REVIEW / ARTÍCULO DE REVISIÓN

An overview of vaccine production against shrimp White Spot Syndrome Virus, effects and the possible impact of this technology in Ecuador

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Abstract: Although aquaculture in Ecuador has a high economic and socio-cultural importance, pathogenic microorganisms affect the development and vitality of crustaceans, fish, and mollusks, reducing their production yields. Among these pathogens, White Spot Syndrome Virus (WSSV) is an invertebrate virus that induces high mortality, generating severe economic losses due to its wide geographical distribution and high infection rate finding the most significant devastation worldwide in the shrimp sector. Although several strategies are described to fight against WSSV, this study points to an updated overview of vaccines used against this virus, including types, effects and large-scale production ways. Thus, this research supplies an analysis of possible treatments based on vaccination to combat the WSSV caused-disease that significantly impacts the aquaculture economy and could be helpful to those working in this field.

Key words: *Whispovirus*, White Spot Syndrome Virus, Shrimp, virus, vaccine, production, Ecuador.

Introduction

In the last 50 years aquaculture industry in Ecuador has become one of the most critical sectors for the domestic economy since more than 40% of Ecuadorian exports are related to this income source[1]. During 2021-2022, shrimp production reached in the country 848,000 MT with a profit of 5323.30 million dollars[2], making the country one of the largest shrimp exporters worldwide. The European Union (EU), Russia, the United States and China are currently the four main destinations for Ecuadorian shrimp exports[3]. However, diverse types of diseases caused by DNA and RNA viruses significantly affect shrimp production. Three types of viruses have been identified that drastically affect farmed shrimps in the country: Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Taura Syndrome Virus (TSV) and White Spot Syndrome Virus (WSSV)[4,5]. All these three viruses in Ecuador caused significant economic and social losses. The primary example is the appearance of WSSV in 1999, which caused a 50% decrease in production and exports during the first years of the incidence, with the subsequent jobs lost in multiple families[4,6,7].

WSSV can infect many aquatic crustaceans, especially decapods, such as marine brackish and freshwater shrimps, sea crabs, crayfish and lobsters[6]. However, neither does it cause problems for human health or food safety nor affects human shrimp consumption while causing a detrimental effect on shrimp farmers' production[8].

World Organisation for Animal Health (WOAH) included White Spot Syndrome Virus in a list of infectious diseases that are considered to be of national socioeconomic and/or public health significance and whose effects on international trade in animals and animal products are not negligible[6,7].

Several approaches have been used to combat the incidence of infectious diseases, including antivirals, prebiotics, plant extracts-based drugs and antibiotics[9-12]. Although several strategies exist to combat WSSV[13-15], this study provides an up-to-date overview of production, effects and types of vaccines against WSSV in shrimp.

Thus, this research supplies an analysis of potential possible treatments and new tools to fight against this disease that significantly impacts the aquaculture economy, not only in our country.

Shrimp immune system and response to vaccines

The innate immune system is pronounced in shrimps to protect them from external agents and pathogenic microorganisms[16]. Crustaceans are generally known not to have a specific immune system[17], which precludes the use of conventional vaccines to treat pathogens. According to Afsharnasab (2014), crustaceans' immune system comprises three defense mechanisms, all needed to defend themselves, as depicted in Figure 1.

The first is the cuticle and skin's physical and chemical defense system encompassing secretions[18-20]. This system is inefficient in protecting the organism from all pathogens because most crustaceans have an open circulatory system. The second line of defense is the cellular one. In the crustacean's world, these cells are called hemocytes and are composed of hyaline, granular and semi-granular cells. Each of them has a significant role in disease prevention. The last one to mention is the humoral defense[21].

Innate immunity is triggered when pathogens are detected by host proteins, such as antimicrobial, coagulation and pattern recognition proteins, which, in turn, activate humoral or cellular effector mechanisms to destroy invading pathogens[22].

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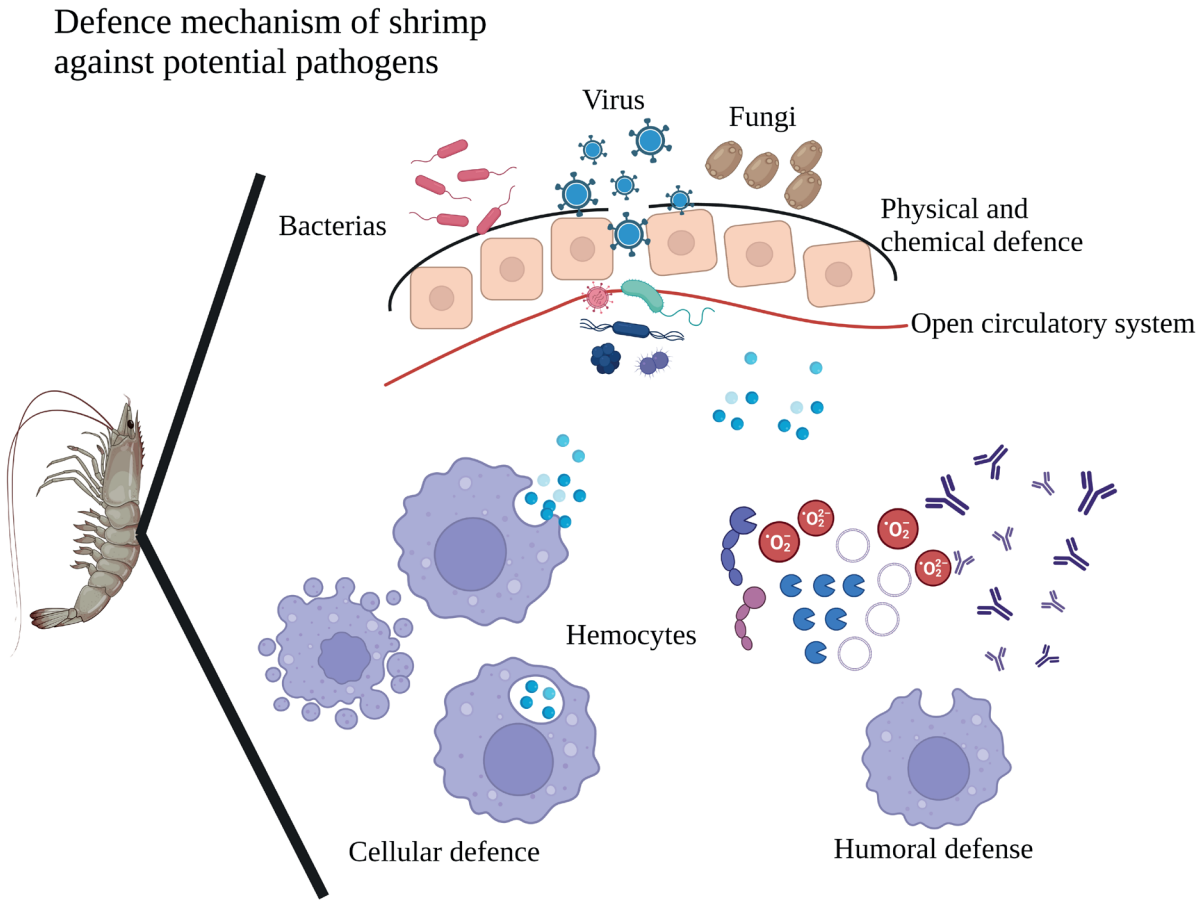

Figure 1. Shrimp defense mechanisms against potential pathogens. The first defense mechanism is the cuticle, and the second consists of cellular defense, including cytotoxicity, coagulation, encapsulation, phagocytosis, melanization, apoptosis and modulation. The third humoral defense mechanism is based in the action of hydrolytic enzymes, agglutinins, coagulation proteins, antimicrobial peptides, oxygen and nitrogen free radicals, and effectors. All three mechanisms act together to eliminate foreign agents[13,17,23,24].

Figure 2 shows the 3D structure of Beta 1,3-Glucan Binding Protein (BGBP) found in plasma, which serves as a protein recognizer in the arthropod immune system[17]. This is in conjunction with the transglutaminase enzyme which is released by hemocytes in the presence of pathogens through receptors[25]. Lectin protein is also represented in the immune system with an antiviral function recognizing WSSV proteins[25,26]. In addition, antimicrobial peptides like Stilicin have antibacterial activity when interacting with the LPS endotoxin of gram-negative and show vigorous activity against filamentous fungi[27].

On the other hand, Alpha 2 macroglobulin, a high molecular mass proteinase, generates opsonization activities against invading pathogens by mediating endocytosis[28]. Penisidins, other essential proteins, are active against Gram-positive bacteria by binding them, causing agglutination, and additionally, in high concentrations, have a good effect against fungi[29]. These are some of the main proteins responsible for humoral immunity[31].

Studies show an alternative memory immune response; however, there are no T cells, B cells or major histocompatibility complex (MHC) molecules[30] in shrimps. Recent experimental data from shrimp and other arthropods have shown that invertebrates own an alternative memory type of immune response. This memory-like peculiarity is called resistant priming[22,31]. With this mechanism, shrimps could improve their defenses after initial pathogenic exposure and then generate better protection after subsequent infections with the same or a different pathogen.

Laboratory tests have shown that vaccinated shrimp and crayfish have improved survival rates following exposure to WSSV[32]. *Penaeus japonicus*, which survived natural and experimental WSSV infections, initially resisted subsequent WSSV exposure. However, these results were not replicated under different conditions - such as temperature, country or type of shrimp[33]. But it is not a treatment that can be applied overnight, mainly because of the unique adaptive immunity of shrimps[34-36].

White spot syndrome virus

Several virus families affect invertebrates; some include DNA viruses[37] such as N*imaviridae, Parvoviridae, Baculoviridae* and *Iridoviridae*[38,39], which has the most significant impact on shrimp farming.

This article focuses on vaccines against the White Spot Syndrome Virus (WSSV), one of the most lethal arthropods viruses worldwide, with a mortality and infectivity rate in shrimp of up to 100%, significantly affecting the larval stage generating large economic losses[4,40].

WSSV is a double-stranded DNA virus with an approximate genome size of 290 to 300 kb, which makes it one of the most complex viruses infecting shrimp[4,40]. Most of its putative translated gene products have no homology with other virus proteins or host cells. Because of this peculiar

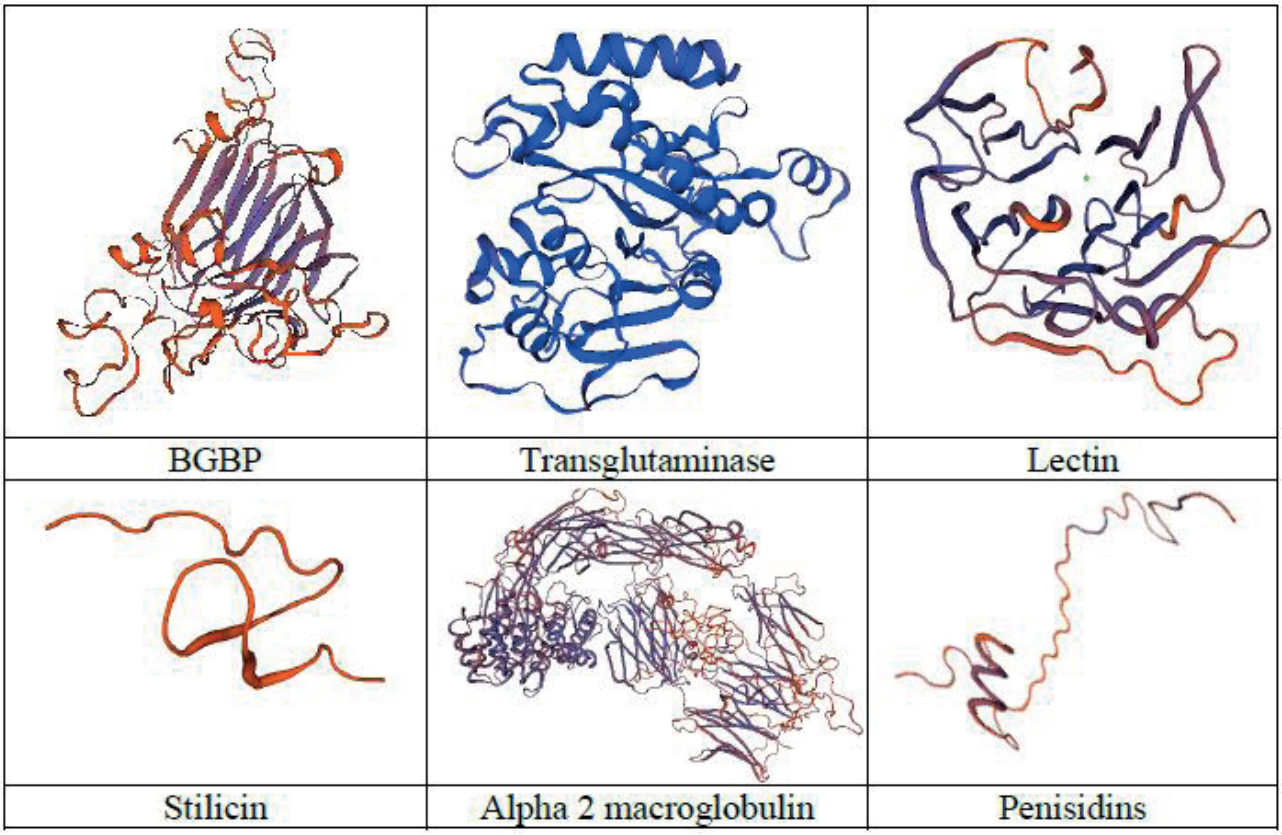

Figure 2. 3D structures of the main proteins involved in pathogen recognition as a humoral defense.

feature, the International Committee on Taxonomy of Viruses (ICTV) classified WSSV in its own family: *Nimaviridae*, within a unique genus *Whispovirus*[40,41].

Impact of WSSV virus on Ecuador's Economy

It is believed that WSSV entered Ecuador by importing contaminated larvae from Panama, spreading to the natural environment and later contaminating all farms. The virus was established between 1999 and 2000[42], causing great economic losses for the producer and the country itself. The National Institute of Fisheries (NIF), attached to the Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP), carries out annual tests[43] in several shrimp farms to determine the presence of different diseases using molecular tests.

There is evidence from the early 1990s that exports generated revenues for the country of around 3.5% of gross domestic product (GDP) on average, rising to almost 4.5% of GDP in 1997, 1998 and 1999[1]. After these years, the White Spot Syndrome epidemic broke out all over the world, and shrimp exports dropped to 2% in 2000 and to less than 1.5% in 2001. The shrimp industry and the Ecuadorian economy suffered significant damage until 2010, when a new increase in the export earnings of this product began reaching higher levels than before due to the control of the shrimp farms before the disease, as seen in Figure 3. Currently, the government conducts annual monitoring that allows the early detection of diseases. It is necessary to point out, that there is no protocol to deal with this virus in case it emerges again[1,44,45].

In August/September 2019, shrimp exports from Ecuador to China significantly dropped due to the presence of WSSV in the shipments; China is the leading importer of Ecuadorian shrimp worldwide. Therefore this problem generated a significant loss in annual profit, affected subsequent trades and caused the suspension of shrimp exports to China from various Ecuadorian companies[48].

As a result of the last infectious trade between Ecuador and China in 2019 a, better product management, constant monitoring and an adequate prevention protocol allowed to control the virus outbreak and thus not generate problems as such, increasing exports to that country[49].

Major vaccines designed to combat infectious diseases in shrimp

Disease-fighting protocol development in shrimp involves the characterization of immune system effectors and understanding defense reactions to potentially lethal pathogens, considering that pathogen-host interactions are constantly changing[49].

Vaccination is a defense mechanism used to enhance the shrimp immune system, which has been studied since the 1990s[9,50,51]. WSSV is one of the most serious pathogens affecting shrimp farming worldwide, so vaccine supplies constitute a significant protective benefit for the shrimp host.

Different vaccines have also been developed to combat the WSSV based on both the capsid and the core proteins, but also virus fragments or even completely inactivated viruses have been used[52,53]. The technologies currently employed are nanoparticles as vectors and gene silencing to prevent virus proteins from binding to shrimp cells generating an efficient immune response[53].

In aquaculture, 3 types of vaccines are commonly used. Live Attenuated Vaccines include a suspension of a live attenuated pathogen that generates a response that does not allow excessive replication despite the ability to multiply in the host[54]. Live vaccines cause an asymptomatic, self-limiting infection. Therefore, the host immune system resem-

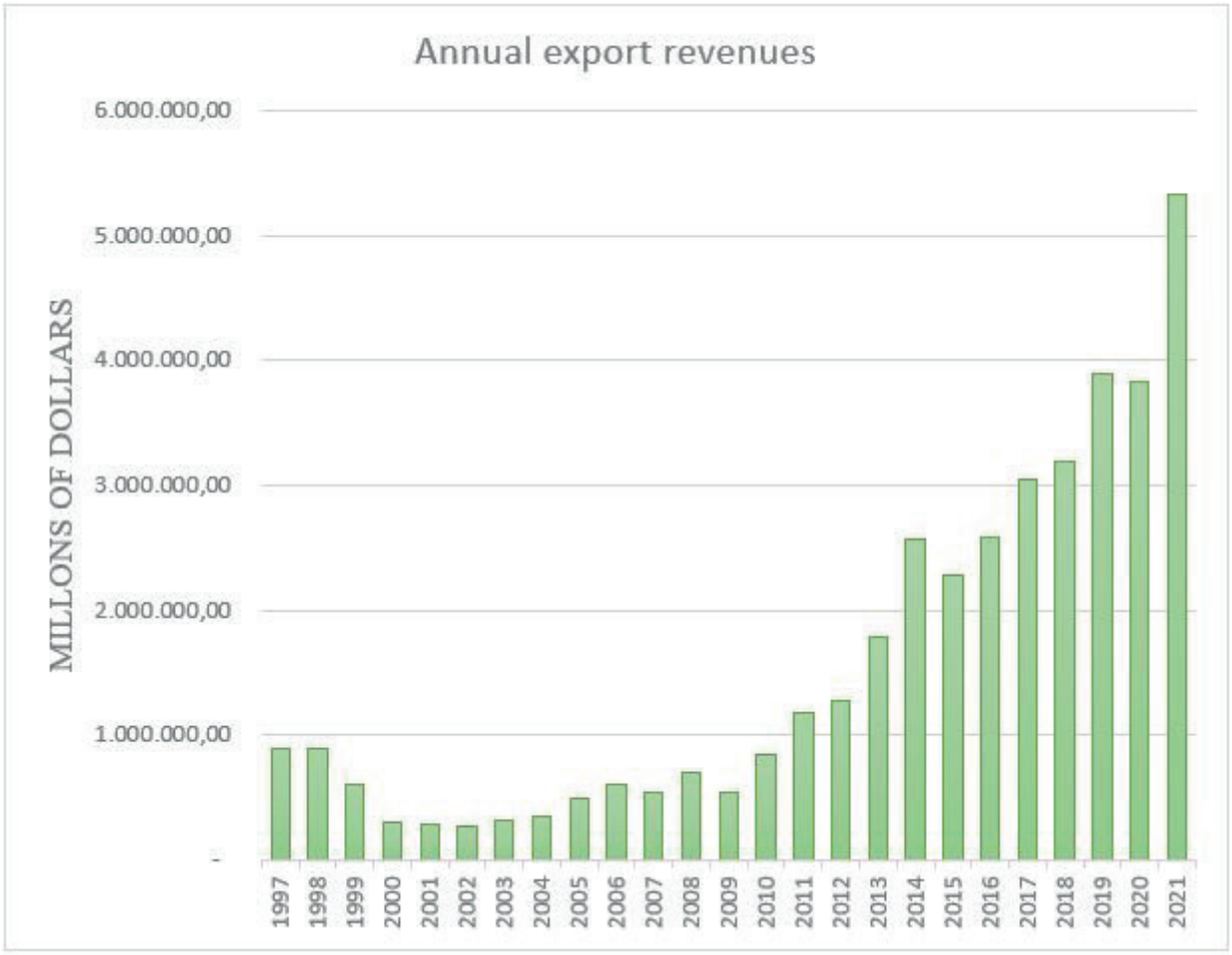

Figure 3. Annual shrimp exports from Ecuador between 1997 - 2021[2,44-47].

bles natural infections in a controlled system[55].

The second type of vaccine is recombinant vaccines which refer to immunogenic proteins or purified epitopes obtained from the pathogens or carriers. These can also be composed of the virus's DNA or dsRNA, as in the Recombinant Infectious Haematopoietic Tissue Necrosis Vaccines[56]. This type of vaccine has been one of the busiest in the last decade, primarily to molecular advances and studies of recombinant virus subunits[57]. More than 40 WSSV structural proteins have been identified[22] and used to manufacture efficient recombinant vaccines. Among these proteins are VP19, VP24, VP26, VP28, Vp36, VP36B, Vp37, VP39. Proteins VP19, VP24, Vp36B and VP39 are found on the WSSV envelope[22,58]. VP15, VP26 and VP36 are proteins found in nucleocapsid[22,59]. Because the structural proteins are the first to act with the host[60], those are considered the basis for neutralization strategies or the most likely candidates for vaccine development.

Particular studies have also shown that shrimp vaccinated with recombinant plasmids or microorganisms carrying a gene for the most studied WSSV coat protein (vp28) could efficiently protect shrimp against WSSV infection[57,61-64].

The third type is the inactivated virus vaccine, prepared from the suspension of completely killed cells of bacteria, viruses, or fungi. This type of vaccine has been successful against different disease-caused agents, such as *Vibrio anguillarum, Vibrio salmonsida*, also used in white shrimp, with good results. These vaccines are produced using chemical and physical (heat and radiation) inactivation methods. The most critical step in the production of such vaccines is inactivation[54].

Lastly, there is another type of vaccine that is not widely used in aquaculture but is commonly used in the veterinary and human area., that is the case of synthetic vaccines manufactured from polypeptides that simulate the primary sequence of antigenic amino acids. Its function is very similar to that which occurs with inactivated viruses[54]. Table 1 shows the type of vaccine, composition, how the active ingredient was obtained and the survival rate for each study.

According to the gathered data, the vaccines with the highest incidence were the envelope protein vaccines VP28[54-56,65]. This protein plays a role in interacting with the host cell surface[64], which has been the most studied since the virus first appeared in 1992[62,63]. VP28 is one of the most critical targets for vaccine manufacture, as it is one of the main WSSV coat proteins and acts as a binding protein, allowing the virus to combine with the shrimp cells and letting it join the cytoplasm[57].

The combination of this protein and others, such as Vp37, an envelope protein that facilitates infection, does not reduce the infection rate. Still, it does allow an improvement in the time of resistance to WSSV. Another of the mixtures is with VP24, as it is the only infection protein that has been shown to interact with the host polymeric immunoglobulin receptor protein (MjpIgR), which can mediate WSSV infection, generating good resistance results[57,66].

Designed specific vaccines against WSSV used in shrimp production

The primary purpose of vaccines is to stimulate the

TYPE OF VACCINE	COMPOSITION OF THE VACCINE	METHOD OF OBTAINING THE ACTIVE SUBSTANCE	METHOD OF ADMINISTRATION	PERCENTAGE OF SURVIVAL SOURCE	SOURCE
Inactivated virus	Gamma-inactivated virus	Gamma-irradiated WSSV virus produced in crab	Immersion	85	54,67–71
				86,66	
			Intramuscular	62	
				57	
				76	
		Gamma-irradiated WSSV produced in shrimp		73	
	Formalin-inactivated virus	Formalin-inactivated virus replicated in shrimp	Immersion	71,2 ± 3,13	31,72,73
			Oral	50	
			Intramuscular	60	
Subunit, recombinant, polysaccharide, and combination vaccines	Recombinant Vp28 and vp37 proteins	Protein cloning in *E. coli* of MrNv-VLP, amplification of VP28 and 37 dsRNA T7 RiboMAX™ Express large-scale RNA production system.	Intramuscular	45	64
	Recombinant VP28 and VP24 Proteins	Protein cloning in *E. coli* with amplification of VP28 and 24	Oral	100	57
	Recombinant VP15 protein	Protein cloning in *E. coli* with VP15 amplification	Intramuscular (2 doses)	80	74,75
				60	
	Recombinant VP28 protein	Protein cloning in *E. coli* with amplification of recombinant VP28	oral	87,10	52,62,63,76–79
				70	
				60	
				50	
			Intramuscular (2 doses)	81	
			Intramuscular	44,99	
				67	
		Use of recombinant *B. subtilis* CotB-VP28 expressing the VP28 protein of WSSV	Oral	70	80,81
				65	
		Use of recombinant filamentous cyanobacteria expressing the VP28 protein of WSSV		28,68	82
	Recombinant VP19 + VP28 proteins	Protein cloning in *E. coli* with recombinant VP19 + VP28 amplification	Oral	71,1	61,83
		Protein cloning in lentiviruses with amplification of VP19 + recombinant VP28	Immersion	86	
	dsRNA	Amplified protease fragments and cloning in *E. coli*	Intramuscular	70	84–87
		The double-stranded RNA corresponding to the vp28 protein-coding gene of WSSV		73	
		The double-stranded RNA corresponds to the genes coding for rr1 and vp28 of WSSV.		93,3 entre 90	
		A partial fragment of C-type lectin cDNA associated with *M. japonicus* stomach virus was amplified by PCR.		75	
	lcppae2	Amplification of the gene encoding WSV056 was amplified from the DNA of the WSSV genome.	Intramuscular	85	88
	CQD with RNA	Total RNA was isolated using TRIzo reagent	Oral	20	89
	Recombinant DNA	Genomic DNA isolated from pleopod tissue in microsatellites	Intramuscular	88,1	90
		Design of two primer pairs using the viral VP24 gene for nested PCR	Oral	50	21
	Recombinant *baculovirus*	Recombinant *baculovirus* viral DNA isolated from recombinant *baculoviruses* containing VP28, VP19 and FL2 using a NucleoS RNA Virus kit	Oral	89,5	91
		Establishment of recombinant vectors harboring VP28 and gene encoding dsRNA specific for rr2 and egfp		64	92
	CotC: Vp26 fusion protein	Recombinant spores (RS) of *B. subtilis*, showing CotC: Vp26 fusion protein (FP)	Oral	100	93
	Recombinant vp39 and vp28 proteins	Protein cloning in *E. coli* with amplification of VP28 and 24	Intramuscular and oral	60 y 50	94
	Recombinant VP28 and VP36B proteins	Protein cloning in *E. coli* with amplification of VP28 and VP36B	Intramuscular (2 doses)	100	58
	Antiviral vp28-siRNA	Sequencing vp28-siRNA randomly organised and mutated at one nucleotide.	Intramuscular	60	95
	Recombinant rVP26 and rVP28 proteins	WSSV DNA from WSSV-infected shrimp tissue by RNase-PEG precipitation method	Oral, immersion, intramuscular	100, 71 y 61	96
	Recombinant VP24 protein	Protein cloning in *E. coli* with amplification of recombinant VP24	Oral	64	97,98
				43	
	Recombinant Wsv477 protein	Protein cloning in *E. coli* with recombinant wsv477 amplification	Intramuscular y oral	40 y 30	99
Synthetic vaccine	Protein pmrab7	Protein cloning in *Agrobacterium tumefaciens* with amplification of pmrab7	Intramuscular	87	100
	Phagocytosis Activating Protein recombinant plasmid (phMGFP-PAP)	Protein cloning in *E. coli* with phMGFP-PAP amplification	Oral	62	68
	Anti-sense constructions	Specific primers to amplify the H3 promoter of *P. monodon* and selected portions of the viral ORFs (structural proteins)	Intramuscular	90	73

Table 1. Efficiency and composition of different vaccine preparations according to the 46 consulted papers.

shrimp's immune system and generate a defense response against WSSV to prevent virus scape and thus reduce its replication and spread[101]. These cell responses against WSSV are given in different ways. Two of them stimulate the cell response by: 1-the presence of biomolecules belonging to the virus and 2-molecules that interfere with the receptors where the virus assembles to the host cell. On the other hand, genetic modifications that provide a protective response by not generating interactions in the cells with the virus[102] also result in good practice.

Among the revised papers, 34 deal with recombinant vaccines, the most used ones based on recombinant proteins from the structural parts of WSSV. The combination of 2 or more structural recombinant proteins[5] generates a higher protection rate against this virus[61,83]. The revised reports also determined that the main type of vaccine is composed of the subunit-recombinant, polysaccharide, and combined subunit vaccines. According to Figure 4, the vaccines mentioned above showed a protection percentage of 73.91%, while other treatments related to both; synthetic or inactivated virus vaccines reached lower protection percentages of only 6.52% and 19.57%, respectively. In this figure is also noticed that the most frequent active principle is the recombinant vp28 protein, reaching 21.74% of incidence.

Experimental conditions are very important in reaching a good performance of any vaccine treatment against WSSV since protection results could change from one experiment to another according to the experimental conditions. Some parameters to take under consideration in this experiment are a) the type of shrimp, b) the form of virus replication referring specifically to the animal used, c) the region in which the study was carried out where the environmental parameters varied and d) the variation of virus infection that can reach mortality levels up to 100%[103,104]. Interestingly, in some research reports, there was no total mortality, mainly due to the resistance some arthropods can generate against WSSV[105].

Also, administration methods at the production level deal with the efficiency in the vaccination methods[99,100]. It is worth noting that the most common method of vaccine administration is intramuscular administration, with a frequency of 48%, followed by oral administration at 42% and finally by immersion at 10%. However, oral vaccine administration is the best and most studied method at the industrial level.

The effect on the immune system produced by the vaccine in shrimp is calculated by the efficiency of the treatment against WSSV, demonstrated by the number of vaccinated animals that survived exposure to the virus; the treatment with the highest efficiency and best protective effect was the intramuscular administration. The treatment with the highest efficiency and best protective effect was the intramuscular route, with 18% of treatments having a survival rate of more than 75%; the oral way had an efficiency of 10% for medicines with a survival rate of more than 75%, and the immersion route had a frequency of 6% for treatments with a survival rate of more than 75% (Figure 5).

It was determined that, in general, the efficiency of the vaccine is between 50% and 75% of shrimp survival rate

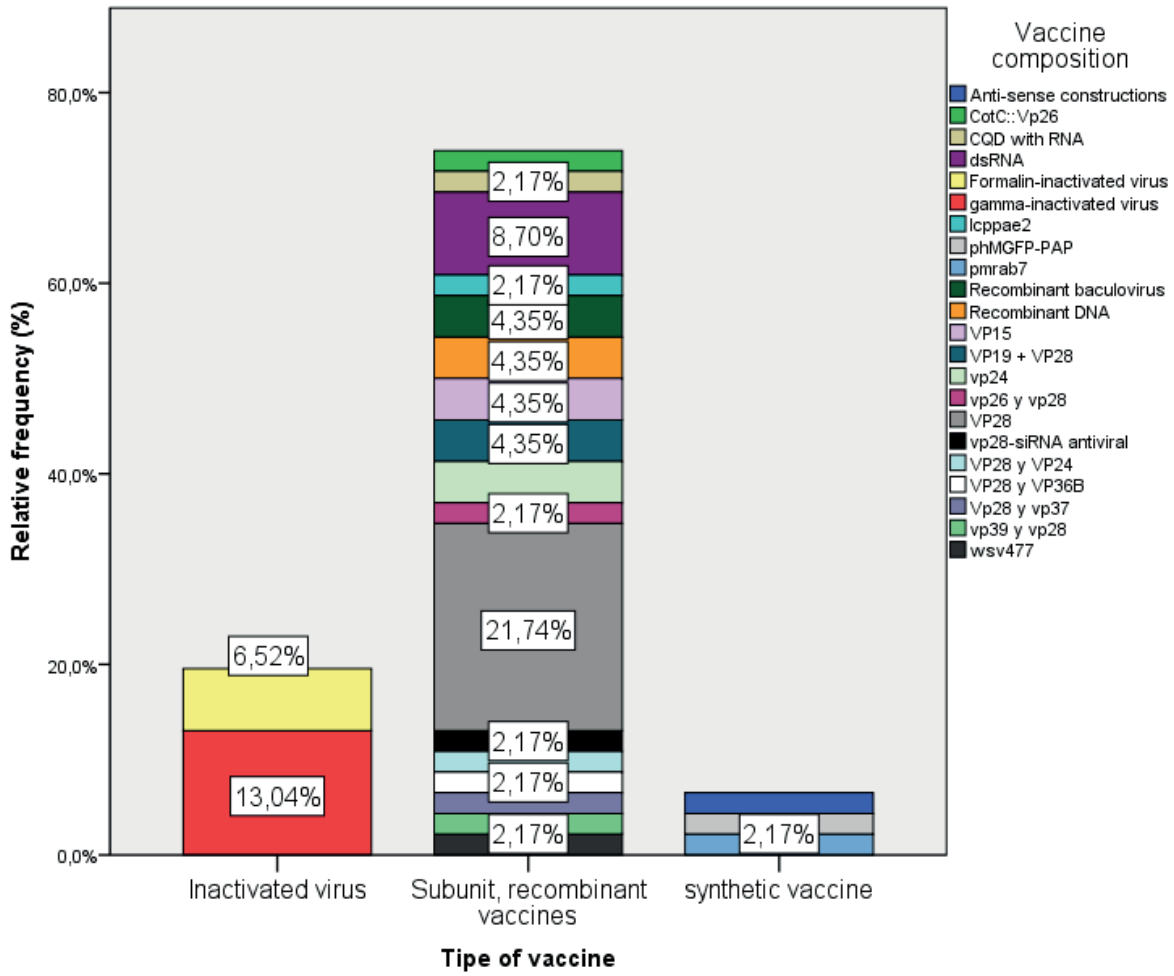

Figure 4. Types and composition of WSSV vaccines in shrimp.

reaching 52% of frequency in the studied articles, followed by others that reached an effectiveness of more than 75% having 34% of frequency, allowing to assert that vaccination is an effective treatment against the virus.

Detailing the efficiency depending on the vaccine composition, it was determined that gamma-inactivated virus is the most effective, reaching a 6% frequency in treatments with a survival rate of 75%, followed by vaccines made with recombinant VP28 protein, which reached a 4% frequency. However, vaccines with recombinant VP28 had the highest frequency of 12% among all treatments, with a survival rate between 50% and 75%.

WSSV vaccine production for *Litopenaeus spp.*

Penaeidae is a crustaceans family of great commercial value[106,107]. Among its different genera, *Litopenaeus* stands out as one of the most important shrimp species in the world industry[108-110]. *Litopenaeus vannamei* is among the principal species of this genus, commonly known as Pacific white shrimp111, the main farming species on the Ecuadorian coast[48]. However, this genus is prone to devastating diseases such as WSSV, which generate significant economic losses, and no commercial cure can eradicate the disease. Table 2 shows recent reports on conditions affecting the Litopenaeus genus, showing some updated general approaches to fighting them.

According to the research reviewed, vaccine manufacturing has been carried out *ex-situ*. Therefore, this technology is still limited to the laboratory level. Further studies on production scale-up should be carried out to reduce costs, maintain product quality and develop *in situ* trials, allowing more accurate data to be generated during shrimp treatment.

Whispovirus vaccines aren't currently being commercialized at large-scale in the industry because of the high degree of variation in response to laboratory-tested vaccines and the high economic value of vaccine development[9,115]. Nevertheless, interest in controlling the devastating effects of the virus on *Litopenaeus vannamei* farms has led to increased interest in producing a vaccine that is efficient and affordable for field application.

The most efficient way to immunize *Litopenaeus spp.* with vaccines is by oral or infusion as it is not productive at the industrial level to apply it intramuscularly as this implies the application of the vaccine organism by the organism.

The vaccine industry and production is a complex activity with risks, which takes place in a harsh environment. Protocols for potential occupational hazards are necessary concerning contamination issues such as, product contamination, cross-contamination, amplification of contaminants, infection of workers and contamination of the environment[116].

The animal vaccines currently available worldwide are developed by the veterinary pharmaceutical industry. Developing a vaccine requires an economic effort that takes years to perfect and guarantees its safety.

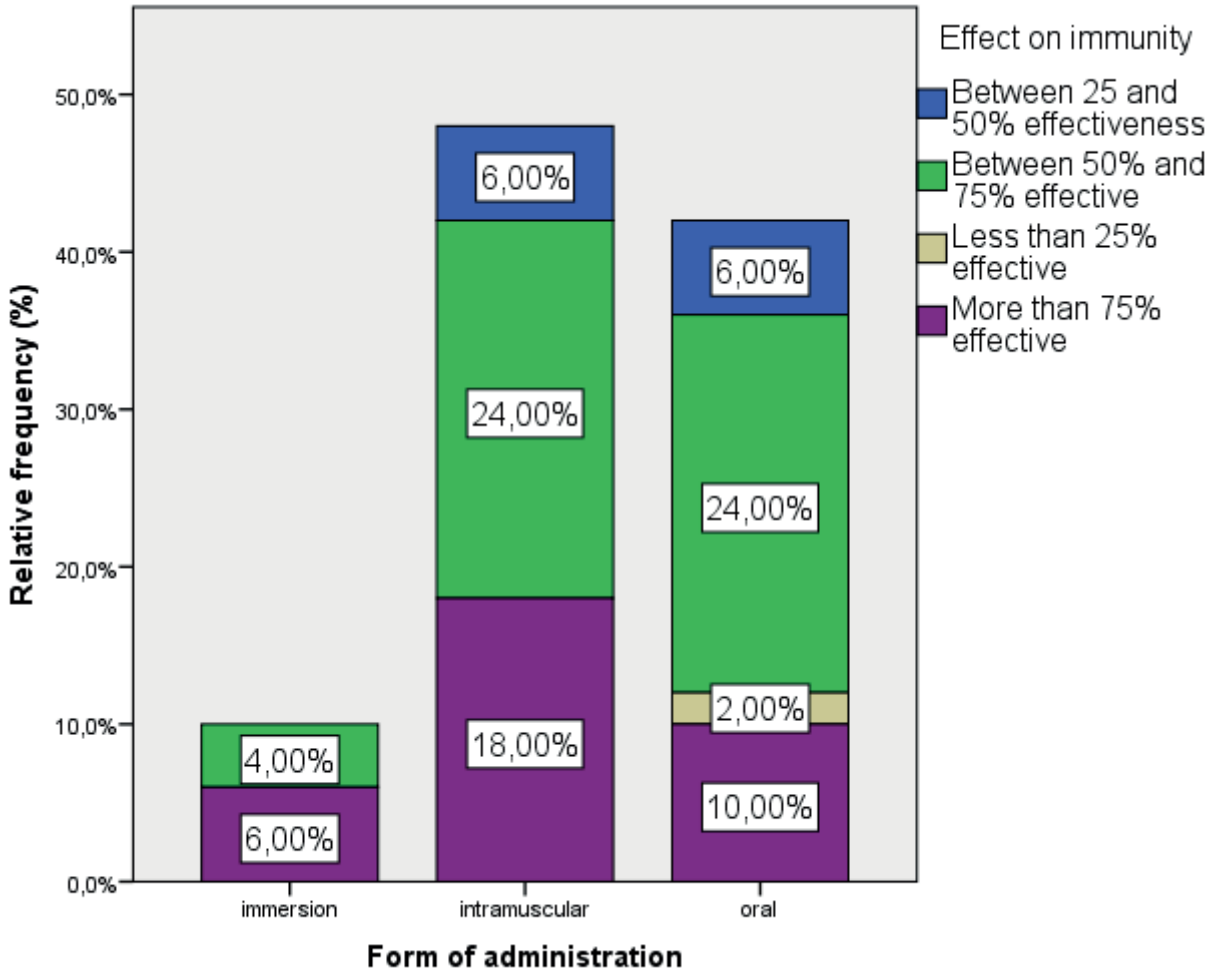

Figure 5. Routes of vaccine administration and effect on the immune system of shrimp.

TITLE	DESCRIPTION	YEAR OF PUBLICATION	REFERENCE
RNA Nanovaccine Protects against White Spot Syndrome Virus in Shrimp	A double-stranded RNA-based nanovaccine was developed as a shrimp disease control with emphasis on the Pacific white shrimp *L. vannamei*.	2022	108
Characterization of *Litopenaeus vannamei* secreted protein acidic and rich in cysteine -like in WSSV infection	Cloned the full-length cDNA sequence of an acidic, cysteine-rich secreted protein from the shrimp *Litopenaeus vannamei* (LvSPARC-L) that encodes 333 amino acids and promotes haemocyte expression.	2021	112
The Active Microbiota of the Eggs and the Nauplii of the Pacific Blue Shrimp *Litopenaeus stylirostris* Partially Shaped by a Potential Vertical Transmission	Analysed the active microbiota associated with *L. stylirsotris* eggs and nauplii, using HiSeq sequencing of the V4 region of the 16S rRNA gene, demonstrating that the microbiota is transmitted vertically at different growth stages.	2022	113
Deciphering the virulent Vibrio *harveyi* causing spoilage in muscle of aquatic crustacean *Litopenaeus vannamei*	The research showed that proven not only viral diseases destroy muscle tissue in crustaceans but also bacterial agents are capable of causing this reaction by changing the microbial composition and that crustaceans could be used as a sensitive broad-spectrum bio detector to indicate the degree of microbial contamination.	2022	114

Table 2. Recent research on WSSV affecting *Litopenaeus spp.* and used fighting strategies.

Industrial development usually starts after laboratory testing that is based on solid academic research. A vaccine can only be made available to the veterinary community once the authorities have granted marketing approval, verifying its effects and potential harm[117].

Industrial development must be seen in an economic context, which is not always the case in academic research so the use of reagents has large economic differences.

Farm Animals' vaccines are produced in large quantities at low cost, while vaccines for companion animals are produced in smaller quantities and sold at higher prices. It should also be taken into account that for-profit companies will generate the development of vaccines for higher incidence diseases or vaccines for high population species[118]. In the case of shrimp, being a species of large-scale production generates interest in aquaculturists, and although *Whispovirus* is sporadic, it generates losses that affect shrimp production during these periods of appearance[42].

Figure 6 shows a production scheme for recombinant protein vaccines that could be used for further implementation in the industry. There is a small amount of commercialization of shrimp vaccines against WVVS. Yet, it is guessed that by having an efficient and replicable treatment in any environment, an industrial process could be implemented for its elaboration and oral administration.

According to figures 4 and 5 of the results obtained from the extracted articles, the production of vaccines with 2 genes has had a higher effectiveness rate. It confers more excellent protection to shrimps, being a process that can be used at the industrial level[61,83].

The bacteria most commonly involved in the replication of recombinant proteins are *Escherichia coli* and *Bacillus subtilis* because of their more efficient replication, procurement and easy genetic manipulation[119-121].

Conclusions

Antibiotics use on shrimp production cause: 1-potential adverse effect on human health[9,122],2-appearance of antibiotic-resistant strains[123,124] and 3-affections on shrimp larvae[125]. Contrarily, vaccine administration to control or lessen the incidence of vibriosis is an attractive choice nowadays.

Vaccination strategies against WSSV, such as inactivated viruses, subunit antigens, and DNA-based vaccines, have shown promise on a laboratory scale. However, drawbacks such as variable efficacy, high manufacturing cost, and limited field applicability need further investigation[126].

A recent study describes a new attractive strategy based on RNAi technologies and polyanhydride nanoparticle-based delivery to develop a nanovaccine[108]. In aquaculture systems, the concept of RNAi-based vaccines has been advocated for several reasons: (a) RNAi functions as an antiviral immune response in shrimp; (b) it is pathogen-specific; and (c) it generates a long-term protective immune response.

On the other hand, another new technology combining vaccines with prebiotics has been shown to maximize the protective efficacy127–129 (Table 1). β-glucans, for example, is a joint prebiotic used in aquaculture and has long been used as an additive in the fish diet to improve the immune response enhancing the innate immune response127,130,131,132.

Despite all these new alternatives to vaccine production and applications, more and more research, mainly on field trials, needs to be carried out to validate further and enhance the vaccine application effectiveness in shrimp.

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Conflicts of Interest

The authors declare no conflict of interest.

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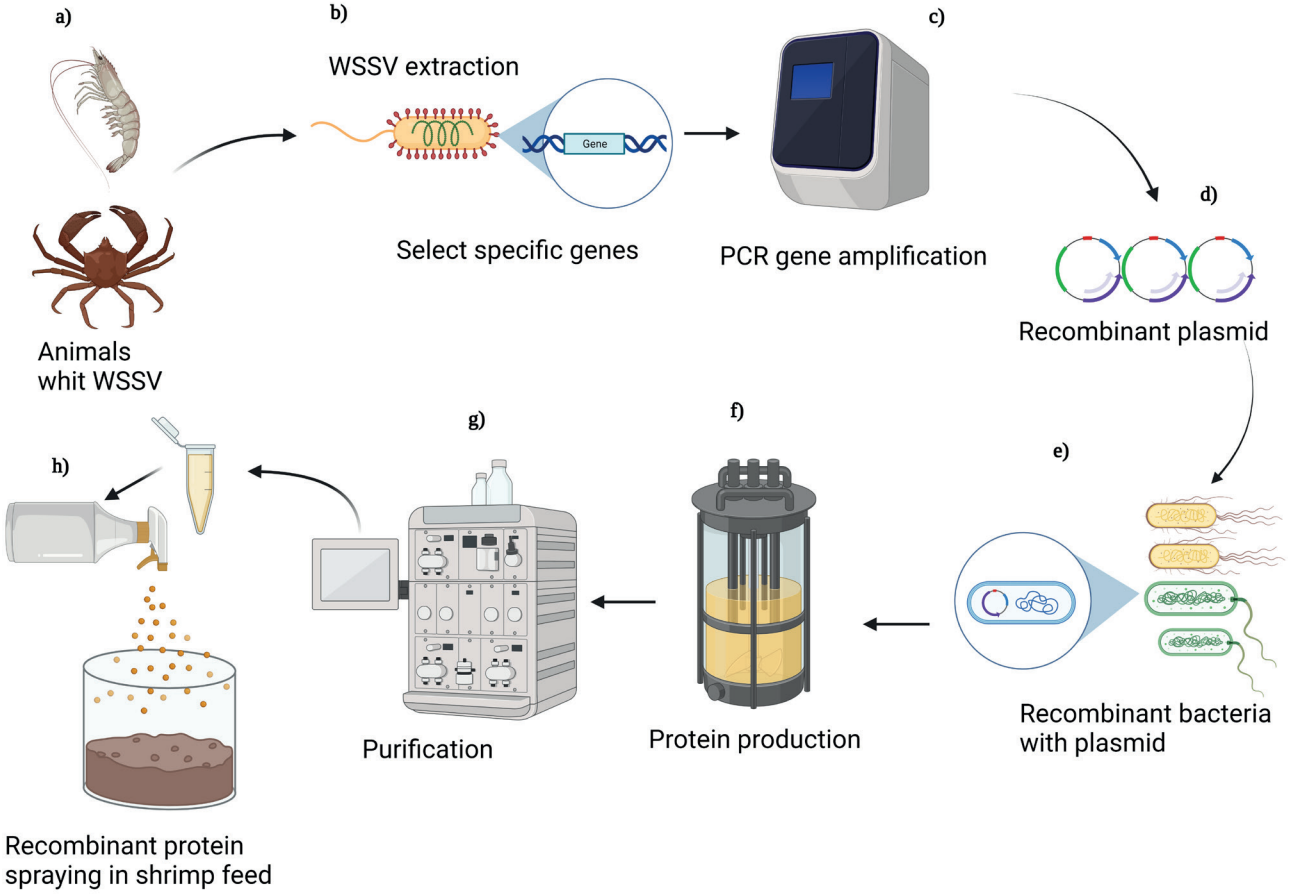

Figure 6. The production process of the oral vaccine in shrimp to combat WSSV. a) The first step is extracting the virus from animal sources such as crabs and shrimps. b) Selection of specific genes for each protein and isolation. c) Amplification. d) Generation of recombinant plasmids. (e) cloning of recombinant bacteria (f) large-scale production and replication in bioreactors of proteins from the recombinant bacteria (g) purification of the medium and extraction of proteins by affinity chromatography (h) combination of purified recombinant proteins with commercial shrimp feed.

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