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Laboratory and Field Assessments of Oral *Vibrio* Vaccine Indicate the Potential for Protection against Vibriosis in Cultured Marine Fishes

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Abstract

Vibriosis is one of the most common threats to farmed grouper; thus, substantial efforts are underway to control the disease. This study presents an oral vaccination against multiple *Vibrio* spp. in a marine fish with double booster immunisation. The *Vibrio harveyi* strain VH1 vaccine candidate was selected from infected groupers *Epinephelus* sp. in a local farm and was formalin inactivated and combined with commercial feed at a 10% ratio (v/w). A laboratory vaccination trial was conducted for seventy days. The induction of IgM antibody responses in the serum of Asian seabass *Lates calcarifer* immunised with the oral *Vibrio harveyi* strain VH1 was significantly ($p < 0.05$) increased as early as week one post-primary vaccination. Subsequent administration of the first and second booster for 5 consecutive days, starting on days 14 and 42, respectively, improved the specific antibody level and reached a highly significant ($p < 0.05$) value at days 35 and 49 before slightly decreasing from day 56 onwards. Antibody titres of the control unvaccinated group remained relatively stable and low throughout the experimental period. At the end of the 70-day vaccination trial, 23 days post final boost, an intraperitoneal challenge with a field strain of *Vibrio harveyi*, *V. alginolyticus*, and *V. parahaemolyticus* was carried out. Our challenge study showed that oral *Vibrio harveyi* strain VH1 vaccine candidate could induce significant protection, with an RPS of 70–80% against different *Vibrio* species. Thereafter, a field trial was conducted in a mariculture farm to study the effect of field vaccination using the oral *Vibrio harveyi* strain VH1 vaccine candidate. A total of 3000 hybrid grouper juveniles were divided into two groups in triplicate. Fish of Group 1 were not vaccinated, while Group 2 were vaccinated with the feed-based vaccine. Vaccinations were carried out on days 0, 14, and 42 via feeding the fish with the vaccine at 4% body weight for 5 consecutive days. At the end of the study period, the fish survival rate was 80% for the vaccinated group, significantly ($p < 0.05$) higher than the 65% seen in the control unvaccinated group. Furthermore, the vaccinated fish showed significantly ($p < 0.05$) better growth performances. Therefore, the oral *Vibrio* vaccine from the inactivated *Vibrio harveyi* strain VH1 is a potential versatile vaccine candidate that could stimulate good immune responses and confer high protection in both Asian seabass, *Lates calcarifer*, and farm hybrid grouper *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*. © 2022 by the authors. Licensee MDPI, Basel, Switzerland.

Author Keywords

Marine fishes; Oral vaccine; *Vibrio harveyi*; Vibriosis

Index Keywords

DNA topoisomerase (ATP hydrolysing) B, formaldehyde, genomic DNA, immunoglobulin M, RNA 16S, unclassified drug, vaccine, vibrio vaccine; antibiotic resistance, antibody production, antibody response, aquaculture, Article, bacterium identification, bacterium isolation, body weight gain, colony forming unit, controlled study, *Epinephelus*, *Epinephelus fuscoguttatus*, *Epinephelus lanceolatus*, fish, food intake, growth, immune response, immunization, laboratory, *Lates calcarifer*, muscle necrosis, nonhuman, polymerase chain reaction, protection, sequence analysis, skin necrosis, skin ulcer, survival, survival rate, vaccination, *Vibrio*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, vibriosis, water quality

Chemicals/CAS

formaldehyde, 50-00-0; immunoglobulin M, 9007-85-6

Tradenames

T100, Biorad, United States; YSI Pro Plus

Manufacturers

Qiagen, Germany; Biorad, United States; HACH Company, United States; Thermo

References

- Ina-Salwany, M.Y., Al-Saari, N., Mohamad, A., Mursidi, F.A., Mohd-Aris, A., Amal, M.N.A., Kasai, H., Zamri-Saad, M.
Vibriosis in Fish: A Review on Disease Development and Prevention
(2019) *J. Aquat. Anim. Health*, 31, pp. 3-22.
[CrossRef] [PubMed]
- Arif, M., Suprpto, H., Sulmartiwi, L.
Sudarno. Bacteria associated with mass mortality of hybrid grouper *Epinephelus* sp. in East Java Province Indonesia
(2016) *Int. J. Fish. Aquat. Stud*, 4, pp. 439-441.
- Nor, N.M., Yazid, S.H.M., Daud, H.M., Azmai, M.N.A., Mohamad, N.
Costs of management practices of Asian seabass (*Lates calcarifer* Bloch, 1790) cage culture in Malaysia using stochastic model that includes uncertainty in mortality
(2019) *Aquaculture*, 510, pp. 347-352.
[CrossRef]
- Mohamad, N., Amal, M.N.A., Yasin, I.S.M., Saad, M.Z., Nasruddin, N.S., Al-Saari, N., Mino, S., Sawabe, T.
Vibriosis in cultured marine fishes: A review
(2019) *Aquaculture*, 512, p. 734289.
[CrossRef]
- Mohamad, N., Roseli, F.A.M., Azmai, M.N.A., Saad, M.Z., Yasin, I.S.M., Zulkipli, N.A., Nasruddin, N.S.
Natural Concurrent Infection of *Vibrio harveyi* and *V. alginolyticus* in Cultured Hybrid Groupers in Malaysia
(2018) *J. Aquat. Anim. Health*, 31, pp. 88-96.
[CrossRef]
- Amalina, N.Z., Santha, S., Zulperi, D., Amal, M.N.A., Yusof, M.T., Zamri-Saad, M., Ina-Salwany, Y.
Prevalence, antimicrobial susceptibility and plasmid profiling of *Vibrio* spp. isolated from cultured groupers in Peninsular Malaysia
(2019) *BMC Microbiol*, 19, pp. 1-15.
[CrossRef] [PubMed]
- Liu, L., Ge, M., Zheng, X., Tao, Z., Zhou, S., Wang, G.
Investigation of *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea* (Richardson) reared in Xiangshan Bay, China
(2016) *Aquac. Rep*, 3, pp. 220-224.
[CrossRef]
- Bao, P., Sun, X., Liu, Q., Zhang, Y., Liu, X.
Synergistic effect of a combined live *Vibrio anguillarum* and *Edwardsiella piscicida* vaccine in turbot
(2019) *Fish Shellfish. Immunol*, 88, pp. 84-90.
[CrossRef]
- Zhang, X.-H., He, X., Austin, B.
***Vibrio harveyi*: A serious pathogen of fish and invertebrates in mariculture**
(2020) *Mar. Life Sci. Technol*, 2, pp. 231-245.
[CrossRef]
- Sudhagar, A., Ferosekhan, S., Linga Prabu, D.
Fish vaccination: A health management tool for Aquaculture
(2016) *Aquafind Aquac. Fish Data-Base*, pp. 1-5.
(accessed on 1 January 2021)

- Colquhoun, D.J., Lillehaug, A.
Vaccination against Vibriosis
(2014) *Fish Vaccination*, pp. 172-184.
John Wiley & Sons, Ltd.: Chichester, UK
- Li, C., Ye, Z., Wen, L., Chen, R., Tian, L., Zhao, F., Pan, J.
Identification of a novel vaccine candidate by immunogenic screening of *Vibrio parahaemolyticus* outer membrane proteins
(2014) *Vaccine*, 32, pp. 6115-6121.
[CrossRef]
- Pang, H., Chen, L., Hoare, R., Huang, Y., Wu, Z., Jian, J.
Identification of DLD, by immunoproteomic analysis and evaluation as a potential vaccine antigen against three *Vibrio* species in *Epinephelus coioides*
(2016) *Vaccine*, 34, pp. 1225-1231.
[CrossRef]
- Lun, J., Xia, C., Yuan, C., Zhang, Y., Zhong, M., Huang, T., Hu, Z.
The outer membrane protein, LamB (maltoporin), is a versatile vaccine candidate among the *Vibrio* species
(2014) *Vaccine*, 32, pp. 809-815.
[CrossRef]
- Mursidi, F.
(2018) *Antigenic Analysis of Outer Membrane Protein of *Vibrio* Species and Development of Versatile Recombinant *vhDnaJ* Vaccine against Vibriosis*,
Universiti Putra Malaysia: Serdang, Malaysia
- Shoemaker, C.A., LaFrentz, B.R., Klesius, P.H.
Bivalent vaccination of sex reversed hybrid tilapia against *Streptococcus iniae* and *Vibrio vulnificus*
(2012) *Aquaculture*,
354–355, 45–49, [CrossRef]
- Soltani, M., Lymbery, A., Song, S.K., Shekarabi, P.H.
Adjuvant effects of medicinal herbs and probiotics for fish vaccines
(2018) *Rev. Aquac*, 11, pp. 1325-1341.
[CrossRef]
- Ismail, M.S., Siti-Zahrah, A., Syafiq, M.R.M., Amal, M.N.A., Firdaus-Nawi, M., Zamri-Saad, M.
Feed-based vaccination regime against streptococcosis in red tilapia, *Oreochromis niloticus* × *Oreochromis mossambicus*
(2016) *BMC Veter-Res*, 12, p. 194.
[CrossRef] [PubMed]
- Hoare, R., Jung, S.-J., Ngo, T.P.H., Bartie, K., Bailey, J., Thompson, K., Adams, A.
Efficacy and safety of a non-mineral oil adjuvanted injectable vaccine for the protection of Atlantic salmon (*Salmo salar* L.) against *Flavobacterium psychrophilum*
(2019) *Fish Shellfish. Immunol*, 85, pp. 44-51.
[CrossRef]
- Mohamad, A., Zamri-Saad, M., Amal, M., Al-Saari, N., Monir, S., Chin, Y., Yasin, I.-S.M.
Vaccine Efficacy of a Newly Developed Feed-Based Whole-Cell Polyvalent Vaccine against Vibriosis, Streptococcosis and Motile Aeromonad Septicemia in Asian Seabass. *Lates calcarifer*
(2021) *Vaccines*, 9, p. 368.
[CrossRef]

- Wohlsen, T., Bates, J., Vesey, G., Robinson, W., Katouli, M.
Evaluation of the methods for enumerating coliform bacteria from water samples using precise reference standards
(2006) *Lett. Appl. Microbiol*, 42, pp. 350-356.
[CrossRef]
- Sulaiman, M.A., Kamarudin, M.S., Romano, N., Syukri, F.
Effects of increasing dietary carbohydrate level on feed utilisation, body composition, liver glycogen, and intestinal short chain fatty acids of hybrid lemon fin barb (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* male ♂)
(2020) *Aquac. Rep*, 16, p. 100250.
[CrossRef]
- Firdaus-Nawi, M., Yusoff, S.M., Yusof, H., Abdullah, S.-Z., Zamri-Saad, M.
Efficacy of feed-based adjuvant vaccine against *Streptococcus agalactiae* in *Oreochromis* spp. in Malaysia
(2012) *Aquac. Res*, 45, pp. 87-96.
[CrossRef]
- Aris, A.M., Saad, M.-Z., Daud, H.M., Yusof, M.T., Yasin, I.S.M.
Vibrio harveyi* protease deletion mutant as a live attenuated vaccine candidate against vibriosis and transcriptome profiling following vaccination for *Epinephelus fuscoguttatus
(2019) *Aquac. Int*, 27, pp. 125-140.
[CrossRef]
- Haldar, S.C.A.S.
Vibrio Related Diseases in Aquaculture and Development of Rapid and Accurate Identification Methods
(2012) *J. Mar. Sci. Res. Dev*, s1, pp. 2-7.
[CrossRef]
- Tookwinas, S.
Review of grow-out techniques under tropical conditions: Experience of Thailand on Seabass
(1989) *Adv. Trop. Aquac*, 9, pp. 737-750.
- Plaza, N., Castillo, D., Pérez-Reytor, D., Higuera, G., García, K., Bastías, R.
Bacteriophages in the control of pathogenic *Vibriosis*
(2018) *Electron. J. Biotechnol*, 31, pp. 24-33.
[CrossRef]
- Bin Park, S., Nho, S.W., Bin Jang, H., Cha, I.S., Kim, M.S., Lee, W.-J., Jung, T.S.
Development of three-valent vaccine against streptococcal infections in olive flounder, *Paralichthys olivaceus*
(2016) *Aquaculture*, 461, pp. 25-31.
[CrossRef]
- Mutoloki, S., Munang'Andu, H.M., Evensen, Ø.
Oral Vaccination of Fish—Antigen Preparations, Uptake, and Immune Induction
(2015) *Front. Immunol*, 6, p. 519.
[CrossRef]
- Pereira, G.D.V., Da Silva, B.C., Vieira, F.D.N., Seiffert, W.Q., Ushizima, T.T., Mouriño, J.L.P., Martins, M.L.
Vaccination strategies with oral booster for surubim hybrid (*Pseudoplatystoma corruscans* × *P. reticulatum*) against haemorrhagic septicaemia
(2015) *Aquac. Res*, 46, pp. 1831-1841.
[CrossRef]

- Zhang, W., Zhu, C., Chi, H., Liu, X., Gong, H., Xie, A., Zheng, W., Wu, Y.
Early immune response in large yellow croaker (*Larimichthys crocea*) after immunization with oral vaccine
(2021) *Mol. Cell. Probes*, 56, p. 101708.
[CrossRef]
- Han, B., Xu, K., Liu, Z., Ge, W., Shao, S., Li, P., Yan, N., Zhang, Z.
Oral yeast-based DNA vaccine confers effective protection from *Aeromonas hydrophila* infection on *Carassius auratus*
(2019) *Fish Shellfish. Immunol*, 84, pp. 948-954.
[CrossRef]
- Giri, S.S., Chi, C., Jun, J.W., Park, S.C.
Use of bacterial subcellular components as immunostimulants in fish aquaculture
(2016) *Rev. Aquac*, 10, pp. 474-492.
[CrossRef]
- Fraser, T., Hansen, T., Mayer, I., Skjæraasen, J.E., Glover, K.A., Sambraus, F., Fjelldal, P.G.
The effect of triploidy on vaccine side-effects in Atlantic salmon
(2014) *Aquaculture*, 433, pp. 481-490.
[CrossRef]
- Amar, E.C., Faisan, J.P., Gapasin, R.S.
Field efficacy evaluation of a formalin-inactivated white spot syndrome virus (WSSV) vaccine for the preventive management of WSSV infection in shrimp grow-out ponds
(2021) *Aquaculture*, 531, p. 735907.
[CrossRef]
- **Effect of incorporating different concentrations of palm oil as adjuvant in fish vaccine**
(2018) *Int. J. Biosci. (IJB)*, 12, pp. 35-41.
[CrossRef]
- Wanasawaeng, W., Tawatsin, A., Sasipreeyajan, J., Poomvises, P., Chansiripornchai, N.
Development of inactivated New-castle disease vaccine using palm oil as an adjuvant
(2009) *Thai J. Vet. Med*, 39, pp. 9-16.
- Roslindawani, M.N., Syafiqah, A.S., Jesse, F.F.A., Effendy, A.W., Zamri-Saad, M.
Recombinant Caseous Lymphadenitis Vaccine with Palm Oil as Adjuvant Enhances the Humoral and Cell-Mediated Immune Responses in Rat Model
(2016) *J. Anim. Health Prod*, 4, pp. 22-25.
[CrossRef]
- Monir, S., Yusoff, S.B.M., Zulperi, Z.B.M., Abu Hassim, H.B., Mohamad, A., Ngoo, M.S.B.M.H., Ina-Salwany, Y.
Haemato-immunological responses and effectiveness of feed-based bivalent vaccine against *Streptococcus iniae* and *Aeromonas hydrophila* infections in hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*)
(2020) *BMC Veter-Res*, 16, pp. 1-14.
[CrossRef]
- Chin, Y.K., Al-Saari, N., Zulperi, Z., Mohd-Aris, A., Salleh, A., Silvaraj, S., Mohamad, A., Ina-Salwany, Y.
Efficacy of bath vaccination with a live attenuated *Vibrio harveyi* against vibriosis in Asian seabass fingerling, *Lates calcarifer*
(2019) *Aquac. Res*, 51, pp. 389-399.
[CrossRef]

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