

Insights on the Use of Vaccines for White Spot Syndrome Virus (WSSV) in Shrimp

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ABSTRACT

White Spot Syndrome Virus (WSSV) is one of the most serious viral diseases affecting shrimp aquaculture, causing rapid mortality and major economic losses worldwide. Several vaccination strategies have been explored to control this pathogen, including subunit vaccines, inactivated whole-virus vaccines, DNA vaccines, antibody-based approaches, and RNA interference. Among these, VP28-based recombinant vaccines and DNA vaccines have shown promising protective effects under experimental conditions. However, challenges related to delivery methods, scalability, and field-level performance still limit their commercial application.

INTRODUCTION

White Spot Syndrome Virus (WSSV) is one of the most devastating pathogens in global shrimp aquaculture. Since the major outbreaks reported in the early 1990s, this rod-shaped dsDNA virus (family *Nimaviridae*, genus *Whispovirus*) has caused severe economic

losses worldwide. Infected shrimp develop white spot disease and typically die within 2–7 days, with mortality rates reaching up to 100%. Despite efforts such as innate immune stimulation, environmental management, pre-exposure strategies, and phytotherapy, no fully effective control method is currently available.

Vaccination has emerged as a promising prophylactic strategy. Although shrimp lack classical antibody-mediated adaptive immunity, they exhibit an alternative immune mechanism involving molecules such as Dscam, identified in *Penaeus vannamei* and *Penaeus monodon*, which can generate immune specificity and memory-like responses. The first successful experimental vaccination trial against WSSV was reported by Witteveldt *et al.* (2004), demonstrating promising prophylactic effects. Subsequently, several vaccine types—including inactivated whole-virus vaccines, recombinant viral protein subunits, and DNA/RNA-based vaccines—have been developed. Although these approaches show encouraging results under experimental conditions, large-scale commercial implementation remains limited. WSSV continues to pose a major threat to shrimp farming, highlighting the urge for effective and scalable vaccination strategies.

Subunit vaccines

WSSV Vaccines developed mostly are WSSV protein subunit vaccines. Nearly 40 different envelope proteins of WSSV have been documented, so far. Most monovalent subunit vaccines are subunits from envelope proteins of WSSV that includes VP28, VP19, VP26, VP24, VP292, and VP466, etc. Nearly 70% of the reports have targeted recombinant VP28 subunit vaccines as this was one of the earliest envelope proteins identified. In addition to this, many multivalent WSSV vaccines have also been prepared efficiently, such as VP28, VP19 and VP28-CotB, etc.

Monovalent subunit vaccine

The WSSV envelope protein VP28 is essential during the early phase of viral infection and it was used to develop WSSV subunit vaccine. Following its initial success, various expression systems have been explored, including bacterial, viral, yeast, and transgenic

platforms. Among these, *Escherichia coli* is the most commonly used and has produced highly effective VP28 vaccines. Other systems such as *Bacillus subtilis*, live bacterial vectors, bacteriophages, recombinant baculovirus (showing 82–87% survival), yeast (high yield and rapid growth), and transgenic silkworm pupae (up to 94% protection) have also demonstrated protective efficacy, though some are less practical for large-scale use. Vaccine effectiveness depends on factors such as dosage, viral challenge load, VP28 structure, buffer system, and route of administration. Injection provides the strongest protection, followed by oral and immersion methods, with oral delivery being the most practical for farm application.

Polyvalent WSSV vaccines

To augment the defence effects of vaccines, polyvalent WSSV vaccines have been created by fusion expression or by merging different monovalent subunit vaccines. For instance, shrimp vaccinated with the fusion proteins rTAT-VP28 showed a considerable increase in the survival rate of 53.6% to 67.8%. When fusion proteins CotB-VP28 were mixed with shrimp feed pellets, the percentage of survival in treated shrimp was higher compared to that of untreated animals. Furthermore, studies revealed that the survival rate of shrimp administered with the CotB-VP28 fusion protein vaccine was comparatively less than that with the CotC, which achieved 100% survival. Hence, CotC could be used to formulate a novel vaccine with the endeavour of obtaining a 100% survival field level.

Inactivated whole WSSV vaccines.

Inactivated whole virus vaccine is the alternative possibility to explore new polyvalent WSSV vaccines. WSSV have been inactivated by heat and binary ethylenimine (BEI), formalin, or gamma irradiation, and this can lead to a better immune response in shrimp

after WSSV infection. After immunizing shrimp using formalin-inactivated viral preparation, a 100% survival rate can be achieved using an oral route of administration. This high survival rate could be obtained from a specific antiviral immune response developed by inactivated WSSV and a non-specific antiviral immune response by other humoral factors. If shrimps were vaccinated with inactivated WSSV along with an immunostimulant, there was a heightened protection rate in a vaccination-dependent manner.

DNA vaccines

DNA vaccines have been extensively explored for controlling WSSV due to their stability, low cost, and ease of production. Studies show that DNA vaccines provide significant protection, often outperforming recombinant subunit vaccines by inducing longer-lasting immunity and higher survival rates. Plasmid DNA can also be vertically transmitted from broodstock to offspring, offering potential for producing pathogen-resistant shrimp lines. Several WSSV gene targets including VP28, ICP11, VP19, and VP24 have been used in DNA vaccine development, with VP28-based constructs achieving survival rates up to 90%. Non-WSSV genes such as PmAV, PAP, and GFP have also been evaluated as targets. Protection is associated with enhanced haemocyte phagocytosis, increased immune-related protein activity, and elevated lysozyme expression. Additionally, chitosan encapsulation improves vaccine immunogenicity and delivery efficiency. Oral delivery systems using bacterial or viral carriers such as *Escherichia coli*, *Salmonella typhimurium* and baculovirus enable effective mass administration through feed, making DNA vaccination a promising strategy for large-scale shrimp production.

Protective antibodies in anti-WSSV infection

Many researchers have investigated the possibilities of using antibodies to target the WSSV envelope proteins that have protective effects in shrimp. Most of the studies have generated a positive response to this question. Using antibodies against envelope proteins VP28, VP19, VP28/18 and LEL, neutralization assays have confirmed that each of these antibodies provides notable protection to shrimp, resulting in an increased survival rate. Antiserum specific to the VP28 protein was observed to be a good reagent for rapid and sensitive detection of WSSV infection. Some of these antibodies have been further advanced to develop immunoassay kits for the detection of WSSV and other viruses such as the lateral-flow, immunochromatographic strip test, and flow-through immunoassays.

RNA interference in the field of WSSV vaccines

Earlier studies have revealed that RNA interference (RNAi) technology could be a potential and emerging approach for preventing WSSV infection. Robalino *et al.* (2004) first studied that the invertebrate immune system can identify dsRNA and stimulate the innate immune response. Based on the above-mentioned research findings, many WSSV genes have been used as target genes for RNAi neutralization of WSSV including VP19, VP28, rr2, rr1, and others. Additionally, RNAi targeting of non-WSSV genes such as PmRab7, PmRab7/PmIAP and GFP were also tested for their ability to prevent WSSV infection. Important factors that must be considered for developing RNAi-based vaccines include selecting the appropriate gene for RNAi, optimizing the immunization program, and the molecular structure and size of the specific dsRNA. (Fajardo *et al.*, 2024).

CONCLUSION

Regardless of several attempts by researchers to develop WSSV vaccines, yet there are no successful commercial vaccines currently available for use in the shrimp culture industry. There are numerous limitations and obstacles involved in developing a WSSV vaccine that is good enough for application in the shrimp farming industry. This article summarises the different vaccines available and the areas which can be further explored to develop a commercial vaccine for farmer's benefit.

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