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Review

Heat Shock Proteins HSP70: Viruses and Potential Role in Shrimp Immunity

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Abstract: Shrimps are under the influence of several environmental factors such as fluctuation of physical and chemical parameters of the water affected by variations in rainfall, temperature, salinity, and pH. These factors have also been identified as risk factors for shrimp disease outbreaks. Despite the high levels of production, shrimp producers suffered significant economic losses in years, mainly due to the presence of diseases that now plague the industry. In particular, viral diseases have had and will continue to have profound impact on industry growth. In response to stress such environmental or pathophysiological, cells are able to up regulating selectively the expression of a protein group known as Heat Shock Proteins (HSPs). In a recent search at the literature we observed a close relation between HSP70 with apoptotic proteins and others stress proteins such HSP60 and HSP90. Moreover, the response of shrimp to viral stress was examined, some of which are correlated to the reactions of HSP70. Thus, the aim of this review is to describe the current knowledge on the status of stress responses in shrimps, particularly HSP70 responses, triggered by viruses.

Keywords: HSP70; Viruses; Shrimp Immunity

1. Introduction

Seafood provides a set of nutrients available, with healthier fats, proteins and vitamin D, among other micronutrients. In coastal regions of developing nations and small island states, 50% of animal protein can come from fishing (FAO 2016). The practice of cultivating penaeid shrimp and their development are of commercial importance and can result in the occurrence of infectious and non-infectious diseases worldwide. Opportunistic microorganisms that are part of the microflora and fauna of the penaeid shrimp cause most of these diseases (Arulmoorthy et al. 2020). Viral pathologies are among the most harmful and lethal diseases described for crustaceans. The knowledge about the genes associated with the viral immune response, as well as the relationship between pathogen and host is still very incomplete. As invertebrates do not have adaptive immunity, they do not have high specificity against invaders. Thus, resistance to disease is based on the innate defense system, which includes a series of coordinated cellular and humoral reactions. Within this set of reactions, Heat Shock Proteins (HSP) is considered the main responses of cell protection against various stressors. There are several studies on the constitutive or inducible responses to thermal shock in HSP70 reported in the literature in the last 20 years.

The scientific literature has been revised for publication records touching of studies for the terms HSP70, shrimp, viral diseases, and immunity within the years 2000 and 2020 and therefore the main results were outlined in Figures 1 and 2. Despite considerable advances in animal virology in recent years and the need for the global aquaculture industry to avoid economic losses, knowledge about aquaculture viruses remains scarce.

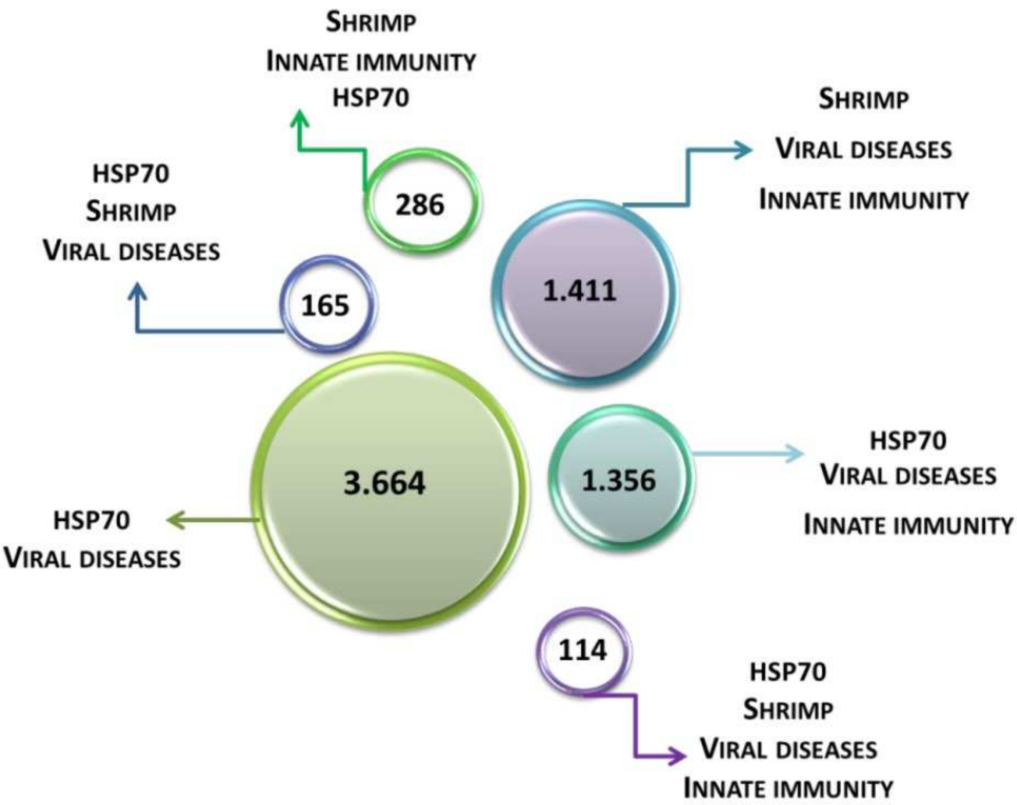

Figure 1. Schematic representation of literature reports in the last 20 years for the terms HSP70, shrimp, viral diseases, and innate immunity**.**

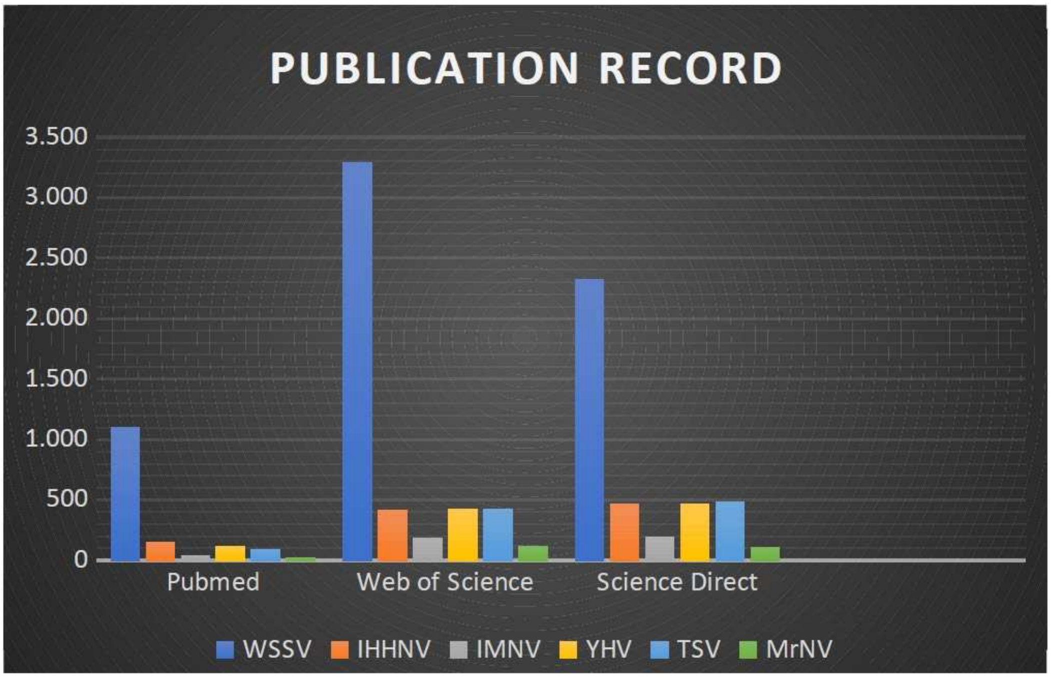

Figure 2. Publication record graphic until August 2020 of the main viral diseases in shrimps worldwide.

HSPs are responses sharply induced by various stressors that denature proteins. In addition to resistance to stress, HSPs are involved in the folding of nascent proteins, in the development of plants and animals, in aging, in adaptation to the environment and in the immune response, demonstrating

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their fundamental importance for cell survival. HSPs are induced in aquatic organisms by perturbations of environmental parameters, contaminants, handling, hormones and biotic stressors (Sung et al. 2011). Perhaps the lack of data related to HSP responses to viral infections in crustaceans is due to the fact that these molecules have a well-established role in studies with thermal stress. The main factors that disturb the production of shrimp are the fluctuations in the physical and chemical parameters of the water caused by precipitation, temperature, salinity and pH (Nguien et al 2020). These factors have also been identified as risk factors for shrimp disease outbreaks associated to climate change events (Tendencia and Verreth 2011). Some immunological or physiological parameters are modulated in response to stressful conditions that negatively affect aquatic organisms and can act as an important indicator of health status in farm animals such as shrimp, where a rapid and accurate assessment of health conditions is essential to ensure the success of cultivation programs. However, non-lethal heat shock protects aquatic organisms against biotic and abiotic stress, perhaps due in part to the buildup of heat shock protein 70 (HSP70) (Sung et al. 2018). Despite the high number of studies involving marine shrimp, most of these studies address species of commercial interest, such as Pacific white shrimp *Litopenaeus vannamei* and *Macrobrachium rosenbergii*.. Both are species with zootechnical interest and a well-established production system. Notably, the number of surveys covering responses to HSP70 and viral diseases has been almost restricted to White Spot Syndrome Virus (WSSV). Among viral pathogens, WSSV remains the most serious for the shrimp industry worldwide. With the intensification of the shrimp farming industry and the international trade in aquatic species, outbreaks of infectious diseases in shrimp-producing regions have become a growing problem (Dorf et al. 2005).

2. Scope of Review

In this review, we describe the main molecules under study, which are related to the immune system discovered by advanced analysis techniques such as genomics and proteomics and the characterization of these immunological molecules that participate in the main defense reactions against pathogenic viruses in crustaceans. We analyzed the history of HSP70 Heat Shock Protein studies that assessed the potential for response to viral diseases in shrimp. We evaluate species, viruses, year of publication, response effectiveness, objectives and efforts, such as optimization and accuracy, and offer recommendations for the development of diagnostic tools for viral diseases in farmed shrimp.

3. Stress physiology and HSP response

Stress may be defined as a physiological disturbance that can be correlated with various abnormalities. The term stress was first used in this context by the physiologist Hans Selye, who found that humans and animals share a specific and consistent pattern of physiological responses to illness or injury (Selye 1936), by which an organism tries to maintain or restore a normal metabolism in face of a physical or chemical force. Living organisms are constantly being subjected to varied situations of stress and the response to these stimuli occurs through changes in cellular metabolism, activating their defense mechanisms. Brett (1958) proposes an additional definition: stress is a change caused by a factor, which exceeds the animal's adaptive responses beyond its normal level, or disrupts normal function in such a way that the chances of survival are reduced. These responses include body attempt to cope with the demands imposed by the disease or lesions.

Marine crustaceans are under the influence of several environmental factors. Among these factors, farmed shrimp are particularly subject to climate change and changes due to agricultural practices that influence the physicochemical water quality. Stress in the aquatic environment as a result of poor environmental quality and intensive agriculture is one of the triggers for the transition from a chronic infectious disease to an acute infection. Stress can modulate the immune capacity of shrimp, as well as causing changes at the biochemical and molecular level. In areas with intensive cultivation system, agricultural effluents are discharged into drainage channels and the chemical and biological contaminants from these effluents recirculate between other rural properties (Kautsky et al. 2000). Abiotic and biotic factors when altered can result in stress for shrimp during the developmental period. Also, these changes in seawater quality can affect animal metabolism, growth, reproduction, immune system and the survival of animals. In fact, shrimps are animals that get stressed easily.

This interpretation of an organism's ability or inability to adjust to a disturbance is consistent with the "General Adaptation Syndrome – GAS" paradigm of Selye (1950), which considers that an organism passes through three stages in response to stress. The first stages are often manifested by measurable physiological changes at different levels of organization, especially at the lowest levels, however the final stage is the maladaptive stage normally related to the development of pathological states, which can alter the body's health condition, eventually resulting in mortality (Barton et al. 2002) in spite of mounting a stress response, also known by "distress" (Selye 1984; Rehman et al. 2017). Organisms and cells can respond to various stress circumstances such as culture system managements, metabolic, or pathophysiological stress by up regulating selectively the expression of a protein group (Bakthisaran et al. 2015). One of the stress responses comprises the action of proteins called HSP, which is one of the primary responses of cell protection (Lindquist and Craig 1988; Sung et al. 2011).

The HSP was first reported by Ritossa (1962), who observed activation of a set of 'puffs' in polytene chromosomes in the salivary glands of Drosophila larvae exposed to sudden elevated temperature or to chemical agents that disturbed oxidative phosphorylation. Afterward, Tissiers et al. (1974) observed that exposure of Drosophila to heat shock resulted in the synthesis of a common set of new proteins called "heat shock proteins" or "stress proteins". The heat shock response has been most extensively studied in Drosophila, but an analogous response has been observed in cells of a broad spectrum of eukaryotes. According to Lindquist and Craig (1988) the heat response is the most highly conserved genetic system known, existing in every organism in which it has been sought, from archaebacteria to eubacteria, from plants to animals.

The HSPs comprises a family of proteins with a high degree of homology. Different HSPs classes are found in various cellular compartments, semiautonomous organelles, mitochondria, and chloroplasts and normally represent 5-10% of the total protein in most cells. Subcellular compartmentalization is a fingerprint of eukaryotic cells (Gabaldón and Pittis 2015). Figure 3 illustrates a schematic representation of a generalized eukaryotic cell showing the main compartments and some HSP location. The size of HSP range from 15 to 110 kDa and they are classified based on the size, site of synthesis, and function. Six structurally conserved distinct classes are: HSP40, HSP60, HSP70, HSP90, HSP100, approximately 17–30 kDa molecular weights referred to as small HSPs (sHSPs). They are expressed both constitutively (cognate proteins) and under stressful conditions (inducible forms) (Danwattananusorn et al. 2011). In addition, HSPs can perform a multitude of housekeeping functions that are essential for cell stability (Srivastava 2002). Under normal conditions, may participate in cellular processes such as protein folding and transport, cell cycle regulation and apoptosis (Mallouk et al. 1999; Johnston et al. 2018), as well as in physiological processes such as embryonic development, gonadal development and spermatogenesis (Binder 2014; Chan et al. 2014). They act as molecular chaperones, maintaining homeostasis and acting against the proteotoxic effects (Huang et al. 2011; de la Vega et al. 2006). Small heat shock proteins (sHSPs) are conserved across species and are key actors in stress tolerance. Several sHSPs exhibit chaperone-like activity to avoid aggregation of target proteins, keeping them in a folding–competent state and refolding them by themselves or with other ATP-dependent chaperones (Bakthisaran et al. 2015).

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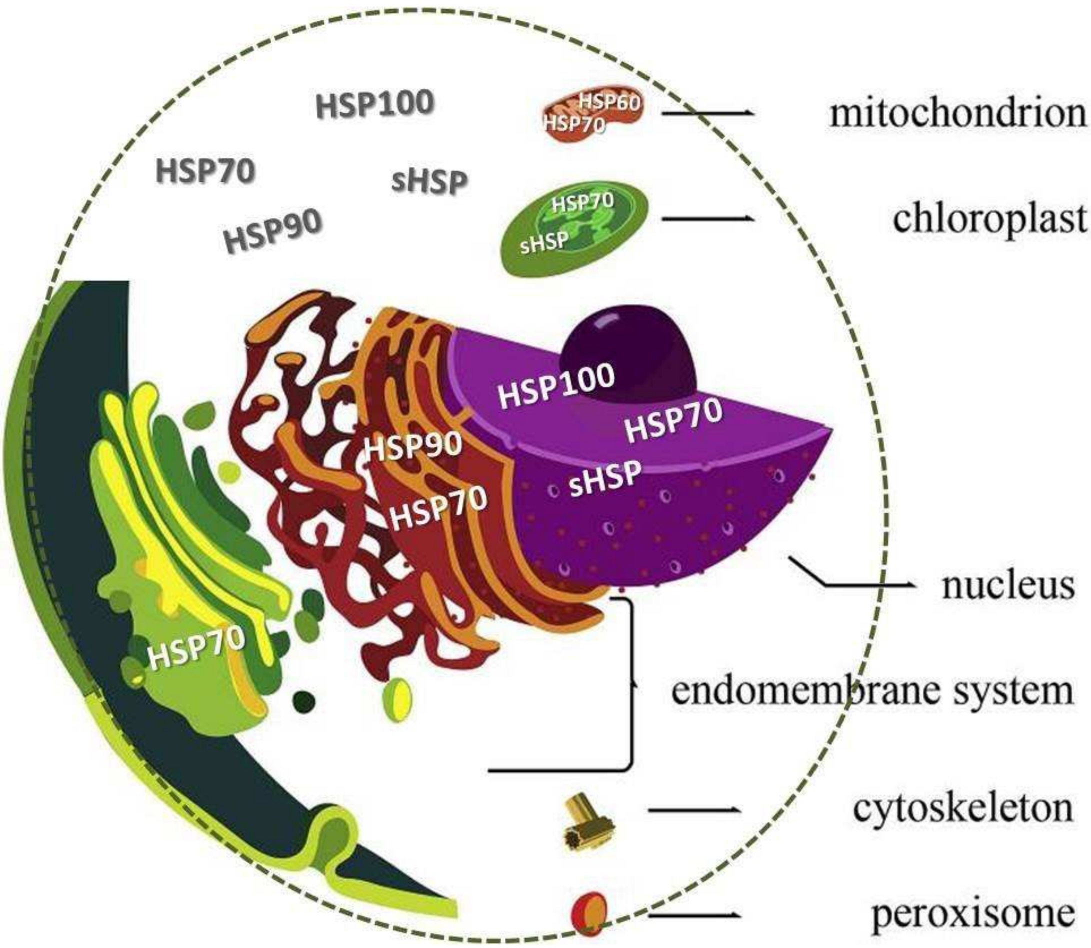

Figure 3. Heat shock proteins location. Schematic representation of a generalized eukaryotic cell. showing the main compartments and some HSP location. Particular organelles were grouped into the single category "endomembrane system" the endoplasmic reticulum Golgi apparatus, lysosomes.

vacuoles, vesicles, and endosomes. Figure modified from Gabaldón & Pittis (2015).

Mainly, the HSP70 family includes the constitutive cytosolic Hsc70 (or HSP73), the stress-induced cytosolic HSP70 (or HSP72), the endoplasmic reticulum Bip (or Grp78), and the mitochondrial mt-HSP70. The HSP70 is composed of two major functional domains according to Lindquist and Craig (1988). The NH2-terminal, very conserved ATPase domain binds ADP and ATP tightly and hydrolyzes ATP, while the COOH-terminal domain is essential for polypeptide binding (Lindquist and Craig 1988; Hartl and Hayer-Hartl 2002). **Thus, HSP70 is an important molecule that acts in the immune defense in response to viral infections in shrimp (Janewanthanakul et al. 2020). On the other hand, a survey in the existing literature shows that little importance has been given to these molecules regarding defense against viruses.**

4. Immune response in viral infections

A wide range of pathogens and parasites can host in decapod crustaceans. However, viral pathogens appear to exercise the most significant restrictions on the growth and survival of these farmer animals. Approximately 60% of the current losses associated with the disease in shrimp aquaculture may be due to viral pathogens, with more than 20% due to bacterial pathogens, while the losses associated with fungal and parasitic agents are lower (Flegel 2006, 2012). Viral infections have been found in wild and cultured crustaceans, since its initial discovery in the 1960s (Vago 1966), more than 50 viruses have been described from several groups of crustaceans. Due to their larger size and pathological characteristics, DNA viruses were mostly described in groups of hosts studied with any level of detail (Takahashi et al. 1994; Stentiford 2008; Behringer et al. 2011). Viruses with RNA genomes, some of which have devastating consequences for cultivated animals, are also being increasingly described, particularly in penaeid shrimp in intensive farming systems (Hasson et al. 1995; Poulos et al. 2006; Lightner 2011).

In general terms, since the beginning of the industry, shrimp farming has been hampered by pathogens that continue to appear despite efforts to create specific pathogen-free (SPF) stocks and specific pathogen resistant (SPR) shrimp. According with Moss et al. (2012) although SPF shrimp are, by definition, free of specifically listed pathogens, SPF shrimp may not be disease free. Also, those authors highlight that SPF shrimp have no innate resistance to any particular pathogen, nor are they innately susceptible. Resistance or susceptibility to disease can be maintained in a shrimp strain through selective breeding, but these characteristics do not influence the status of the SPF.

Shrimps that have compromised immunity As a result of abiotic stress shrimps may be more susceptible to disease and infection, which can lead to mass mortality. To protect against pathogens the defense mechanisms of crustaceans depend completely on the innate immune system (Barracco et al. 2014).. Crustaceans do not have an adaptive immune system, as present in vertebrates, thus, there is no participation of antibodies and memory cells as protection highly specific and long-term (Barracco et al. 2014). The carapace or exoskeleton is the first line of defense. In addition, part of the gastrointestinal is coated with chitin and consists of an acidic environment and full of digestive enzymes and antimicrobial effectors (Soonthornchai et al. 2010). In general, The best known immune defenses in crustaceans are those linked to haemolymph, fluid tissue responsible for transporting nutrients, excreta, hormones, oxygen, and immune system components. The comprehension of innate immune response in shrimps against invading pathogens provides important information for establishing effective methods to control these and, potentially, those of related emerging infectious diseases (Tassanakajon et al. 2013).

Despite the multiple defense mechanisms against viruses, viral diseases continue to be frequent in aquatic environment and shrimp frmas. They represent one of the most significant infectious diseases and can be destroyed by the innate immune response (Machado et al. 2004). A wide range of life stages of shrimp may be susceptible to certain viral infections causing mortality, slow growth, and deformations (Rahman et al. 2007). Shrimp production by aquaculture has been seriously impacted by diseases during the last decades. Among the several viral problems that affect shrimp, there are seven main ones: White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Infectious Myonecrosis Virus (IMNV), Macrobrachium rosenbergii Nodavirus (MrNV), Taura Syndrome Virus (TSV) and Yellow Head Virus (YHV) (Seibert and Pinto 2012), actually part of the OIE listed viral diseases of shrimp (Marques et al. 2006; OIE 2016). Together, the top five viral pathogens (WSSV, IHHNV, IMNV, TSV and YHV) can represent additional annual losses of around $ 500 million, causing a total annual loss of $ 1.5 billion (or 15% of production). If we consider the rates of loss of production by WSSV (10% of the total) and the five main viral diseases (15% of the total), it is equivalent to the total that is imported into the United States and the European Union combined (Stentiford et al. 2012).

White spot syndrome virus (WSSV): is classified in the *Nimaviridae* family as the only known member of the W*hispovirus* genus, comprise a large, circular, double-stranded DNA genome with ~ 300,000 nucleotides (van Hulten et al. 2001). In 1992 WSSV appeared in shrimp farms in northern Taiwan causing massive mortality. A rapidly replicating and extremely virulent pathogen, is the most serious disease-causing agent in shrimp aquaculture worldwide (OIE 2016). The WSSV is spreading across the world causing production breaks and economic losses. One of the last records was in the mud shrimp *Austinogebia edulis* in Taiwan (Zhu et al. 2019).

Infectious hypodermal and hematopoietic necrosis virus (IHHNV): the first widespread epizooty that seriously affected the commercial penaeid shrimp industry and was first reported in Hawaii on blue shrimp *Penaeus stylirostris* in 1980 (Lightner et al. 1983; Lightner 2011). IHHNV possesses a single-stranded linear DNA genome comprising 4,100 nucleotides and is classified as a member of the *Parvoviridae* family that is not formally classified but likely to be a member to the genus *Brevidensovirus* (Shike et al. 2000). In addition, recent studies suggest that IHHNV may act as an immunostimulant that triggers the shrimp's immune response against viral infection (Jariyapong et al. 2019a).

Infectious myonecrosis virus (IMNV): is likely to be a member to *Totiviridae* family, and the first member of this family to infect a host other than fungus or protozoan (Nibert 2007; Poulos et al. 2006). Is a non-enveloped virion with 40 nm in diameter whose icosahedral capsid has fibrous protrusions (Nibert 2007; Takahashi et al. 2000). The long non-segmented double-stranded RNA genome (dsRNA) contains 7,560 nucleotids. Is the most recently emergent virus that infects Pacific white shrimps *Litopenaeus vannamei*. Epizootic events due to IMNV infection were initially reported in 2002 in Northeastern Brazil (Lightner et al. 2006). IMNV is an emerging virus from Penaeid shrimp. It is endemic to Brazil and Indonesia, with a potential chance of spreading across borders. The OIE lists it as one of the main viral pathogens for crustaceans and has initiated active surveillance (Prasad et al. 2017).

Macrobrachium rosenbergii Nodavirus (MrNV): the virus belongs to the family of Nodaviridae (Hameed et al. 2004), which is a small icosahedral, non-enveloped virus of 27 to 30 nm in diameter, causes white tail disease (WTD) in Giant freshwater prawn *Macrobrachium rosenbergii* (Citarasu et al. 2019). The viral genome contains two segments of positive-sense, single-stranded RNA (ssRNA) with 1146 nucleotides. The first report was in the Guadeloupe Island in the French West Indies in 1997 (Arcier et al. 1999). The latest studies show that white tail disease infection caused by MrNV, occurs only in nauplii, and not in adults. The functions related to the immune system derived from hematopoietic tissue and haemocyte homeostasis in adult shrimp are believed to play roles in resistance to viral infection. (Jariyapong et al. 2019b).

Taura syndrome virus (TSV): a member of the *Dicistroviridae* family, comprising of a small, non-enveloped, icosahedral virion with 31-32 nm in diameter. Its genome comprises 10,250 nucleotides in a single-stranded linear RNA of positive polarity (Mari et al. 2002). One of the most important pathogens affecting farm-reared shrimp was first reported in 1992 in *Litopenaeus vannamei* collected from shrimp farms in Taura River, Ecuador (Lightner et al. 1995; Lightner 2011). The mechanisms of TSV transmission occur by cannibalism of infected moribund or dead shrimp by healthy shrimp in a pond or in an experimental tank. The current TSV control methods emphasize biosecurity at the farm level and the utilization of SPF, TSV-resistant shrimp for stocking (Dhar and Allnut 2008).

Yellow head virus (YHV): is classified in the *Roniviridae* family, genus *Okavirus*, within the *Nidovirales* order (Wongteerasupaya et al. 1995). YHV genome is a positive-sense single-stranded RNA of approximately 26,662 nucleotides (Sittidilokratna et al. 2008) and is one of six known genotypes in the yellow head complex of viruses, was the cause of the second most serious viral epizootic of penaeid shrimp that occurred in Thailand in 1990 in cultured *Penaeus monodon* (OIE 2016). Moreover, Gill-associated virus (GAV) was assigned as the type species of *Okavirus* genus in *Roniviridae* based on the evolutionary divergence of GAV and YHV from other nidoviruses (Gorbalenya 2008; Gorbalenya et al. 2006). Interestingly, their rod-shaped enveloped virions being distinct from the spherical enveloped virions formed by viruses in either the *Coronaviridae* or the *Arteriviridae* families of the *Nidovirales* known to exist at that time. Suppositions about vertical transmission come by analogy with GAV and can be very accurate. However, the prevalence of YHV in healthy shrimp appears to be far lower than for GAV and other genotypes, and it is unclear how it maintains a cycle of natural infection (Walker and Sittidilokratna 2008).

Shrimp haemocyte iridescent virus (SHIV): one of the last viral diseases discovered and still little studied, according to a review by Arulmoorphy et al. (2020), was first identified and described by Qiu et al. (2017). A large icosahedral virus classified in the family *Iridoviridae* with diameters in the range of 120 to 300 nm, reaching up to 350 nm, this new iridescent virus does not belong to the five known genera of this family (*Iridovirus, Chloriridovirus, Ranavirus, Lymphocystivirus,* and *Magalocytivirus*). The SHIV virion core comprises a single linear double stranded DNA (dsDNA) molecule of 140–303 kbp. This family of viruses has a wide range of hosts, including from invertebrates (such as insects) to pecilothermic vertebrates, such as fish, amphibians and reptiles (Jancovich et al. 2012).

Several molecules concerning immunology in penaeid shrimp have been discovered, and can assist the animal defense against viral pathologies, most through the analysis of expressed sequence libraries, microarray studies, biochemical, genomic and proteomic approaches (e.g., Aoki et al. 2011; Hirono et al. 2011; Robalino et al. 2009; Somboonwiwat et al. 2010; Liu et al. 2011; Valentim-Neto et al. 2014; Valentim-Neto et al. 2015; Chen et al. 2016; Ren et al. 2019). Rowley and Pope (2012) mention that the immune system of invertebrates is composed of a cellular and a humoral part that are closely related. The main effector cells of this immune system in crustaceans are haemolymph cells or haemocytes, while the hepatopancreas is responsible for the biosynthesis of some humoral factors. These factors may be naturally occurring and/or formed after antigenic stimulation (Ratcliffe et al. 1985). In shrimp, as in other crustaceans, there appear to be three main types of haemocytes (blood cells), hyaline cells, semi-granular cells and granular cells (called granulocytes by some authors). Immune molecules include antimicrobial peptides, serine proteinases and inhibitors, phenoloxidases (PO), oxidative enzymes, coagulable proteins, pattern recognition proteins (PRP), lectins, Toll receptors, HSPs and other humoral factors that can participate in the shrimp's innate immune system (Tassanakajon et al. 2013; Janewanthanakul et al. 2020).

The modulation of some immunological or physiological parameters, as a response to a viral infection stress, may function as an important indicator of health status in farmed animals such as shrimps, where a rapid and precise assessment of health conditions is crucial to ensure the success of farm programs.

5. HSP70 and cell death

For successful viral replication within an individual cell, a remarkable cascade of interactions between the virus and the host is required, beginning at the first involvement of the cell receptor until the final release of progeny virions. Verbruggen et al. (2016) have list a variety of molecular mechanisms to response and even prevent virus infectivity responses in host. These mechanisms involve some of genes related to heat shock responses. Shrimp showed high induced genes like Caspase, Ubiquitin and HSP in severity viral load, shown a role play of responsive proteins in pathological processes. Further HSPs also play a role in apoptosis mechanism.

Apoptosis, a programmed cell death, is considered an important mechanism in the development, homeostasis and cell defense that inhibits viral multiplication and eliminates infected cells in multicellular organisms (Tschopp et al. 1998; Everett and McFadden 1999; Koyama et al. 2000). The apoptosis mechanism of infected cells is triggered by cytolytic cells activated during the antiviral immune response or may be a direct result of viral infection, if apoptosis occurs at the early stage of viral infection, i.e., before complete viral replication, the production of progeny virions will be severely hampered, limiting virus spread in the host. Consequently, many viruses have developed various strategies that inhibit apoptosis during virus infection, thereby prolonging cell viability until sufficient progeny viruses have been produced (Tschopp et al. 1998; Hay and Kannourakis 2002). However, the viruses could intentionally induce apoptosis at the late stage of viral infection in order to facilitate the assembly or release of progeny virus, or to promote the spread of progeny virus within a host without triggering inflammatory responses (Best and Bloom 2004; Best 2008). Some proteins that were predominantly anti-apoptotic were HSP27, HSP70, and HSP90, whereas HSP60 is pro-apoptotic (Murthy and Ravishankar 2016). HSP90 and HSP70 are critical to the folding and assembly of other cellular proteins and are also involved in regulation of kinetic partitioning between folding, translocation, and aggregation within the cell (Roberts et al. 2010).

HSP70 genes in aquatic invertebrates have also been experimentally shown to decrease the mortality rate of certain organisms (Ferreira et al. 2017; Yan et al. 2010). In *L. vannamei*, several HSP (HSP10, HSP27, HSP30, HSP60, HSP70 and HSP90) were consistently or specifically expressed in response to bacterial or viral infection (Chaurasia et al. 2016; Junprung et al. 2017; Yuan et al. 2017), and was demonstrate that HSC70 reduced protein aggregation and mortality after viral infection (Yuan et al. 2017). In the same way HSP70 was predominantly induced in hepatopancreas and gills of *Fenneropenaeus chinensis* when the shrimp were exposed to heat shock and *Vibrio anguillavium*-challenged stresses (Zhenyu et al. 2004). Under WSSV treated condition, three HSP (HSP21, HSP70

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and HSP90) genes were commonly expressed in all examined tissue suggesting that all three forms of *HSP* gene products were required to maintain cellular homeostasis (Venkatesan and Hammed 2014).

The expression of *HSP70* was increased as a part of an immune response against *Vibrio harvey* in *P. monodon* (Rungrassamee et al. 2010). According to Valentim-Neto et al. (2014) was observed that the shrimp positive to IHHNV or WSSV infection with changes in HSP70 fold had a higher rate of survival during the period of cultivation in comparison with others. Also, an association between resistance to TSV and *HSP70* gene polymorphisms among Asian commercial *L. vannamei* populations has been reported by Zeng et al. (2008). In addition to that, induction of *HSP70* gene transcription caused by increasing the water temperature to 32°C, during WSSV infection, suggests a connection between this gene and WSSV resistance in shrimp (Lin et al. 2011).

Xu et al. (2009) propose HSP70 is one of the binding partners of VP28 through virus infection and its expression was enhanced by WSSV infection at the early stage of the process. Like other animal viruses, WSSV infection induces apoptosis. Leu et al. (2013) propose a model for apoptotic interaction between white spot syndrome virus and shrimp. The basic sequence of events is as follows: first, when a WSSV infection occurs, cellular sensors detect the invading virus, and activate signaling pathways that lead to (1) the expression of pro-apoptosis proteins, like Caspase modulators, and (2) mitochondrial changes, including the induction of mitochondrial membrane permeabilization and increased oxidative stress.

Caspases are central effectors in apoptosis cascade. In marine shrimp *Marsupenaeus japonicus* was found to be significantly upregulated in survivors of WSSV-challenged shrimp, suggesting that it might be involved in shrimp antiviral immunity (Wang et al. 2008). Likewise, Ubiquitin protein has been associated with several processes, including cellular progression, organelles biogenesis, transcriptional regulation, antigen processing, and apoptosis according with Chen et al. (2008) and Shen et al. (2009). Induction of this gene has been expressed in studies with WSSV- infected shrimp (Wang et al. 2006; He et al. 2005) shown to play an important role in viral latency regulation.

WSSV infection can induce characteristic signs of apoptosis (nuclear fragmentation, chromosomal DNA fragmentation and increased activity of caspases) in different tissues. Apoptosis is also known as a defense mechanism against the TSV and YHV viruses. The occurrence of a rapid and progressive spread of apoptosis in infected *P. monodon* with YHV is the main cause of dysfunction and death of the host (Flegel and Sritunyalucksana 2011). Given this, the induction of apoptosis only becomes effective as an antiviral response if it is triggered in an early stage of infection, which may limit the production of particles and reduce or eliminate the spread of the viral progeny to other tissues (Xu et al. 2014).

Janewanthanakul et al. (2020), report that HSP70 enhanced the expression of the key gene in the prophenoloxidase (proPO) activating system but reduced the expression of caspase2 and inhibitor of apoptosis proteins (IAP) in WSSV-infected *L. vannamei*. These results suggested that the HSP70 is an important molecule involved in antiviral defense in shrimp presumably via modulating the proPO system and apoptosis.

The health of aquatic species is dependent on interactions between the environment, pathogens, and the host itself. Different aspects related to each one of them can contribute to impact the interaction among pathogen and host (Moser 2005). Farmed animals can coexist with potential pathogens with little or no impact on production (Bachère 2003), while some pathogens dramatically impact production, like the WSSV. Hyperthermal, hypoxic or hyposmotic conditions, for example, have been recognized as triggers for the transition from chronic to acute viral infections in shrimp populations (de la Vega et al. 2007; de la Vega et al. 2006; Liu et al. 2006). This transition appears to be the result of a reduction in the immunity and defense capacity of shrimp (Hall and van Ham 1998; Le Moullac and Haffner 2000).

6. Concluding remarks

Current progress in aquaculture is a reality and biosafety practices to prevent outbreaks of shrimp disease are a necessity. Stress in aquatic animals can occur due to several factors, including opportunistic diseases, climate changes and increased stocking density, which leads to the appearance of physiological disorders. Marine crustaceans, such as shrimp, are under the influence of these factors, which can cause pathologies, including some viral diseases. Within this framework, several techniques are being developed to minimize the impacts, among them the study of HSP70. Several authors have disclosed in important research that this protein can help to fight infections and the animal immune defense. However, we are still at the beginning of this process and other studies and measures are extremely important to improve understanding these animals health status, use of certified broodstock, regular monitoring of aquaculture ponds, developing good management practices and the safe maintenance of aquaculture crops, avoiding economic losses.

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