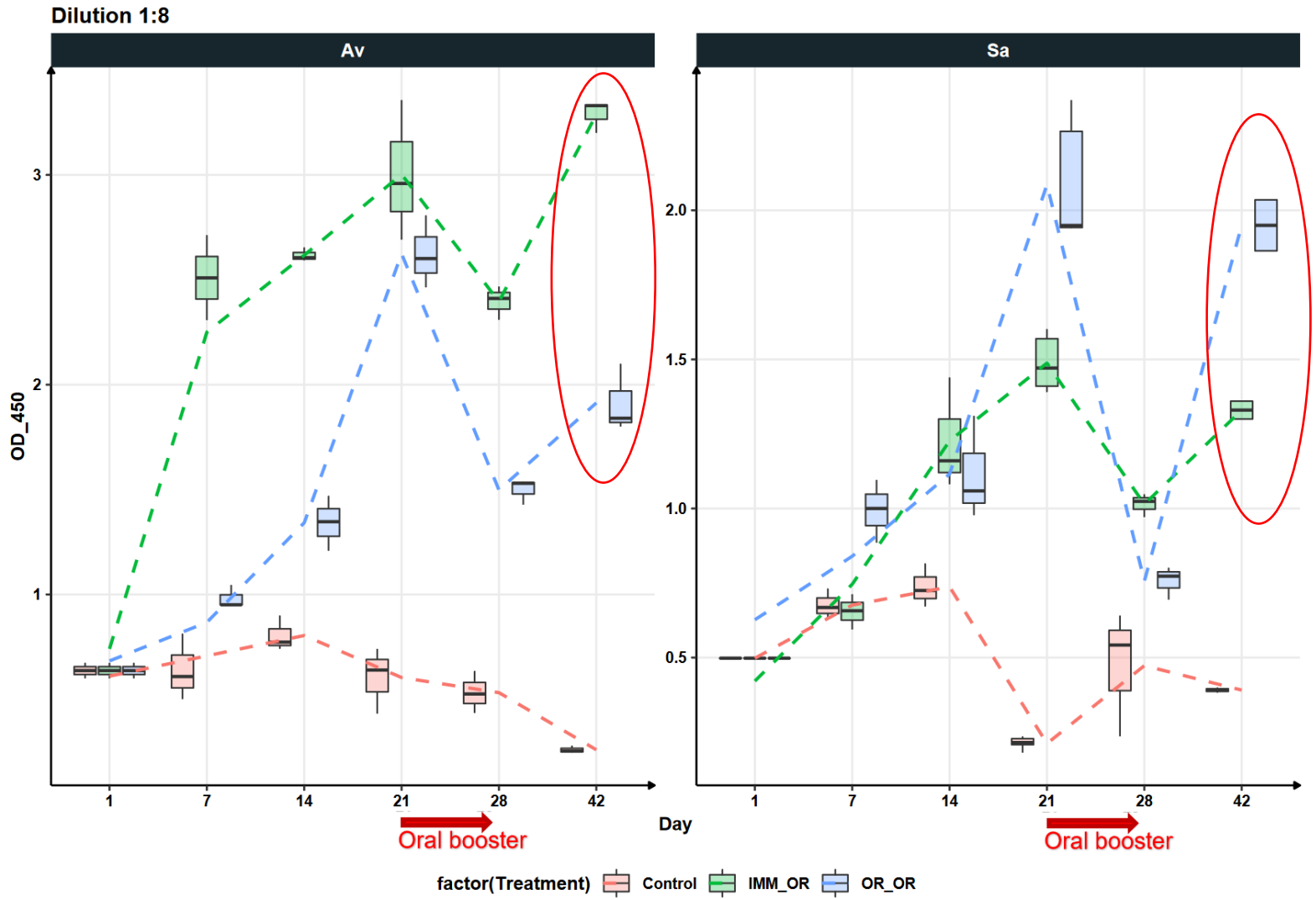
CHALLENGE TRIAL 2 : IMMERSION WITH AEROMONAS VERONII



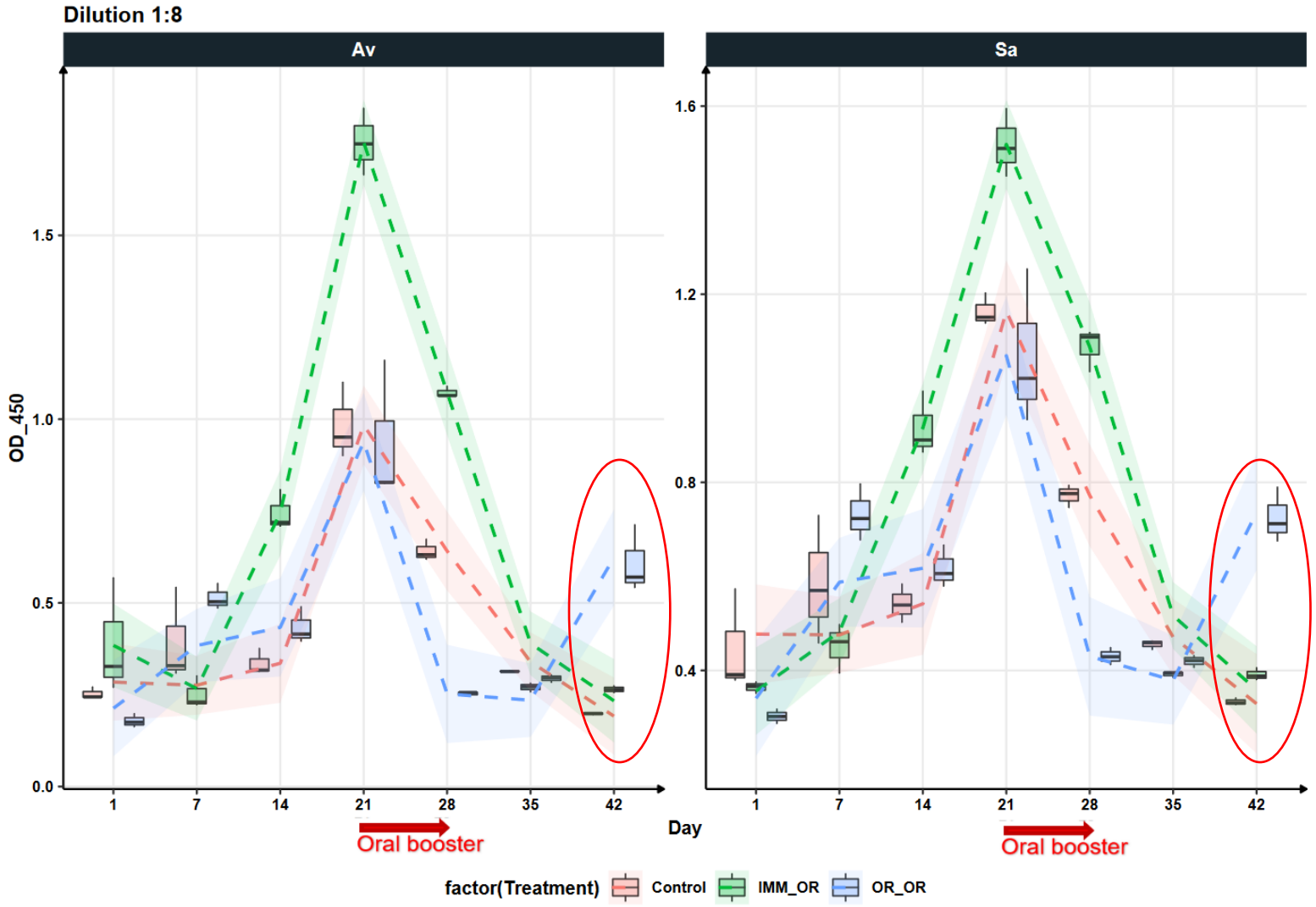
87.5 % survival with immersion – oral vaccination against Av

0 % survival with oral – oral vaccination against Av

ANTIGEN-SPECIFIC IGM IN THE HEAD OF NILE TILAPIA FINGERLINGS



ANTIGEN-SPECIFIC IGM IN THE GUT OF NILE TILAPIA FINGERLINGS



5 CONCLUSIONS

- **Oral bivalent** inactivated vaccine **with booster dose** (OR+OR) **stimulates specific IgM** against both *S.agalactiae* and *A.veronii.*

- **Bath immersion** inactivated bivalent vaccine with **oral booster dose** (IM+OR) **stimulates specific IgM** against both *S.agalactiae* and *A.veronii.*

- **Only IM+OR is effective** at protecting fingerling Nile tilapia from *S.agalactiae* and *A.veronii* infections.

>> Recommendation: **IM+OR bivalent vaccine is a simple, inexpensive, yet effective immunization of fingerlings in small to medium scale farms and nurseries.**

THANK YOU FOR YOUR ATTENTION

Any questions?

You can find me at:

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