







RESEARCH ARTICLE

REVISIED Transfer of maternal immunity using a polyvalent vaccine and offspring protection in Nile tilapia, *Oreochromis niloticus* [version 3; peer review: 1 approved, 1 approved with reservations]

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V3 First published: 24 Sep 2021, 10:966
<https://doi.org/10.12688/f1000research.52932.1>
 Second version: 15 Jun 2022, 10:966
<https://doi.org/10.12688/f1000research.52932.2>
 Latest published: 09 Mar 2023, 10:966
<https://doi.org/10.12688/f1000research.52932.3>

Abstract




Background: Vaccination is an effective and alternative means of disease prevention, however, it cannot be conducted on the offspring of fish. For this process to take place, the transfer of maternal immunity must be implemented. This study aims to determine the effectiveness of transferring immunity from the broodstock to the offspring using a polyvalent vaccine against *Aeromonas hydrophila*, *S. treptococcus agalactiae*, and *Pseudomonas fluorescens* in Nile tilapia, *Oreochromis niloticus*.


Methods: Nile tilapia broodstock, with an average weight of 203g (\pm SD 23 g) was injected with a vaccine used as a treatment. Example include *A. hydrophila* monovalent (MA), *S. agalactiae* monovalent (MS), *P. fluorescens* monovalent (MP), *A. hydrophila* and *S. agalactiae* bivalent (BAS), *A. hydrophila* and *P. fluorescens* bivalent (BAP), *P. fluorescens* and *S. agalactiae* bivalent (BPS), and *A. hydrophila*, *S. agalactiae*, and *P. fluorescens* polyvalent vaccines (PAPS). While the control was fish that were injected with a PBS solution. The broodstock's immune response was observed on the 7th, 14th, 21st, and 28th day, while the immune response and challenge test on the offspring was conducted on the 10th, 20th, 30th, and 40th day during the post-hatching period.

Result: The application of PAPS in broodstock could significantly induce the best immune response and immunity to multiple diseases compared to other treatments. The RPS of the PAPS was also higher than the other types of vaccines. This showed that the transfer of immunity from the broodstock to the Nile tilapia offspring could protect it against bacterial diseases such as *A. hydrophila*, *S. agalactiae*,

Open Peer Review

Approval Status  

	1	2
version 3 (revision) 09 Mar 2023		
version 2 (revision) 15 Jun 2022	 view	
version 1 24 Sep 2021	 view	 view

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and *P. fluorescens*.

Conclusion: The application of PAPS *A. hydrophila*, *S. agalactiae*, *P. fluorescens* vaccines increased the broodstock's immune response and it was transferred to their offsprings. They were able to produce tilapia seeds that are immune to diseases caused by *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*.

Keywords

Aeromonas hydrophila, bivalent vaccine, monovalent vaccine, Pseudomonas fluorescens, Streptococcus agalactiae.

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Author roles: **Amrullah A:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Methodology, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Wahidah W:** Data Curation, Project Administration, Validation; **Ardiansyah A:** Data Curation, Validation, Writing – Review & Editing; **Indrayani I:** Formal Analysis, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The authors are grateful to the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for funding this study through the 2019 National Strategic Research Scheme (No.: 004/PL.22.7.1/SP-PG/2019). *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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How to cite this article: Amrullah A, Wahidah W, Ardiansyah A and Indrayani I. **Transfer of maternal immunity using a polyvalent vaccine and offspring protection in Nile tilapia, *Oreochromis niloticus* [version 3; peer review: 1 approved, 1 approved with reservations]** F1000Research 2023, 10:966 <https://doi.org/10.12688/f1000research.52932.3>

First published: 24 Sep 2021, 10:966 <https://doi.org/10.12688/f1000research.52932.1>

REVISED Amendments from Version 2

In the 2nd revision, there are several sentence improvements (rephrase). In the research methodology section, there was a revision of the writing of pool dimensions and an explanation of the vaccine production method. In the blood collection, a revision was carried out, where the blood collection became the Caudal Vein in the broodstock, the revision of the number of seeds that were ground was 5 individuals/unit, the revision of the effendor used, and the centrifugation time was 5 mL and 5 minutes respectively. In the agglutination test, it was clarified that the addition of antigen volume was 25 μ L in the control and vaccine treatment, and in the challenge test the seeds were immersed with pathogenic bacteria for 24 hours. In the results of the study, there was an improvement in Figure 1. Meanwhile, in the discussion, there was an additional explanation that offspring from unvaccinated broodstocks had native immunity, but all parameters of the immune response were very low compared to offspring from vaccinated brood stocks. It was also explained that the immune response of offspring with high maternal immunity compared to controls lasted until the 30th day.

Any further responses from the reviewers can be found at the end of the article

Introduction

Tilapia was originally considered to be more resistant to bacterial, parasitic, fungus, and viral diseases than other species of cultivated fish. However, they are found to be susceptible to bacterial and parasitic diseases¹⁻³, particularly during the offspring phase⁴. Globally, the control of bacterial disease mostly uses antibiotics that are proven not environmentally friendly⁵⁻⁷. Some common diseases of tilapia found in several Southeast Asian countries including Indonesia are *Streptococcus agalactiae*, *Aeromonas hydrophila*, *Edwardsiella ictaluri*, *Flavobacterium columnaris*, and *Pseudomonas fluorescens* infections⁸⁻¹⁰. In addition to the bacterial disease, a new disease has emerged called Tilapia Lake Virus (TiLV) disease whose specific host is tilapia, causing disease outbreaks with high mortality rates in several Southeast Asian countries such as Thailand¹¹ and Malaysia¹².

Among the various methods of disease control, vaccination is one of the most effective ways, which is commonly used^{5,13-16}. The administration of vaccines is meant to produce antibodies that could improve the immunity of tilapia^{3,5}. Unfortunately, they could not be administered to the offspring of fish because the organs that form the immune response are not yet fully developed, therefore they are unable to produce antibodies^{7,13-17}. Tilapia fry was not able to produce their own immune system at the age of less than 21 days¹⁸, Immune systems of *Xenopus laevis* develop within 2 weeks of age¹⁹, while Indian major carp develop within 3 weeks of age²⁰.

An effective solution to the aforementioned issue is the application of maternal immunity transfer. This is the transfer of immunity from broodstock to offspring, by which immunoglobulin (IgM type) are transferred through eggs^{19,21,22}. Maternal immunity has been shown to improve the fish

offspring's immunity against pathogens in the early phases of their lives²³⁻²⁶.

This process is usually carried out using monovalent vaccines²⁷⁻³⁰. However, a polyvalent vaccine would be more effective because it could control multiple diseases^{3,31,32} especially using a formalin-killed vaccine with low production cost compared to other types of vaccines³. Though the effectiveness has been known, the application of polyvalent vaccines to confer maternal immunity in offspring has not been extensively investigated, particularly in Nile tilapia (*O. niloticus*).

The transfer of maternal immunity using polyvalent vaccine for *S. agalactiae*, *Lactococcus garvieae*, and *Enterococcus faecalis* has been studied by Abu-elala *et al.*³³, and three vaccine strains for *S. agalactiae* by Nurani *et al.*³⁴. The types of bacterial diseases studied in the aforementioned studies are very limited even though Nile tilapia often suffer from them in fish farms and hatcheries³⁵. Besides being infected by *S. agalactiae*^{29,34-36}, Nile tilapia are often infected by *A. hydrophila*^{9,35,37} and *P. fluorescens*^{37,38} leading to high mortality, including in Indonesia. Therefore, this study aimed to examine maternal immunity transfer using the polyvalent vaccine for *S. agalactiae*, *A. hydrophila*, and *P. fluorescens* (PAPS). It was expected that the broodstock could pass their immunity to their offspring, making them resistant to the three diseases (*A. hydrophila*, *S. agalactiae*, and *P. fluorescens* bacteria), and also the production of tilapia offspring could also be increased. Furthermore, this study aimed to determine the effectiveness of the transfer of immunity induced by PAPS against *A. hydrophila*, *S. agalactiae*, and *P. fluorescens* from the Nile tilapia (*O. niloticus*) broodstock to their offspring and the protection against *S. agalactiae*, *A. hydrophila*, and *P. fluorescens* infections.

Methods**Experimental animal**

Nile tilapia broodstock, obtained from the Ompo Inland Hatchery, Soppeng, Indonesia, with an average weight of 203g (\pm SD 23 g) were used as experimental animal. They were kept in spawning ponds (25X30X1.2 LxWxD) and fed ad libitum with pellets that have a protein content of 30% in the mornings and afternoons. Also, 25% of the water was replaced daily. One week after the fish spawned, they were harvested and a large number of Nile tilapia broodstock at gonad developmental stage 2 were obtained.

Vaccine production

Pure isolates of the *A. hydrophila*, *S. agalactiae*, and *P. fluorescens* bacteria were obtained from the Research and Development of Fish Disease Control Installation, Ministry of Marine Affairs and Fisheries, Depok, Indonesia. Vaccine production was carried out by harvesting bacteria aged 24 hours, which were cultured on TSA media. The yields were then put into 100 mL of PBS with a bacterial density of 10¹⁰ cfu/mL measured by the McFarland method. Further, it

was killed with formalin according to the results of Amrullah *et al.*³⁹, *S. agalactiae* and *P. fluorescens* were inactivated in 1% formalin, while *A. hydrophila* was inactivated with 0.6%³⁹. Later, stirred and incubated for 24 hours at 4°C. After 24 hours of incubation, a vaccine safety test was carried out using the sterilization method. Finally, the vaccine was diluted at a dose of 10⁷ cfu/mL and was ready to be used for the vaccination of tilapia broodstock.

Vaccine treatments and administration

The vaccine treatments consist of (1) a monovalent vaccine against *A. hydrophila* (MA), (2) a monovalent vaccine against *P. fluorescens* (MP), (3) a monovalent vaccine against *S. agalactiae* (MS), (4) a bivalent vaccine against *A. hydrophila*, *P. fluorescens* and (BAP), (5) a bivalent vaccine against *A. hydrophila* and *S. agalactiae* (BAS), (6) a bivalent vaccine against *P. fluorescens* and *S. agalactiae* (BPS), (7) a polyvalent vaccine against *A. hydrophila*, *P. fluorescens* and *S. agalactiae* (PAPS), and (8) the control, fish injected with PBS solution. However, only the female broodstock was vaccinated.

The vaccination method used was intramuscular (*i.m.*)^{40,41} by injecting between the first and second scales of the dorsal fin and was administered at a dose of 0.4 mL/kg of fish (± 0.08 mL/fish). After the fish were vaccinated, a booster with the same dose as the initial vaccination was later administered on the 7th day. The fish were anesthetized using MS-222 (Sigma) before injection.

The gonad developmental stage 2 fish post-vaccination were reared using 3×3 m cages and installed in dirt ponds 25×30×1.2 (L×W×D). Furthermore, 20 broodstock were reared per cage, consisting of 15 females and 5 males. The fish were fed with pellets at a dose of 4%/day in the morning, at midday, and in the afternoon. The water was replaced daily at a rate of 5%/day. The fish would spawn after being reared for approximately 4 weeks.

Broodstock and larvae immune response

Following vaccinations, the fish's immune response was observed on the 7th, 14th, 21st, and 28th day by collecting caudal vein blood samples. The immune response parameters were the antibody titer using the direct agglutination method⁴², total leukocyte^{9,34,43}, phagocytic^{44,45} and lysozyme activities^{27,34,45,46}.

Random blood sampling from the offspring was conducted on each treatment group on the 10th, 20th, 30th, and 40th day post-hatching period. Serum was collected by grinding 5 offspring in effendorf tube for 5 μ L with PBS-tween at a ratio of 4:1. It was then centrifuged at 6000 rpm. Furthermore, the serum in the second layer of the centrifugation result was harvested and stored at 47°C for 30 minutes to inactivate the complements⁴⁷. It was then stored for agglutination titer and lysozyme activity. The direct agglutination test on both broodstocks and offspring was carried out by adding 25 μ L of whole-cell antigen⁴⁸ of *A. hydrophila*, *P. fluorescens*, and *S. agalactiae* (10⁷ cfu/mL) bacteria into the well, starting from the 1st well to the 12th well. It was found that the last well showed an agglutination reaction. The agglutination test

used 3 types of bacteria at once; *A. hydrophila*, *S. agalactiae*, *P. fluorescens*

Challenge procedures

The offspring challenge test was conducted on the 10, 20, 30, and 40 days old during the post-hatching period. It was carried out by dividing the fish into 7 groups based on the type of vaccine administered plus one unvaccinated. Challenge tests on all treatments were carried out using three types of pathogenic bacteria; *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*. This test was carried out by placing 20 offsprings into containers containing 4 liters of water and then they were immersed for 24 h in water containing pathogenic bacteria at a dose of 2.1×10⁸ cfu/mL according to their relative treatments, each conducted triplicate. To observe the effectiveness of the vaccine, the relative percentage survival (RPS) was calculated^{49,50} on the 14th day post-challenge test.

Data analysis

The data for the specific and non-specific immune response and RPS were analyzed statistically and with Duncan's test (IBM SPSS Statistic 21; Chicago, IL, USA).

Results

Broodstock total leukocyte dan phagocytic activity post-vaccination

In general, the different types of vaccines at each period of post-vaccination had a significant effect ($P < 0.05$) on the broodstock's total leukocyte (Figure 1), and phagocytic activity (Figure 2). The follow-up test showed that the fish vaccinated with PAPS had the highest total leukocyte (7.56–10.70×10⁶ cell/mm³) and phagocytic activity (8.33–19.33%), followed by those vaccinated with bivalent and monovalent vaccines, while the lowest was found in control (total leukocyte was 7.40–7.86×10⁶ cell/mm³, phagocytic activity was 9.00–9.33%).

Broodstock and offspring agglutination titers

The broodstock's antibody (Table 1) increased, especially after the booster, except in the unvaccinated fish. After the peak, the broodstock's immune response remained high up to day 28 even though there was a tendency for it to decrease. All the types of vaccines at each point of time had a significant effect ($P < 0.05$) on the agglutination titer in the broodstock. The Duncan's follow-up test showed that the vaccinated broodstock had a higher agglutination titer than the unvaccinated fish. Also, the highest significant value was found in the vaccinated fish with PAPS (1.67–6.67), followed by those vaccinated with the bivalent and monovalent vaccines, while the lowest was in the control (1.33–1.67). Offspring from unvaccinated broodstocks have native immunity, hence in the agglutination test occurs agglutination, but it is very low and does not show an increase, and has not been able to control infections.

Based on the effect of the vaccine on the broodstock's immune response, the agglutination titer in the offspring from the vaccinated broodstock at ages 10, 20, 30, and 40 days was higher than unvaccinated ($P < 0.05$). The follow-up test showed that PAPS was more effective in increasing the

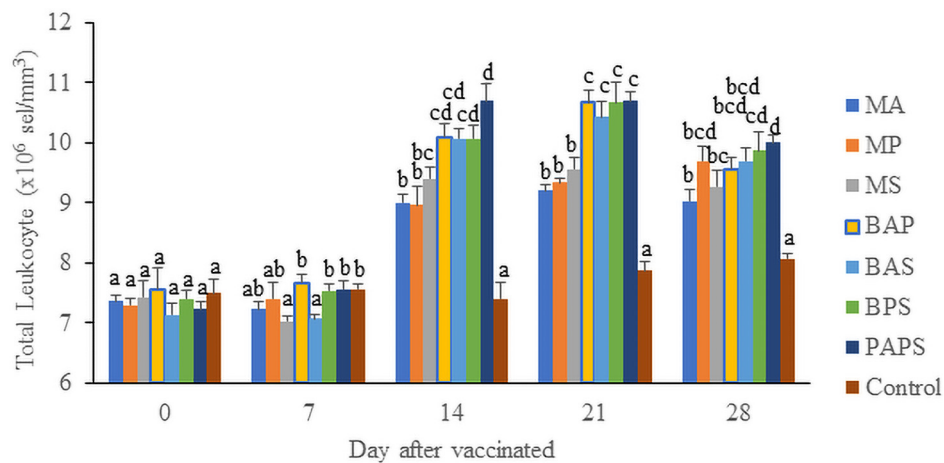


Figure 1. Total leukocyte of tilapia broodstock after the vaccination with various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae*, P: *P. fluorescens*. Values with different superscripts a,b indicate that their corresponding means are significantly different ($P < 0.05$) according to one-way ANOVA followed by Duncan's test.

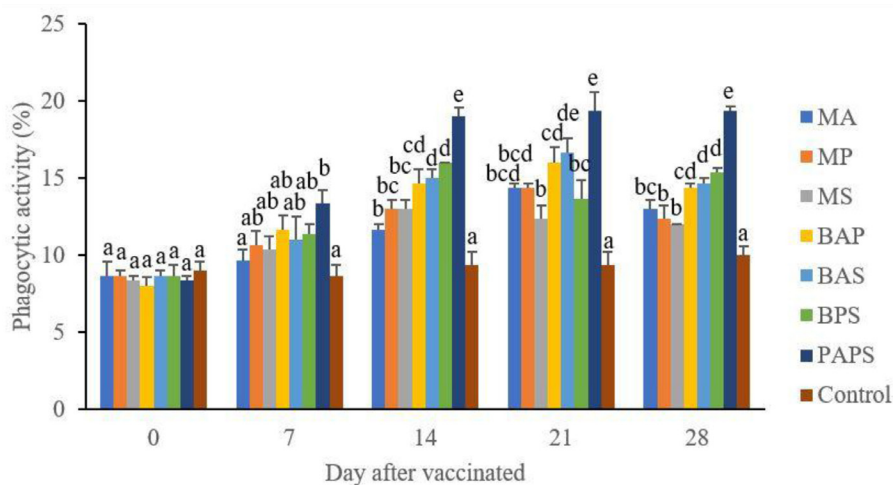


Figure 2. The phagocytic activity in the tilapia broodstock after being vaccinated with the various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae*, P: *P. fluorescens*. Values with different superscripts a,b indicate that their corresponding means are significantly different ($P < 0.05$) according to one-way ANOVA followed by Duncan's test.

agglutination titer in the offspring (6.33–3.00) than the bivalent and monovalent vaccines. The results showed that the administration of vaccines in tilapia broodstock had a significant effect on the maternal immunity transfer to the offsprings that were up to 30 days old (Table 2).

Broodstock and offspring lysozyme activity

The lysozyme activity of broodstock vaccinated with PAPS (29.87–103.08 U/mL) was higher than other vaccines, and the lowest was in broodstock that was not vaccinated (27.65–33.89 U/mL) ($P < 0.05$) (Figure 3). Generally, the offspring from the broodstock vaccinated with PAPS had a higher lysozyme activity (77.81–43.11 U/mL) than those of other treatments ($P < 0.05$) up to the 30th day, the lowest was in the control

(20.29–20.24 U/mL). The results showed that the application of PAPS in tilapia broodstock could increase lysozyme activity transferred to the offsprings (Figure 4).

RPS of offspring post-challenge

Offsprings that were 10, 20, 30, and 40 days old from the vaccinated broodstock had higher RPS than those from the unvaccinated broodstock after being challenged with bacteria. The offsprings from the broodstock that were vaccinated with PAPS had the highest RPS when challenged with 3 bacteria simultaneously (a combination between *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*) (Table 3) up to day 30. The RPS of the offspring vaccinated with PAPS were 86,11% (10 days old), 78,95% (20 days old) and 56,41% (30 days

Table 1. The agglutination titer in Nile tilapia broodstock after being vaccinated with various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae*, P: *P. fluorescens*. Values with different superscripts a,b indicate that their corresponding means are significantly different ($P<0.05$) according to one-way ANOVA followed by Duncan's test.

Type of vaccine	Day after vaccinated (day)				
	0	7	14	21	28
MA	1.67±0.33 ^a	2.00±0.00 ^a	3.33±0.33 ^a	3.67±0.3 ^{bc}	3.67±0.33 ^{bc}
MP	1.67±0.33 ^a	2.67±0.33 ^a	3.67±0.33 ^a	3.33±0.33 ^{bc}	3.33±0.33 ^b
MS	1.33±0.33 ^a	2.33±0.33 ^a	3.33±0.33 ^a	3.00±0.00 ^b	3.33±0.33 ^b
BAP	2.00±0.58 ^a	2.33±0.33 ^a	4.33±0.33 ^{ab}	4.33±0.33 ^c	4.67±0.33 ^{bc}
BAS	1.67±0.33 ^a	2.33±0.33 ^a	4.33±0.33 ^{ab}	4.33±0.33 ^c	4.33±0.88 ^{bc}
BPS	1.67±0.67 ^a	2.33±0.33 ^a	4.33±0.33 ^{ab}	4.33±0.33 ^c	5.00±0.58 ^c
PAPS	1.67±0.33 ^a	3.67±0.33 ^b	5.33±0.33 ^b	6.67±0.33 ^d	6.67±0.33 ^d
Control	1.67±0.33 ^a	1.67±0.33 ^a	1.33±0.33 ^a	1.33±0.33 ^a	1.67±0.33 ^a

Table 2. The agglutination titer of tilapia offspring from maternal immunity produced by various types of vaccines at the ages of 10, 20, 30 and 40 days post-hatching (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae*, P: *P. fluorescens*. Values with different superscripts a,b indicate that their corresponding means are significantly different ($P<0.05$) according to one-way ANOVA followed by Duncan's test.

Type of vaccine	Day post-hatching (day)			
	10	20	30	40
MA	4.00±0.58 ^{ab}	3.67±0.33 ^{bc}	1.67±0.33 ^a	1.33±0.33 ^a
MP	4.00±0.00 ^{ab}	3.67±0.33 ^{bc}	1.67±0.33 ^a	1.33±0.33 ^a
MS	3.67±0.33 ^b	3.33±0.33 ^b	2.33±0.33 ^{ab}	1.33±0.33 ^a
BAP	4.67±0.33 ^{ab}	4.67±0.33 ^c	2.33±0.33 ^{ab}	1.67±0.33 ^a
BAS	5.00±0.58 ^c	4.33±0.33 ^{bc}	2.33±0.33 ^{ab}	1.67±0.33 ^a
BPS	4.33±0.33 ^{ab}	4.33±0.33 ^{bc}	2.33±0.33 ^{ab}	1.33±0.33 ^a
PAPS	6.33±0.33 ^d	5.67±0.33 ^d	3.00±0.33 ^b	1.67±0.33 ^a
Control	1.67±0.33 ^a	1.67±0.33 ^a	1.67±0.33 ^a	1.33±0.33 ^a

old). The immune response generated through maternal immunity only lasts up to 30 days and in the end, the immune response will be formed by the body of the offspring itself.

Discussion

Efforts to produce seeds that are immune to several diseases were the best alternative to increasing Nile tilapia production. Furthermore, PAPSs for *A. hydrophila*, *S. agalactiae*, and *P. fluorescens* were able to improve the broodstock's immune response which was then transferred to the offspring. This process was carried out in order to produce offspring that possess both lysozyme and antibodies and a high survival

rate post-challenge test using pathogenic bacteria. This was better than the other treatments that made use of the bivalent and monovalent vaccines.

The results from the observation of the broodstock for 28 days showed that the total leukocyte (Figure 1), phagocytic (Figure 2), antibody titer (Table 1), and lysozyme activity (Figure 3), started to increase in week two post-vaccination. The broodstock vaccinated with PAPS showed a higher increase in the immune response compared to the others that were vaccinated with the bivalent, monovalent vaccines, and was the lowest in the unvaccinated broodstock^{28,30,33,34,51}. This

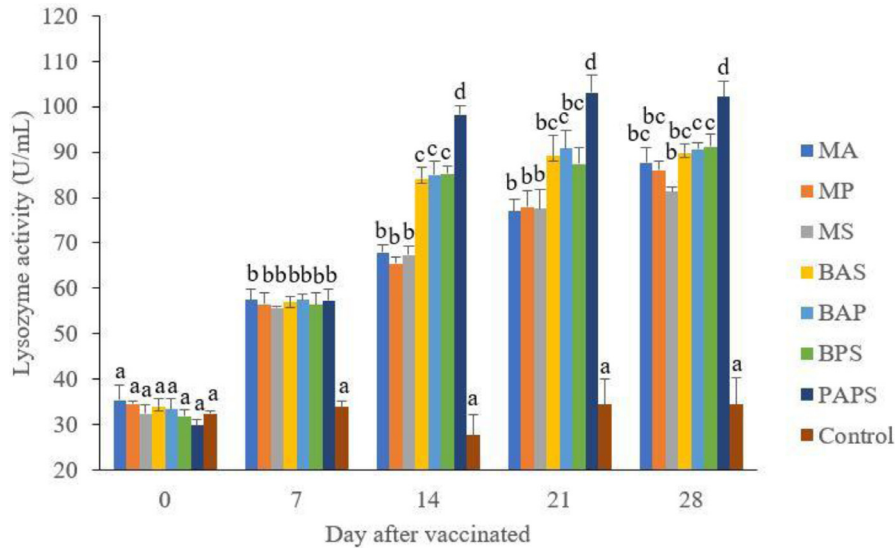


Figure 3. The lysozyme activity in the tilapia broodstock after being vaccinated with the various types of vaccines (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae*, P: *P. fluorescens*. Values with different superscripts a,b indicate that their corresponding means are significantly different ($P<0.05$) according to one-way ANOVA followed by Duncan's test.

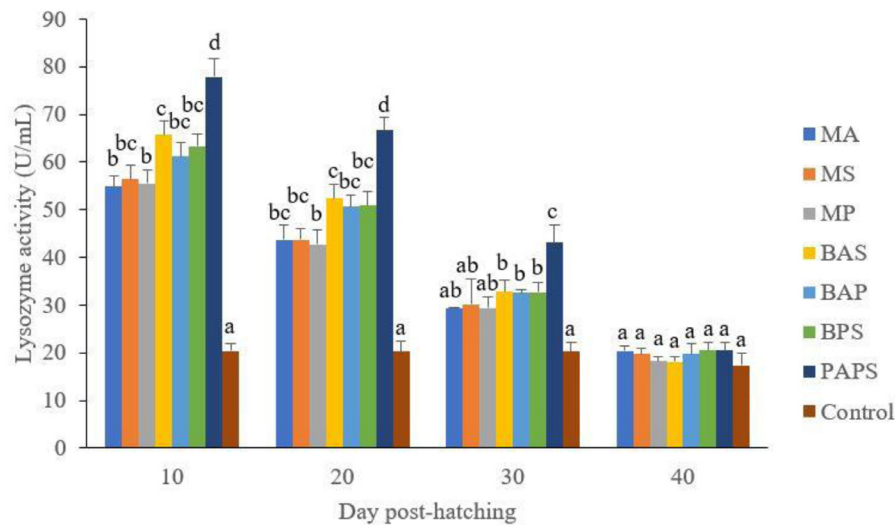


Figure 4. The lysozyme activity of tilapia offspring from maternal immunity produced by various types of vaccines at the ages of 10, 20, 30 and 40 days post-hatching (mean±SE). M: monovalent, B: Bivalent, P: Polyvalent vaccine, A: *A. hydrophila*, S: *S. agalactiae*, P: *P. fluorescens*. Values with different superscripts a,b indicate that their corresponding means are significantly different ($P<0.05$) according to one-way ANOVA followed by Duncan's test.

showed that PAPS could increase the Nile tilapia broodstock's immune response better than the other treatments.

The offspring produced from the broodstock that were vaccinated with PAPS had the highest antibodies (Table 2) and lysozyme activity (Figure 4) up to the 30th day post-hatching period and was the lowest in the offsprings from the unvaccinated broodstock ($P<0.05$). This demonstrated that their strong immune response was transferred to their offsprings^{27–29,33,34,52} through the egg yolk⁵³.

The results from the challenge test using pathogenic bacteria (Table 3) showed that the offsprings that were produced using PAPS had a higher RPS compared to those from the offsprings produced from broodstocks that were treated using the monovalent and bivalent vaccines ($P<0.05$). This further showed that the vaccine treatment had adequately protected the fish from bacterial diseases with an RPS that was greater than 60% up to the 30th day post-hatching period⁴⁹. RPS of the offspring vaccinated with formalin-inactivated vaccine in this study was higher at same time and lasted

Table 3. The Relative Percentage Survival (RPS) of tilapia offspring from maternal immunity produced by various types of vaccines at the ages of 10, 20, 30 and 40 days post-hatching. The offspring were produced by broodstock vaccinated with various types of vaccines through intramuscular (i.m.) injection (mean±SE).

Type of vaccine	Day post-hatching (day)			
	10	20	30	40
MA	66.67±4.81 ^a	55.26±5.26 ^a	41.03±2.56 ^a	14.29±4.96 ^a
MP	61.11±2.78 ^a	50.00±6.96 ^a	41.03±2.56 ^a	14.29±4.96 ^a
MS	63.89±2.78 ^a	52.63±4.56 ^a	43.59±2.56 ^a	17.14±2.86 ^a
BAP	72.22±2.78 ^a	60.53±4.56 ^a	46.15±4.44 ^{ab}	11.43±7.56 ^a
BAS	69.44±2.78 ^a	60.53±4.56 ^a	46.15±4.44 ^{ab}	14.29±4.95 ^a
BPS	69.44±7.35 ^a	57.89±6.96 ^a	43.59±2.56 ^a	11.43±2.86 ^a
PAPS	86.11±2.78 ^b	78.95±2.63 ^b	56.41±5.13 ^b	20.00±2.86 ^a

longer than the findings of Nurani *et al.*³⁴ on days 10 and 20, closely similar to the Sukenda *et al.*¹⁸ and Pasaribu *et al.*⁵⁴, but higher on day 20. The high RPS in the offspring during the challenge test using pathogenic bacteria in PAPS treatment was due to the broodstock's high number of leukocytes, phagocytic activity, the amount of antibody, and lysozyme activity transferred to the offsprings for protection against diseases. Meanwhile, in the control (unvaccinated), it only relies on immunity transferred naturally from the broodstock, whereas in the vaccinated broodstock, the offspring also get immunity from the broodstock which is induced by the vaccine. The existence of vaccine induction in the broodstocks can increase the total leukocytes, phagocytic activity, antibodies, and lysozyme activity of the offspring which are higher than the offspring produced from unvaccinated broodstocks. Thus, in the challenge test, the immune response of the vaccinated offspring is sufficient to control bacterial attacks, while the control offspring have not been able to control bacterial attacks. Compared to the Abu-elala *et al.*³³ study, the offspring RPS was higher and could last up to 3 months, whereas in this study, the PAPS vaccine was lower and only lasted up to days 30. The low RPS of the PAPS vaccine can be improved by the use of adjuvants, the use of quality tilapia broodstock, proper nutrition in terms of quality and quantity, and the application of biosecurity in the hatchery³³.

The role of leukocytes which consist of neutrophils, lymphocytes, and monocytes, is to infiltrate the infected area for rapid protection⁵⁵, stimulating the production of antibodies through the recognition of foreign bodies, including vaccines and pathogens during the challenge test in this study. The phagocytic activity occurs during phagocytosis, which involves antibodies and complements during opsonization. Furthermore, the total leukocyte parameter increases in line with other immune responses, such as the antibacterial lysozyme, which triggers the complement system and phagocytic cells⁵⁶⁻⁵⁸. It encourages phagocytosis by activating leukocytes and polymorphonuclear macrophages or through opsonization⁵⁹. The high number of leukocytes and a large amount of lysozyme

in the treatment using PAPS which is similar to an infection by a pathogen indicated the success of PAPS in triggering the fish's immune system when developing an immune response.

The offsprings produced by the broodstock that were vaccinated with PAPS were protected from infections by *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*. However, the monovalent vaccines only protected the offsprings from one type of bacteria. This is one of the advantages of applying PAPS. The results of this study revealed that the application of PAPS produced broodstock and offspring with better immune responses than the bivalent and monovalent vaccines. Therefore, the development of a polyvalent vaccine is more prudent than that of bivalent or monovalent because of its ability to target more than one species of bacteria^{31,51,52,60-63}. The use of this type of vaccine caused the fish to respond to multiple antigens and form an immune response, thereby making it a strategic method in controlling bacterial diseases commonly found in culture and breeding environments^{33,34,52,64}. Additionally, the application of polyvalent vaccines is more practical than the monovalent containing only one type of antigen. This showed that PAPS provided the most effective protection against diseases caused by pathogenic bacteria that often affect fishes, and thus is an ideal candidate for developing a polyvalent vaccine against bacterial infection.

Conclusion

The results show that the application of the polyvalent vaccine against *A. hydrophila*, *S. agalactiae*, and *P. fluorescens* increased the antibody, lysozyme, total leukocytes, and phagocytic activity in Nile tilapia broodstock which was transferred to their offsprings, leading to a high RPS during the challenge test. Therefore, it is possible to produce seeds of Nile tilapia that are immune to diseases caused by *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*. This process could be carried out through the vaccination of the broodstocks using a polyvalent vaccine against *A. hydrophila*, *S. agalactiae*, and *P. fluorescens*.

Ethical statement

Research using fish in Indonesia has not been regulated and therefore it does not require animal ethics. However, this research has received approval from the Ministry of Education and Culture of the Republic of Indonesia (No.: 004/PL.22.7.1/SP-PG/2019). In addition, this study applies the principle of the International Animal Welfare standards including the assurance of fish welfare during maintenance and the use of drugs during sampling.

Data availability

Underlying data

OSF: Underlying data for ‘**Transfer of maternal immunity using a polyvalent vaccine and offspring protection in**

Nile tilapia, *Oreochromis niloticus*’. <https://doi.org/10.31219/osf.io/cnqdg65>

The project contains the following underlying data:

Data on broodstock immune response, offspring immune response, and offspring RPS in tilapia, *O. niloticus* can be accessed on OSF

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgments

Special gratitude also goes to the Director of Pangkep State Polytechnique of Agriculture, South Sulawesi, Indonesia for allowing the sample analysed in the laboratory.

References

- Klesius P, Shoemaker C, Evans J: **Streptococcus: a worldwide fish health problem**. In: *8th international symposium on tilapia in aquaculture*. 2008; 83–107. [Reference Source](#)
- Ismail MS, Syafiq MR, Siti-Zahrah A, et al.: **The effect of feed-based vaccination on tilapia farm endemic for streptococcosis**. *Fish Shellfish Immunol.* 2017; **60**: 21–24. [PubMed Abstract](#) | [Publisher Full Text](#)
- Shirajum Monir M, Yusoff SM, Mohamad A, et al.: **Vaccination of Tilapia against Motile *Aeromonas* Septicemia: A Review**. *J Aquat Anim Health*. American Fisheries Society, 2020; **32**(2): 65–76. [PubMed Abstract](#) | [Publisher Full Text](#)
- Yue F, Zhou Z, Wang L, et al.: **Maternal transfer of immunity in scallop *Chlamys farreri* and its trans-generational immune protection to offspring against bacterial challenge**. *Dev Comp Immunol.* 2013; **41**(4): 569–77. [PubMed Abstract](#) | [Publisher Full Text](#)
- Laith AA, Abdullah MA, Nurhafizah WWI, et al.: **Efficacy of live attenuated vaccine derived from the *Streptococcus agalactiae* on the immune responses of *Oreochromis niloticus***. *Fish Shellfish Immunol.* 2019; **90**: 235–243. [PubMed Abstract](#) | [Publisher Full Text](#)
- Munang'andu HM, Salinas I, Tafalla C, et al.: **Editorial: Vaccines and Immunostimulants for Finfish**. *Front Immunol.* 2020; **11**: 573771. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Yassine T, Khalafalla MM, Mamdouh M, et al.: **The enhancement of the growth rate, intestinal health, expression of immune-related genes, and resistance against suboptimal water temperature in common carp (*Cyprinus carpio*) by dietary paraprobiotics**. *Aquac Reports.* 2021; **20**: 100729. [Publisher Full Text](#)
- Chitmanat C, Lebel P, Whangchai N, et al.: **Tilapia diseases and management in river-based cage aquaculture in northern Thailand**. *J Appl Aquac.* 2016; **28**(1): 9–16. [Publisher Full Text](#)
- Hardi EH, Nugroho RA, Kusuma IW, et al.: **Borneo herbal plant extracts as a natural medication for prophylaxis and treatment of *Aeromonas hydrophila* and *Pseudomonas fluorescens* infection in Tilapia (*Oreochromis niloticus*)** [version 2; peer review: 2 approved, 1 approved with reservations]. *F1000Res.* 2019; **7**: 1847. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kayansamruaj P, Areechon N, Unajak S: **Development of fish vaccine in Southeast Asia: A challenge for the sustainability of SE Asia aquaculture**. *Fish Shellfish Immunol.* 2020; **103**: 73–87. [PubMed Abstract](#) | [Publisher Full Text](#)
- Surachetpong W, Janetanakit T, Nonthabenjawan N, et al.: **Outbreaks of tilapia lake virus infection, Thailand, 2015–2016**. *Emerg Infect Dis.* 2017; **23**(6): 1031–1033. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Abdullah A, Ramly R, Ridzwan MSM, et al.: **First detection of tilapia lake virus (TiLV) in wild river carp (*Barbonymus schwanenfeldii*) at Timah Tasoh Lake, Malaysia**. *J Fish Dis.* 2018; **41**(9): 1459–1462. [PubMed Abstract](#) | [Publisher Full Text](#)
- Nayak SK: **Current prospects and challenges in fish vaccine development in India with special reference to *Aeromonas hydrophila* vaccine**. *Fish Shellfish Immunol.* 2020; **100**: 283–299. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wang Q, Ji W, Xu Z: **Current use and development of fish vaccines in China**. *Fish Shellfish Immunol.* 2020; **96**: 223–234. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zhao Z, Zhang C, Lina Q, et al.: **Single-walled carbon nanotubes as delivery vehicles enhance the immunoprotective effect of an immersion DNA vaccine against infectious spleen and kidney necrosis virus in mandarin fish**. *Fish Shellfish Immunol.* 2020; **97**: 432–439. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zhang Z, Liu G, Ma R, et al.: **The immunoprotective effect of whole-cell lysed inactivated vaccine with SWCNT as a carrier against *Aeromonas hydrophila* infection in grass carp**. *Fish Shellfish Immunol.* 2020; **97**: 336–343. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zapata A, Diez B, Cejalvo T, et al.: **Ontogeny of the immune system of fish**. *Fish and Shellfish Immunol.* 2006; **20**(2): 126–36. [PubMed Abstract](#) | [Publisher Full Text](#)
- Sukenda, Rahman, Nisaa K, et al.: **The efficacy of *Streptococcus agalactiae* vaccine preparations, administered to tilapia broodstock, in preventing streptococcosis in their offspring, via transfer of maternal immunity**. *Aquac Int.* 2018; **26**: 785–798. [Publisher Full Text](#)
- Poorten TJ, Kuhn RE: **Maternal transfer of antibodies to eggs in *Xenopus laevis***. *Dev Comp Immunol.* 2009; **33**(2): 171–5. [PubMed Abstract](#) | [Publisher Full Text](#)
- Swain P, Dash S, Bal J, et al.: **Passive transfer of maternal antibodies and their existence in eggs, larvae and fry of Indian major carp, *Labeo rohita* (Ham.)**. *Fish Shellfish Immunol.* 2006; **20**(4): 519–527. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zapata AG, Torroba M, Varas A, et al.: **Immunity in fish larvae**. *Dev Biol Stand.* 1997; **90**: 23–32. [PubMed Abstract](#)
- Magnadottir B, Lange S, Gudmundsdottir S, et al.: **Ontogeny of humoral immune parameters in fish**. *Fish Shellfish Immunol.* 2005; **19**(5): 429–439. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wang H, Ji D, Shao J, et al.: **Maternal transfer and protective role of antibodies in zebrafish *Danio rerio***. *Mol Immunol.* 2012; **51**(3–4): 332–6. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zhang S, Wang Z, Wang H: **Maternal immunity in fish**. *Dev Comp Immunol.* 2013; **39**(1–2): 72–8. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gilman CL, Soon R, Sauvage L, et al.: **Umbilical cord blood and placental mercury, selenium and selenoprotein expression in relation to maternal fish consumption**. *J Trace Elem Med Biol.* 2015; **30**: 17–24. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Itoi S, Suzuki M, Asahina K, et al.: **Role of maternal tetrodotoxin in survival of larval pufferfish**. *Toxicon.* 2018; **148**: 95–100. [PubMed Abstract](#) | [Publisher Full Text](#)

27. Hanif A, Bakopoulos V, Dimitriadis GJ: **Maternal transfer of humoral specific and non-specific immune parameters to sea bream (*Sparus aurata*) larvae.** *Fish Shellfish Immunol.* 2004; **17**(5): 411–35.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Nisaa K, Sukenda, Junior MZ, et al.: **Fry tilapia (*Oreochromis niloticus*) antibody improvement against *Streptococcus agalactiae* through broodstock vaccination.** *Pakistan J Biotechnol.* 2017.
[Reference Source](#)
29. Liu X, Sun W, Jian S, et al.: **Passive protective ability of the outer membrane protein PF1380 of *Pseudomonas fluorescens* against the major pathogenic bacteria of freshwater aquaculture in fish.** *Aquac Rep.* 2022; **22**: 100985.
[Publisher Full Text](#)
30. Wang J, He RZ, Lu GL, et al.: **Vaccine-induced antibody level as the parameter of the influence of environmental salinity on vaccine efficacy in Nile tilapia.** *Fish Shellfish Immunol.* 2018; **82**: 522–530.
[PubMed Abstract](#) | [Publisher Full Text](#)
31. Cheng ZX, Chu X, Wang S, et al.: **Six genes of ompA family shuffling for development of polyvalent vaccines against *Vibrio alginolyticus* and *Edwardsiella tarda*.** *Fish Shellfish Immunol.* 2018; **75**: 308–315.
[PubMed Abstract](#) | [Publisher Full Text](#)
32. Hoare R, Jung SJ, Ngo TPH: **Efficacy and safety of a non-mineral oil adjuvanted injectable vaccine for the protection of Atlantic salmon (*Salmo salar* L.) against *Flavobacterium psychrophilum*.** *Fish Shellfish Immunol.* 2019; **85**: 44–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Abu-Elala NM, Samir A, Wasfy M, et al.: **Efficacy of Injectable and Immersion Polyvalent Vaccine against Streptococcal Infections in Broodstock and Offspring of Nile tilapia (*Oreochromis niloticus*).** *Fish Shellfish Immunol.* 2019; **88**: 293–300.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Nurani FS, Sukenda, Nuryati S: **Maternal immunity of tilapia broodstock vaccinated with polyvalent vaccine and resistance of their offspring against *Streptococcus agalactiae*.** *Aquac Res.* 2020; **51**(4): 1513–1522.
[Publisher Full Text](#)
35. Ekasari J, Rivandi DR, Firdausi AP, et al.: **Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance.** *Aquaculture.* 2015.
[Publisher Full Text](#)
36. Mo XB, Wang J, Guo S, et al.: **Potential of naturally attenuated *Streptococcus agalactiae* as a live vaccine in Nile tilapia (*Oreochromis niloticus*).** *Aquaculture.* 2020; **518**: 734774.
[Publisher Full Text](#)
37. Hal AM, El-Barbary MI: **Gene expression and histopathological changes of Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila* and *Pseudomonas fluorescens*.** *Aquaculture.* 2020; **526**: 735392.
[Publisher Full Text](#)
38. Mahmoud MMA, El-Lamie MMM, Kilany OE, et al.: **Spirulina (*Arthrospira platensis*) supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (*Oreochromis niloticus*) challenged with *Pseudomonas fluorescens*.** *Fish Shellfish Immunol.* 2018; **72**: 291–300.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Amrullah, Baga I, Jaya AA: **Offspring production of Nile resistant to Streptococcosis and Motile Aeromonad Septicemia to increase productivity of tilapia in South Sulawesi.** *MP3EI Research Final Report 2017.* Department of Aquaculture, Pangkep State Polytechnic of Agriculture, **107**.
40. Kanlis G, Suzuki Y, Tauchi M, et al.: **Immunoglobulin in Oocytes, Fertilized Eggs, and Yolk Sac Larvae of Red Sea Bream.** *Fish Sci.* 1995; **61**(5): 787–790.
[Publisher Full Text](#)
41. Silva BC, Martins ML, Jatobá A, et al.: **Hematological and immunological responses of Nile tilapia after polyvalent vaccine administration by different routes.** *Pesqui Vet Bras.* 2009; **29**(11): 874–880.
[Publisher Full Text](#)
42. Anderson DP: **Fish immunology: Book 4.** (TFH Publications, Inc., 1974).
[Reference Source](#)
43. Blaxhall PC, Daisley KW: **Routine haematological methods for use with fish blood.** *J Fish Biol.* 1973; **5**(6): 771–781.
[Publisher Full Text](#)
44. Anderson D, Siwicki A: **Basic hematology and serology for fish health programs.** In: ed: Shariff M, Arthur JR, Subasinghe RP. *Diseases in Asian aquaculture II.* in *Fish Health Science.* Asian Fisheries Society, Manila, 1995.
[Reference Source](#)
45. Cavalcante RB, Telli GS, Tachibana L, et al.: **Probiotics, Prebiotics and Synbiotics for Nile tilapia: Growth performance and protection against *Aeromonas hydrophila* infection.** *Aquac Rep.* 2020; **19**: 100343.
[Publisher Full Text](#)
46. Tachibana L, Telli GS, Dias DDC, et al.: **Effect of feeding strategy of probiotic *Enterococcus faecium* on growth performance, hematologic, biochemical parameters and non-specific immune response of Nile tilapia.** *Aquac Reports.* 2020; **16**: 100277.
[Publisher Full Text](#)
47. Sakai DK: **Opsonization by fish antibody and complement in the immune phagocytosis by peritoneal exudate cells isolated from salmonid fishes.** *J Fish Dis.* 1984; **7**(1): 29–38.
[Publisher Full Text](#)
48. Hardi EH, Sukarti K, Agriandini M, et al.: **The comparative studies of Borneo plant extracts to increases vaccine efficacy in tilapia, *Oreochromis niloticus*.** *J Akuakultur Indones.* 2018; **17**(2): 158–167.
[Publisher Full Text](#)
49. Amend DF: **Potency testing of fish vaccines.** *Dev Biol Stand.* 1980; **49**: 447–454.
[Reference Source](#)
50. Xu H, Xing J, Tang X, et al.: **The effects of CCL3, CCL4, CCL19 and CCL21 as molecular adjuvants on the immune response to VAA DNA vaccine in flounder (*Paralichthys olivaceus*).** *Dev Comp Immunol.* 2020; **103**: 103492.
[PubMed Abstract](#) | [Publisher Full Text](#)
51. Li H, Chu X, Peng B, et al.: **DNA shuffling approach for recombinant polyvalent OmpAs against *V. alginolyticus* and *E. tarda* infections.** *Fish Shellfish Immunol.* 2016; **58**: 508–513.
[PubMed Abstract](#) | [Publisher Full Text](#)
52. Grindstaff JL: **Maternal antibodies reduce costs of an immune response during development.** *J Exp Biol.* 2008; **211**(Pt 5): 654–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
53. Swain P, Nayak SK: **Role of maternally derived immunity in fish.** *Fish Shellfish Immunol.* 2009; **27**(2): 89–99.
[PubMed Abstract](#) | [Publisher Full Text](#)
54. Pasaribu W, Sukenda S, Nuryati S: **The efficacy of Nile tilapia (*Oreochromis niloticus*) broodstock and larval immunization against *Streptococcus agalactiae* and *Aeromonas hydrophila*.** *Fishes.* 2018; **3**(1): 16.
[Publisher Full Text](#)
55. Risjani Y, Yuniarta, Couteau J, et al.: **Cellular immune responses and phagocytic activity of fishes exposed to pollution of volcano mud.** *Mar Environ Res.* 2014; **96**: 73–80.
[PubMed Abstract](#) | [Publisher Full Text](#)
56. Jollès P, Jollès J: **What's new in lysozyme research? Always a model system, today as yesterday.** *Mol Cell Biochem.* 1984; **63**(2): 165–89.
[PubMed Abstract](#) | [Publisher Full Text](#)
57. Grinde B, Lie Ø, Poppe T, et al.: **Species and individual variation in lysozyme activity in fish of interest in aquaculture.** *Aquaculture.* 1988; **68**(4): 299–304.
[Publisher Full Text](#)
58. Panase P, Saenphet S, Saenphet K: **Visceral and serum lysozyme activities in some freshwater fish (three catfish and two carps).** *Comp Clin Path.* 2017; **26**: 169–173.
[Publisher Full Text](#)
59. Saurabh S, Sahoo PK: **Lysozyme: An important defence molecule of fish innate immune system.** *Aqua Res.* 2008; **39**(3): 223–239.
[Publisher Full Text](#)
60. Nikoskelainen S, Verho S, Järvinen S, et al.: **Multiple whole bacterial antigens in polyvalent vaccine may result in inhibition of specific responses in rainbow trout (*Oncorhynchus mykiss*).** *Fish Shellfish Immunol.* 2007; **22**(3): 206–17.
[PubMed Abstract](#) | [Publisher Full Text](#)
61. Peng B, Ye JZ, Han Y, et al.: **Identification of polyvalent protective immunogens from outer membrane proteins in *Vibrio parahaemolyticus* to protect fish against bacterial infection.** *Fish Shellfish Immunol.* 2016; **54**: 204–10.
[PubMed Abstract](#) | [Publisher Full Text](#)
62. Park S, Nho SW, Jang HB, et al.: **Development of three-valent vaccine against streptococcal infections in olive flounder, *Paralichthys olivaceus*.** *Aquaculture.* 2016; **461**: 25–31.
[Publisher Full Text](#)
63. Peng B, Lin XP, Wang SN, et al.: **Polyvalent protective immunogens identified from outer membrane proteins of *Vibrio parahaemolyticus* and their induced innate immune response.** *Fish Shellfish Immunol.* 2018; **72**: 104–110.
[PubMed Abstract](#) | [Publisher Full Text](#)
64. Plant KP, LaPatra SE: **Advances in fish vaccine delivery.** *Dev Comp Immunol.* 2011; **35**(12): 1256–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
65. Amrullah: **Transfer of maternal immunity project.** *Center for Open Science.* 2021.
<http://www.doi.org/10.31219/osf.io/cnqgd>

Open Peer Review

Current Peer Review Status: ? ✓

Version 2

Reviewer Report 04 August 2022

<https://doi.org/10.5256/f1000research.128337.r140937>

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Please refer to the highlights in the [attached pdf](#).

P3: Intro

Para 1

Streptococcus agalactia, Aeromonas hydrophila, Edwardsiella ictaluri, Flavobacterium columnaris, and Pseudomonas fluorescens are the pathogens. Disease refer to the infection, for examples Streptococcus agalactiae infection, Aeromonas hydrophila infection. Please revise. Consider 'Streptococcus agalactiae, Aeromonas hydrophila, Edwardsiella ictaluri, Flavobacterium columnaris, and Pseudomonas fluorescens infections'.

Tilapia lake virus is the pathogen, the disease is tilapia lake virus disease.

Para 2

'The offspring of fish' instead of 'their offspring' because 'they' refers to 'vaccines'

Para 4

It isn't applying polyvalent vaccines in offspring through maternal immunity, but 'application of polyvalent vaccines to confer maternal immunity in offspring'. Please rephrase.

Methods

Para 1, experimental animal

What size was the spawning ponds that you managed to replace 25% of water daily? Please provide the dimension (LxWxD).

Para 2, vaccine production

The test vaccine. Please provide reasons why *S.agalactiae* and *P. fluorescens* were inactivated with 1% formalin while *A.hydrophila* inactivated with 0.6%?

P4: Para 1, vaccine treatments and administration

Did you vaccinate both female and male broodstocks, or only female? Please mention.

Revise 'However, before being injected with the vaccines, they were first anesthetized using MS-222, Sigma.'

Consider: The fish were anesthetized using MS-222 (Sigma) prior to injection.

Para 2

25x30x1.2 m (LxWxD)

Para 3

Broodstock and larvae immune response.

Intramuscular blood samples? Not caudal vein?

Para 4

Instead of 'post-spawning period', 'post-hatching period' will better reflect the offspring size. Please revise.

How many offspring was ground in a tube, and what kind of tube was it?

Why was there a range of 5-10 minutes centrifugation time? If the samples were centrifuged for different lengths of time, would it have affected the parameters later? Please clarify.

Inactivate the complements or components?

What type of antigen? Whole-cell antigen?

Para 5: Challenge procedures

How long was the immersion in water containing pathogenic bacteria?

P4 Results

Broodstock total leukocyte dan phagocytic activity post vaccination

PAPs did not result in highest total leukocyte in different time frames. BAP was highest on day 14.

P5, para 1: Broodstock and offspring agglutination titers

Please provide explanation as to why the unvaccinated fish (control) also show agglutination titer (although lower).

Para 4, RPS of offspring post-challenge

86.11% (10 days old), 78.95% (20 days old) and 56.41% (30 days old)

Please provide explanation as to why the offspring of PAPS vaccinated broodstock encountered drops of RPS from 10 days old to 30 days old.

P5, Discussion, para 1

Was carried out in order to produce...

P7, Para 1

The statement 'in the control (unvaccinated), there was no transfer of immunity from the mother' is not generally true. If the unvaccinated broodstocks have acquired immunity from prior infections, the immunity will have been passed on to the offspring as maternal immunity. It is only true that the offspring of the unvaccinated broodstocks did not have the vaccine-induced maternal immunity.

The statement 'the offspring hasn't been able to produce their own immune response, so the total leukocyte, phagocytic activity, antibody, and low lysozyme activity' is not true. The offspring of the unvaccinated broodstocks did show immune response to the bacterial challenge but at the lower level compared with vaccinated group.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquatic animal health, microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 28 January 2022

<https://doi.org/10.5256/f1000research.56264.r101122>

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The work is clearly and accurately presented. It is interesting research and I hope they can further study for farm application. However, the other serious bacteria pathogen is missing. Please add more review about *Flavobacterium columnare*. In addition, the viral pathogen doesn't be mentioned. It seems survival rates were quite low after bacterial challenge. Please discuss about low survival and how to improve it.

This work, of course, has academic merit. This study was well designed, the details of the methods are enough and they could be replicated, and the statistical analysis was appropriate. However, please discuss more about the negative control. No challenge test for control groups? All the source data underlying the results were available to ensure full reproducibility and the conclusions are drawn adequately and supported by the results. However, I just wonder about the TiLV problem? Do you plan to produce vaccines?

In addition to the previous comments, **enclosed** is the manuscript with some additional comments.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: fish immunology, fish diseases, aquaculture, aquaculture extension

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 December 2021

<https://doi.org/10.5256/f1000research.56264.r98048>

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Summary

The study examined the transfer of vaccine-induced maternal immunity in Nile tilapia, *Oreochromis niloticus* against *Aeromonas hydrophila*, *Streptococcus agalactiae* and *Pseudomonas fluorescens*. The protective effects of monovalent, bivalent and polyvalent vaccines were compared. The relative percentage survival in immersion challenges, agglutination titers and lysozyme activities indicated that the polyvalent vaccine induced significantly better immune response compared with the bivalent, monovalent and unvaccinated groups.

Part of the introduction is rather brief. Suggestion for improvement as follows:

1. Provide more references on vaccination in tilapia. The following two contain some of the relevant information
<https://doi.org/10.1002/aah.10099>
<https://doi.org/10.1016/j.fsi.2019.04.052>
2. Until which stage of offspring is the immune system not ready for immune response? Juvenile? Please elaborate more.
3. What types of Ig are transferable through eggs? Please elaborate.

Part of the method description is rather brief and lacks references. Suggestion for improvements as follows:

1. Provide the reference for the two formalin concentrations used for inactivation of bacteria.
2. Mention the site of IM injection and provide the reference.
3. Mention the final bacterial concentration (cfu/mL) in the vaccines used at 0.4 mL/ kg.
4. Mention the size of the dirt ponds.
5. Detail the antigen preparation for direct agglutination test. Was it monovalent, bivalent or polyvalent?

Please see some additional annotations [here](#).

References

1. Shirajum Monir M, Yusoff SM, Mohamad A, Ina-Salwany MY: Vaccination of Tilapia against Motile *Aeromonas* Septicemia: A Review. *J Aquat Anim Health*. **32** (2): 65-76 [PubMed Abstract](#) | [Publisher](#)

Full Text

2. Laith AA, Abdullah MA, Nurhafizah WWI, Hussein HA, et al.: Efficacy of live attenuated vaccine derived from the *Streptococcus agalactiae* on the immune responses of *Oreochromis niloticus*. *Fish Shellfish Immunol.* 2019; **90**: 235-243 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquatic animal health, microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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