COMBINATION BIOLOGICS FOR TREATMENT AND DISEASE MANAGEMENT IN AQUATIC ORGANISMS, COMPOSITIONS AND METHODS THEREOF

FIELD OF INVENTION

[001] The present disclosure broadly relates to the field of prophylactic and therapeutic interventions for infections in aquatic organisms. In particular, the disclosure relates to a chimeric antigen comprising a polypeptide of white spot syndrome virus (WSSV) or fragment thereof, and a virus-like particle (VLP)-forming domain. It further relates to a feed composition comprising the chimeric antigen and a dsRNA, methods of preparation, and uses thereof.

BACKGROUND OF THE INVENTION

[002] Shrimp farming makes a significant contribution to the global agricultural and international trade. In 2021, 4.45 million tons of shrimps were produced, and 3.35 million tons were part of the international trade worldwide. The United States of America imported 0.8 million tons of shrimp, valued ~8 billion USD (*GLOBEFISH Highlights - International Markets for Fisheries and Aquaculture Products* 2022), while India contributes to ¼ th of total global shrimp trade *(Patil et al. 2021*).

[003] Although profitable, the investment including capital cost and crop maintenance cost in a shrimp farm is very high. Roughly, the investment for maintaining a crop is double the expected profit *(Prawn Farming Project Report, Cost, Profits Guide-Agri Farming, 2018).* This makes the adaptation of methods and procedures to minimize loss of crop of utmost importance for shrimp farmers. One of the common reasons for shrimp crop loss are infections. The economic loss due to viral diseases alone has been estimated to be 6 billion USD *(Rizan, N., Yew, C.Y., Niknam, M.R. et al. Electronic Properties of Synthetic Shrimp Pathogens-derived DNA Schottky Diodes. Sci Rep* ***8****, 896 (2018). https://doi.org/10.1038/s41598-017-18825-6),* and white spot syndrome virus (WSSV) infections are estimated to be causative for ⅔ rd of the loss caused by viral

infections *(Stentiford, G.D.; Neil, D.M.; Peeler, E.J.; Shields, J.D.; Small, H.J.; Flegel, T.W.; Vlak, J.M.; Jones, B.; Morado, F.; Moss, S.; Lotz, J.; Bartholomay, L.; Behringer, D.C.; Hauton, C.; and Lightner, D.V., "Disease will limit future food supply from the global crustacean fishery and aquaculture sectors" (2012). Publications, Agencies and Staff of the U.S. Department of Commerce. 350)*. In 2018-2019 a survey indicated that 0.77 million tons of shrimp was lost due to WSSV infection in India alone. Various measures are taken to save the shrimp crop from the infections, such as farm management, creation of disease resistant shrimps, and vaccination of shrimps.

[004] Vaccination of shrimps have been attempted using various approaches that include sub-unit protein vaccines, whole virus inactivated vaccines, DNA vaccines, and RNA vaccines. However, there is not a gold-standard mechanism of shrimp vaccination available yet, as there is limited availability of data on the protections these vaccinations provide in field trials and also there is variability in the laboratory trials. Therefore, there is a need for optimized protection strategies of shrimps against infections; and thereby develop a solution that is industrially scalable and effective.

SUMMARY OF INVENTION

[005] In an aspect of the present disclosure, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide is selected from the group consisting of: (a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain; and (b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain.

[006] In an aspect of the present disclosure, there is provided a composition comprising the chimeric antigen as disclosed herein.

[007] In an aspect of the present disclosure, there is provided a feed composition for an aquatic organism, comprising: (a) the chimeric antigen as disclosed herein, or the composition as described herein; and (b) a double stranded ribonucleotide.

[008] In an aspect of the present disclosure, there is provided a nucleotide encoding the chimeric antigen as disclosed herein.

[009] In another aspect of the present disclosure, there is provided a recombinant construct comprising the nucleotide as disclosed herein, operably linked to a promoter.

[0010] In an aspect of the present disclosure, there is provided an expression vector comprising the construct as disclosed herein.

[0011] In an aspect of the present disclosure, there is provided a recombinant host cell comprising the vector as disclosed herein.

[0012] In an aspect of the present disclosure, there is provided a method for preparing the chimeric antigen as disclosed herein, wherein the method comprises: (a) providing the host cell as disclosed herein, in a culture medium; (b) culturing the host cell for a period in the range of 48 to 72 hours at a temperature in the range of 25 to 35 °C, to obtain the chimeric antigen.

[0013] In an aspect of the present disclosure, there is provided a method for producing the feed composition as disclosed herein, wherein the method comprises providing the chimeric antigen as disclosed herein or the composition as disclosed herein and a carrier feed; and mixing to obtain the feed composition.

[0014] In an aspect of the present disclosure, there is provided a method for treating an aquatic organism, comprising administering the chimeric antigen as disclosed herein, or the composition as disclosed herein, or the feed composition as disclosed herein, to the aquatic organism.

[0015] These and other features, aspects, and advantages of the present subject matter will be better understood with reference to the following description. This summary is provided to introduce a selection of concepts in a simplified form. This summary is not intended to identify key features or essential features of the disclosed subject matter, nor is it intended to be used to limit the scope of the disclosed subject matter.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS

[0016] The following drawings form a part of the present specification and are included to further illustrate aspects of the present disclosure. The disclosure may be better understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

[0017] Figure 1 depicts the percentage survival of shrimps injected with VP28 and chimeric antigen, 10 days after the WSSV challenge, in accordance with the embodiments herein.

[0018] Figure 2 depicts the percentage survival of untreated shrimps and shrimps treated with the feed composition having chimeric antigen or dsRNA, after 7 days of WSSV challenge, in accordance with the embodiments herein.

[0019] Figure 3 depicts the percentage survival of the untreated shrimps and shrimps treated with the feed composition having chimeric antigen and/or dsRNA, after 8 days of WSSV challenge, in accordance with the embodiments herein.

[0020] Figure 4 depicts the percentage survival of the untreated or chimeric antigen treated shrimps, 8 days after the WSSV challenge, in accordance with the embodiments herein.

[0021] Figure 5 is a schematic representation illustrating the expression cassette to be integrated in yeast, in accordance with the embodiments herein.

[0022] Figure 6 is schematic representation illustrating the *E.coli* vector and yeast vector, with expression cassette.

[0023] Figure 7 is a schematic representation illustrating chimeric antigens FA1, FA2, SP1 to SP 3, and MP1 to MP11, in accordance with the embodiments herein.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Those skilled in the art will be aware that the present disclosure is subject to variations and modifications other than those specifically described. It is to be understood that the present disclosure includes all such variations and modifications. The disclosure also includes all such steps, features, compositions,

and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any or more of such steps or features.

Definitions

[0025] For convenience, before further description of the present disclosure, certain terms employed in the specification, and examples are delineated here. These definitions should be read in the light of the remainder of the disclosure and understood as by a person of skill in the art. The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[0026] The articles "a", "an" and "the" are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[0027] The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included. It is not intended to be construed as "consists of only".

[0028] Throughout this specification, unless the context requires otherwise the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps.

[0029] The term "including" is used to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

[0030] The term" w/w", as used herein, refers to percentage by weight, relative to the weight of the total composition, unless otherwise specified.

[0031] The term "white spot syndrome virus", as used herein refers to a virus of the family *Nimaviridae.* It is a large DNA virus, and it is the only assigned member of the family. It is the causative agent of white spot syndrome disease. The terms "white spot syndrome virus", or "WSSV" are used interchangeably in the present disclosure.

[0032] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or

equivalent to those described herein can be used in the practice or testing of the disclosure, the preferred methods, and materials are now described. All publications mentioned herein are incorporated herein by reference.

[0033] Embodiments herein provide a chimeric antigen for prophylactic and therapeutic treatment of aquatic organisms. The chimeric antigen, as disclosed herein, is capable of providing protection against infections in aquatic organisms. The chimeric antigen, according to embodiments herein, comprises a polypeptide of white spot syndrome virus (WSSV) protein, or fragment thereof, and a virus like particle (VLP)-forming domain. Embodiments herein further provide a feed composition comprising the chimeric antigen; and a double stranded ribonucleotide corresponding to a target gene of a virus. Further, embodiment herein provide methods for preparing the chimeric antigen and the feed composition; and a method of treating aquatic organisms.

[0034] The composition, as disclosed in the embodiments herein, is capable of addressing various challenges associated with preventing or treating infections in aquatic organisms, especially in farmed aquatic organisms. The disclosed feed composition, according to embodiments herein, is a combination biologic that provides protection to aquatic organisms against infections, such as WSSV infection. The composition provides immunity against WSSV infection using two mechanisms. One of the mechanisms involves exposing the aquatic organism to the chimeric antigen so that the immune system is activated, the other is to provide a dsRNA to generate RNA interference (RNAi) response specific for WSSV. The composition is preferably administered via oral delivery, preferably by coating on the surface of the aquatic organism feed which act as carrier. The manufacturing of this feed composition does not require setting up a specialized facility as it can be easily manufactured in a fermentation facility.

Chimeric antigen

[0035] Embodiments herein provide a chimeric antigen comprising a polypeptide of white spot syndrome virus (WSSV) protein, or fragment thereof; and a virus like particle (VLP)-forming domain.

[0036] The term "chimeric antigen", as used herein, refers to a genetically engineered antigen. Typically, a chimeric antigen includes one or more moieties such as polypeptides, peptides, linker residues, and/or other molecules linked to form a single molecule. According to embodiments herein, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and virus like particle (VLP)-forming domain. The polypeptide of white spot syndrome virus (WSSV) protein is linked to the VLP forming domain by one or more linkers.

[0037] The polypeptide of white spot syndrome virus (WSSV) protein may be linked to the virus like particle (VLP)-forming domain at the N- terminal and/or C-terminal end of the polypeptide of white spot syndrome virus (WSSV) protein.

[0038] In an embodiment, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide selected from the group consisting of: (a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain; and (b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain.

Polypeptide of white spot syndrome virus (WSSV) protein

[0039] Embodiment of the chimeric antigen as disclosed herein include a polypeptide of white spot syndrome virus (WSSV) protein. The terms "polypeptide," and "peptide", used interchangeably herein, refer to a polymer of amino acid residues. The term "fragment", as used herein, refers to a polypeptide sequence that is a portion of a full-length protein. The fragment may possess biological activity similar to that of the full-length protein, for example, a fragment may contain one or more epitopes, that invokes a similar immune response as that of the full-length protein.

[0040] The term "polypeptide of white spot syndrome virus (WSSV) protein", as used herein, refers to a polypeptide of WSSV envelope protein, or fragment thereof,

selected from VP 28, VP 26, VP 19, or combination thereof. VP 28, VP 26, and VP 19 are major proteins found on the envelope of WSSV. Embodiments of the chimeric antigen, according to the present disclosure, may comprise the full-length polypeptide of VP 28, VP 26, or VP 19; a fragment of the VP 28, VP 26, or VP 19; or a combination of fragments of VP 28, VP 26, and VP 19. Fragments of VP 28, VP 26, and VP 19 are referred to herein as VP 28 peptide, VP 26 peptide and VP 19 peptide, respectively. Many fragments have been identified by the present inventors and disclosed in the present disclosure, and embodiments herein may include single fragments or combination of such fragments. For example, the chimeric antigen may include a single VP 28 peptide or a combination of VP 28 peptide, VP 26 peptide, and VP 19 peptide. Various such combinations and single peptides (i.e. antigenic repertoire or "AR"), capable of invoking an immune response, have been disclosed herein. While the present disclosure provides various chimeric antigen designs of the single peptides and combinations by way of illustrations, it is understood that various other combinations and single peptide chimeric antigens may be achieved in light of the present disclosure. All such combinations and single peptide chimeric antigens are understood to be included within the scope of the present invention.

[0041] In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein has at least 80%, at least 97%, at least 98%, at least 99%, or 100% sequence identity sequence identity to a sequence selected from SEQ ID NO. 1 (AR1), SEQ ID NO. 2 (AR2), SEQ ID NO. 3 (AR3), SEQ ID NO. 4 (AR4), SEQ ID NO. 5 (AR5), SEQ ID NO. 6 (AR6), SEQ ID NO. 7 (AR7), SEQ ID NO. 8 (AR8), SEQ ID NO. 9 (AR9), SEQ ID NO. 10 (AR10), SEQ ID NO. 11 (AR11), SEQ ID NO. 12 (AR12), SEQ ID NO. 13 (AR13), SEQ ID NO. 14 (AR14), SEQ ID NO. 15 (AR15), SEQ ID NO. 16 (AR16), SEQ ID NO. 17 (AR17), SEQ ID NO. 18 (AR18), SEQ ID NO. 19(AR19), SEQ ID NO. 20 (AR20), SEQ ID NO. 21 (AR21), SEQ ID NO. 22 (AR22), SEQ ID NO. 23 (AR23), SEQ ID NO. 24 (AR24), SEQ ID NO. 25 (AR5), or combination thereof.

[0042] In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein has at least 80%, at least 97%, at least 98%, at least 99%, or 100% sequence

identity sequence identity to a sequence selected from SEQ ID NO. 1 to SEQ ID NO. 25 (AR5), or combination thereof.

[0043] In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein is a full-length polypeptide of VP28, and has a sequence as set forth in SEQ ID NO.1.

[0044] In some embodiments, the polypeptide of white spot syndrome virus (WSSV) protein is a selected from a VP28 peptide, a VP26 peptide, a VP19 peptide, or combination thereof.

[0045] In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein is a selected from a VP28 peptide, a VP26 peptide, a VP19 peptide, or combination thereof, wherein the VP28 peptide has at least 80% sequence identity to a sequence selected from SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, or combination thereof, wherein the VP26 peptide has at least 80% sequence identity to a sequence selected from SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, or combination thereof, wherein the VP19 peptide has at least 80% sequence identity to a sequence selected from SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof.

[0046] The combination of VP28 peptide, VP26 peptide, and VP19 peptide may be achieved by using one or more linkers. The term "linker", as used herein, refers to a short segment of peptide that are used for connecting with other peptides or polypeptides. The terms "linker", and "linker peptide" are used interchangeably throughout the present disclosure. In an embodiment, the linker may be selected from linker L1, linker L2, linker L3, linker L4, or combination thereof.

[0047] The linker, according to embodiments herein, may be a linker of amino acid residue length in the range of 1 to 50 amino acids, preferably 1 to 30 or 1 to 20 amino acid residues length, having a sequence for example of "GGSGLQ" and "GS". The sequence of linker peptide may vary. In an embodiment, the linker is having a sequence selected from "GGGGSGGGGSGGGGSG",

"KESGSVSSEQLAQFRSLD", "EGKSSGSGSESKST" , "GSAGSAAGSGEF" or combination thereof. In light of the present disclosure, it is understood that various alternatives and modification to linkers may be practised within the scope of the present invention by a person of skilled in the art.

[0048] In an embodiment, the linkers may be selected from linker L1, linker L2, linker L3, linker L4, or combination thereof, wherein the linker L1 has an amino acid sequence as set forth in SEQ ID NO. 42; the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43; the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44; and the linker L4 has an amino acid sequence as set forth in SEQ ID NO. 45.

[0049] In some embodiments, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP28 peptide, a VP26 peptide and a VP19 peptide, linked by one or more linkers. The VP28 peptide, VP26 peptide and VP19 peptide may be linked suitably, for eg: as VP28 peptide-VP26 peptide-VP19 peptide, VP26 peptide-VP28 peptide-VP19 peptide, VP26 peptide-VP28 peptide- VP19 peptide, VP19 peptide-VP28 peptide- VP26 peptide, VP19 peptide-VP26 peptide- VP28 peptide, and so on. Accordingly, in an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a VP28 peptide linked to a VP26 peptide which is further linked to a VP19 peptide. In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a VP26 peptide linked to a VP28 peptide which is further linked to a VP19 peptide. In yet another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a VP19 peptide linked to a VP26 peptide which is further linked to a VP28 peptide.

[0050] In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP28 peptide, a VP26 peptide, and a VP19 peptide, wherein the VP28 peptide has at least 80% sequence identity to a sequence selected from SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, or combination thereof, wherein the VP26 peptide has at least 80% sequence identity to a sequence selected from SEQ ID NO. 12, SEQ ID NO. 13,

SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, or combination thereof, wherein the VP19 peptide has at least 80% sequence identity to a sequence selected from SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof.

[0051] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP28 peptide linked to a VP26 peptide, and the VP26 peptide linked to a VP19 peptide, wherein the VP28 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 9, SEQ ID NO. 8, and SEQ ID NO. 11, wherein the VP26 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 14, and SEQ ID NO. 12, wherein the VP19 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 23, SEQ ID NO. 25, and SEQ ID NO. 24.

[0052] n an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 9, SEQ ID NO. 8, and SEQ ID NO. 11, wherein the VP26 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 14, and SEQ ID NO. 12, wherein the VP19 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 23, SEQ ID NO. 25, and SEQ ID NO. 24, wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

[0053] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 linked to a VP28 peptide having a sequence as set forth in SEQ ID NO. 6, and the VP28 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25.

[0054] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 linked to a VP28 peptide having a sequence as set forth in SEQ ID NO. 6 by a linker L2, and the VP28 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

[0055] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP19 peptide having a sequence as set forth in SEQ ID NO. 23 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 15, and the VP26 peptide is linked to the VP28 peptide having a sequence as set forth in SEQ ID NO. 10.

[0056] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP19 peptide having a sequence as set forth in SEQ ID NO. 23 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 15 by a linker L2, and the VP26 peptide is linked to the VP28 peptide having a sequence as set forth in SEQ ID NO. 10 by a linker L3; wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

[0057] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination selected from the group consisting of: (a) a VP28 peptide having a sequence as set forth in SEQ ID NO. 6 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 16 by a linker L2, and the VP26 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 23 by a linker L3; (b) a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 linked to a VP28 peptide having a sequence as set forth in SEQ ID NO. 6 by a linker L2, and the VP28 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; (c) a VP28 peptide having a sequence as set forth in SEQ ID NO. 9 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 16 by a linker L2, and the VP26 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; (d)

a VP28 peptide having a sequence as set forth in SEQ ID NO. 9 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 by a linker L2, and the VP26 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; (e) a VP19 peptide having a sequence as set forth in SEQ ID NO. 23 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 15 by a linker L2, and the VP26 peptide is linked to the VP28 peptide having a sequence as set forth in SEQ ID NO. 10 by a linker L3; (f) a VP28 peptide having a sequence as set forth in SEQ ID NO. 11 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 14 by a linker L2, and the VP26 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; (g) a VP28 peptide having a sequence as set forth in SEQ ID NO. 9 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 16 by a linker L2, and the VP26 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; (h) a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 linked to a VP28 peptide having a sequence as set forth in SEQ ID NO. 6 by a linker L2, and the VP28 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3; and (i) a VP28 peptide having a sequence as set forth in SEQ ID NO. 8 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 14 by a linker L2, and the VP26 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 24 by a linker L3, wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

[0058] In another embodiment, the polypeptide of white spot syndrome virus (WSSV) protein is selected from the group consisting of VP28 having a sequence as set forth in SEQ ID NO. 1; VP28 peptide having a sequence as set forth in SEQ ID NO. 10; VP26 peptide having a sequence as set forth in SEQ ID NO. 14; and VP26 peptide having a sequence as set forth in SEQ ID NO. 15.

[0059] The VP 28 peptides having sequences as set forth in SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, or SEQ ID NO. 11, according to the present

disclosure, are fragments of the WSSV envelope protein VP 28, wherein each of the peptide fragments are antigenic and capable of triggering an immune response in an organism upon administration.

[0060] The VP 26 peptide having sequences as set forth in SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, or SEQ ID NO. 17, according to the present disclosure, are fragments of the WSSV envelope protein VP 26 protein, wherein each of the peptide fragments are antigenic and capable of triggering an immune response in an organism upon administration.

[0061] The VP 19 peptide having sequences as set forth in SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, or SEQ ID NO. 25, according to the present disclosure, are fragments of the WSSV envelope protein VP 19, wherein each of the peptide fragments is antigenic and capable of triggering an immune response in an organism upon administration.

[0062] The polypeptide of white spot syndrome virus (WSSV) protein, according to embodiments herein is further linked to the VLP forming domain by one or more linkers.

Virus-like particle (VLP) forming domain

[0063] The term "virus-like particle (VLP)-forming domain", as used herein, refers to polypeptides or combination of polypeptides that can self-assemble to create particles having virus-like morphology. Expression and self-assembly of the VLP-forming domain can take place in various living or cell-free expression systems.

[0064] According to embodiments herein, the VLP-forming domain is fused with the polypeptide of white spot syndrome virus (WSSV) protein and is capable of self-assembling to form a VLP. The VLP thus formed exposes the polypeptide of white spot syndrome virus (WSSV) protein on its surface, without requiring any process of encapsulation, and elicits an immune response.

[0065] The VLP-forming domain may be derived from Hepatitis B core (HBc) antigen (HBcAg), Macrobrachium rosenbergii nodavirus (MrNv), rabbit haemorrhagic disease virus (RHDV_VP60), structural protein of Norovirus

(NoV_VP1), iridovirus capsid protein (O-MCP), or red-spotted grouper nervous necrosis virus (RGNNV) capsid protein.

[0066] In an embodiment, the VLP-forming domain comprises a polypeptide having at least 80%, 98%, 98%, 99%, or 100% sequence identity to a sequence selected from SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28, SEQ ID NO. 56, SEQ ID NO. 57, SEQ ID NO. 58, SEQ ID NO. 59, SEQ ID NO. 60, or combination thereof.

[0067] In an embodiment, the virus like particle (VLP)-forming domain comprises (a) a first VLP polypeptide (VLP1) and a second VLP polypeptide (VLP2). In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein is flanked between a first VLP polypeptide (VLP1) and second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain. In an embodiment, the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end, to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain.

[0068] In an embodiment, the virus like particle (VLP)-forming domain comprises (a) a first VLP polypeptide (VLP1) having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and a second VLP polypeptide (VLP2) having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60.

[0069] In an embodiment, the virus like particle (VLP)-forming domain comprises (a) a first VLP polypeptide (VLP1) having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO. 26, and a second VLP polypeptide (VLP2) having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO. 27.

[0070] In another embodiment, the virus like particle (VLP)-forming domain comprises (a) a first VLP polypeptide (VLP1) having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO. 59, and a second VLP polypeptide (VLP2) having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO. 60.

[0071] In another embodiment, the virus like particle (VLP)-forming domain comprises (a) a first VLP polypeptide (VLP1) having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO. 57, and a second VLP polypeptide (VLP2) having at least 80% sequence identity to an amino acid sequence as set forth in SEQ ID NO. 58.

[0072] In an embodiment, the virus like particle (VLP)-forming domain is a third VLP polypeptide (VLP3) having at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56.

[0073] In an embodiment, the polypeptide of WSSV protein is linked to the VLP-forming domain without compromising the ability to form VLPs and displaying the polypeptide of WSSV protein on the VLP surface.

[0074] The chimeric antigen, according to embodiments herein, may further comprise one or more signal peptides, for eg; polyhistidine tags (his-tag) of sequence "HHHHHH". In an embodiment, the chimeric antigen further comprises a His-tag attached to the C-terminal end.

[0075] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide is selected from the group consisting of: (a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain, wherein the first VLP polypeptide (VLP1) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60; and (b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain, wherein the third VLP polypeptide (VLP3) has at least 80% sequence

identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56, and wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence selected from SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof.

[0076] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide is selected from the group consisting of: (a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence selected from SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof, wherein the first VLP polypeptide (VLP1) has at least 80% sequence identity to an 26amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60 ; and (b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain, wherein the third VLP polypeptide (VLP3) has at least 80% sequence

identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 95% sequence identity to a sequence selected from SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof.

[0077] In an embodiment, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 10, SEQ ID NO. 14, and SEQ ID NO. 15, wherein the first VLP polypeptide (VLP1) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60. In an embodiment, the polypeptide of WSSV protein is linked to VLP1 by a linker L1 having an amino acid sequence as set forth in SEQ ID NO. 42, and wherein the polypeptide of WSSV protein is linked to VLP2 by a linker L1 or L4, wherein the linker L1 has an amino acid sequence as set forth in SEQ ID NO. 42, and the linker L4 has an amino acid sequence as set forth in SEQ ID NO. 45.

[0078] In a preferred embodiment, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least

80% sequence identity to a set forth in SEQ ID NO. 1, wherein the first VLP polypeptide (VLP1) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60. In an embodiment, the polypeptide of WSSV protein is linked to VLP1 by a linker L1 having an amino acid sequence as set forth in SEQ ID NO. 42, and wherein the polypeptide of WSSV protein is linked to VLP2 by a linker L1 having an amino acid sequence as set forth in SEQ ID NO. 42. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 29 (FA1).

[0079] In another embodiment, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NO. 10, SEQ ID NO. 14, and SEQ ID NO. 15, wherein the first VLP polypeptide (VLP1) has at least N80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60. In an embodiment, the polypeptide of WSSV protein is linked to VLP1 by a linker L1 having an amino acid sequence as set forth in SEQ ID NO. 42, and wherein the polypeptide of WSSV protein is linked to VLP2 by a linker L4 having an amino acid sequence as set forth in SEQ ID NO. 45. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO. 31(SP1), SEQ ID NO. 32 (SP2) and SEQ ID NO. 33 (SP3).

[0080] In an embodiment, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein linked at the N-terminal

to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain, wherein the third VLP polypeptide (VLP3) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence as set forth in SEQ ID NO. 1.

[0081] In an embodiment, the chimeric antigen comprises a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain, wherein the third VLP polypeptide (VLP3) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence as set forth in SEQ ID NO. 1, wherein the polypeptide of WSSV protein is linked to VLP3 by a linker L1 having an amino acid sequence as set forth in SEQ ID NO. 42. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 30 (FA2).

[0082] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 9, SEQ ID NO. 8, and SEQ ID NO. 11, wherein the VP26 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 14, and SEQ ID NO. 12, wherein the VP19 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 23, SEQ ID NO. 25, and SEQ ID NO. 24, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

[0083] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 9, SEQ ID NO. 8, and SEQ ID NO. 11, wherein the VP26 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 14, and SEQ ID NO. 12, wherein the VP19 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 23, SEQ ID NO. 25, and SEQ ID NO. 24, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

[0084] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 6, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 16, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 23, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 34.

[0085] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19

peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 9, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 16, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 25, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 36.

[0086] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 9, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 17, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 25, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 37.

[0087] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 11, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 14, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 25, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 39.

[0088] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 8, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 12, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 25, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 40.

[0089] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 8, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 14, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 24, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 41.

[0090] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 linked to a VP28 peptide having a sequence as set forth in SEQ ID NO. 6 by a linker L2, and the VP28 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a

linker L3, wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 35.

[0091] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 9, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 16, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 25, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 61.

[0092] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP26 peptide linked to a VP28 peptide by a linker L2, and the VP28 peptide linked to a VP19 peptide by a linker L3, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 17, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 6, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 25, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 62.

[0093] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white

spot syndrome virus (WSSV) protein comprises a combination of: a VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3, wherein the VP28 peptide has an amino acid sequence as set forth in SEQ ID NO. 8, wherein the VP26 peptide has an amino acid sequence as set forth in SEQ ID NO. 14, wherein the VP19 peptide has an amino acid as set forth in SEQ ID NO. 24, and wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 63.

[0094] In an embodiment, there is provided a chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a VP19 peptide having a sequence as set forth in SEQ ID NO. 23 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 15 by a linker L2, and the VP26 peptide is linked to the VP28 peptide having a sequence as set forth in SEQ ID NO. 10 by a linker L3, wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44. In an embodiment, the chimeric antigen comprises a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 38.

[0095] In an embodiment, there is provided a chimeric antigen, wherein the chimeric antigen comprises a polypeptide having an amino acid sequence selected from a group consisting of SEQ ID NO. 29, SEQ ID NO. 30, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 33, SEQ ID NO. 34, SEQ ID NO. 35, SEQ ID NO. 36, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 61, SEQ ID NO. 62, and SEQ ID NO. 63.

Composition

[0096] Embodiments herein include a composition comprising the chimeric antigen. The composition, as disclosed herein, is capable of inducing an immune response in aquatic organisms.

[0097] In an embodiment, the composition comprises a chimeric antigen having a polypeptide comprising a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide is selected from the group consisting of: (a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain; and (b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain.

[0098] In an embodiment, the composition comprises a chimeric antigen, wherein the chimeric antigen comprises a polypeptide selected from the group consisting of: (a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence selected from SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof, wherein the first VLP polypeptide (VLP1) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60 ; and (b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain, wherein the third VLP polypeptide (VLP3) has at least 80% sequence identity to an amino acid sequence

selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56, wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 95% sequence identity to a sequence selected from SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof.

[0099] In an embodiment, the composition comprises the chimeric antigen having a polypeptide of amino acid sequence selected from a group consisting of SEQ ID NO. 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 61, SEQ ID NO 62, and SEQ ID NO 63.

[00100] The composition may further include at least one excipient, diluent, carrier, or their combinations. In an embodiment, the composition is an injectable formulation. In an embodiment, the composition is an oral formulation. Various excipient, diluent and carriers are known and may be used in embodiments herein. In an embodiment, the excipient, diluent, and carrier are suitable for preparing injectable or oral formulations of the chimeric antigen.

Feed composition

[00101] Embodiments herein include a feed composition for an aquatic organism. The term "aquatic organism", as used herein, refers to aquatic vertebrates and invertebrates, fresh water or marine organism. In particular, the aquatic organisms are farmed aquatic invertebrates of commercial value and in need of protection against infections. In an embodiment, the aquatic organism is selected from fish, shrimp, prawn, crab, snail, krill, lobster, squid, clam, mussel, or scallop.

[00102] In some embodiments, the feed composition comprises the chimeric antigen as disclosed herein, or the composition comprising the chimeric antigen as disclosed herein; and a double stranded ribonucleotide corresponding to a target

gene of a virus. The feed composition may further comprise a carrier feed. Accordingly, in some embodiments, the feed composition comprises the chimeric antigen disclosed herein; a double stranded ribonucleotide corresponding to a target gene of a virus; and a carrier feed.

[00103] In some embodiments, the feed composition comprises the composition disclosed herein; a double stranded ribonucleotide corresponding to a target gene of a virus; and a carrier feed.

[00104] In an embodiment, the feed composition comprises a chimeric antigen having a polypeptide of amino acid sequence selected from SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 61, SEQ ID NO 62, SEQ ID NO 63, or combination thereof; and the double stranded ribonucleotide (dsRNA), wherein the dsRNA comprises a nucleotide having at least 80% sequence identity to a sequence selected from SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, or combination thereof.

[00105] In an embodiment, the feed composition comprises the chimeric antigen having a polypeptide of amino acid sequence selected from SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 61, SEQ ID NO 62, and SEQ ID NO 63 or combination thereof; the double stranded ribonucleotide (dsRNA), wherein the dsRNA comprises a nucleotide having at least 80% sequence identity to a sequence selected from SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, or combination thereof; and a carrier feed.

[00106] In an embodiment, the feed composition comprises a combination of the chimeric antigen having a polypeptide of amino acid sequence as set forth in SEQ ID NO 29; the double stranded ribonucleotide (dsRNA), wherein the dsRNA comprises a nucleotide having at least 80% sequence identity to a sequence as set forth in SEQ ID NO 46, preferably mixed with a feed carrier. The feed carrier may be any basal feed given to aquatic organisms. In an example, ShrimpVet basal feed

may be used as a feed carrier. Alternatively, any commercially available feed may be used to coat the feed composition.

[00107] In an embodiment, the feed composition is prepared by mixing a diluted feed composition with a binder (for eg: Carboxymethyl Cellulose) to obtain a solution; and mixing the solution with the feed carrier (for eg: by slowly shaking by hand continuously for about 5 to 10 mins) to allow coating of the feed carrier with the feed composition; and drying, preferably at about 37 to 40°C for 1 to 2 hours.

Double stranded ribonucleotide (dsRNA)

[00108] Embodiments herein provide a double stranded ribonucleotide corresponding to a target gene of a virus. The double stranded ribonucleotide is capable of causing interference in the expression of a target gene in a virus, for eg: WSSV by RNA interference (RNAi) or RNA silencing, according to embodiments herein. The term "interference in the expression" as used herein, refers to either inhibition or disruption of the expression of the target gene of a virus, such that the aquatic organism is protected from infections.

[00109] In some embodiments, the target gene is selected from the group consisting of *vp28* gene, *vp26* gene, *vp19* gene, *wsv069* gene and *rr2* gene of white spot syndrome virus.

[00110] In an embodiment, the double stranded ribonucleotide comprises a nucleotide having at least 80%, 98%, 99%, or 100% sequence identity to a sequence selected from SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, or combination thereof.

[00111] In an example according to embodiments herein, the dsRNA is having at least 80% sequence identity to a sequence as set forth in SEQ ID NO 46 and is capable of interfering with the expression of the target gene *vp 28* in WSSV. In an example according to embodiments herein, the dsRNA is having at least 80% sequence identity to a sequence as set forth in SEQ ID NO 47 and is capable of interfering with the expression of the target gene *wsv069* in WSSV. In an example according to embodiments herein, the dsRNA is having at least 80% sequence identity to a sequence as set forth in SEQ ID NO 48 and is capable of interfering

with the expression of the target gene *vp 26* in WSSV. In an example according to embodiments herein, the dsRNA is having at least 80% sequence identity to a sequence as set forth in SEQ ID NO 49 and is capable of interfering with the expression of the target gene *rr2* in WSSV.

[00112] The double stranded ribonucleotides (dsRNA), as disclosed herein, may be administered individually or in combination along with the chimeric antigen.

Carrier feed

[00113] Embodiments herein provide a feed composition comprising a carrier feed. The term "carrier feed", as used herein, refers to any substance used as feed for aquatic organisms. It includes substances that provide nutrition to aquatic organisms and are generally included as part of feed compositions. It further includes substances that are essential for maintaining the stability of the composition.

[00114] In an embodiment, the carrier feed is selected from a protein, a lipid, a pH modifier, a bulking agent, a polymer, an antioxidant, a preservative, a chelating agent, a vitamin, a mineral, an amino acid, or combinations thereof.

[00115] Embodiments herein include nucleotide encoding the chimeric antigen disclosed herein. In an embodiment, the nucleotide encodes a polypeptide having an amino acid sequence selected from a group consisting of SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 61, SEQ ID NO 62, and SEQ ID NO 63.

[00116] In an embodiment, the nucleotide encoding the chimeric antigen has at least 80%, 98%, 99%, or 100% sequence identity to a sequence selected from a group consisting of SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, SEQ ID NO 53, SEQ ID NO 54, and SEQ ID NO 55.

[00117] The present disclosure also provides a recombinant construct comprising the nucleotide sequence of the chimeric antigen as disclosed herein and operably linked to a promoter. Various inducible and constitutive promoters are

known in the art and may be used in embodiments herein. In an embodiment, the recombinant construct comprises the nucleotide encoding the polypeptide of the chimeric antigen, described herein, operably linked to a Gal1 promoter, lac promoter, araBAD promoter, T7 promoter, trp, tac & trc promoters, TEF1, PGK1, GPD, ADH1, TPI1, FBA1, PYK1, HXT7, RNR2, GAL10, MAL, HSP82, CUP1, MET25, PHO5, DLD3, CLB2, CUP1, SPO13, DSE4, PAOX1, PAOX2, PDAS1, PTEF1, PYPK1, EYK1, XPR2, POX2, or ICL1.

[00118] Further, the present disclosure provides expression vectors comprising the recombinant construct disclosed in various embodiments herein. Various vectors are known in the art and may be used. In an embodiment, the vector is selected from pUC, pCDNA, pPro, or pBKS.

[00119] Further, the present disclosure provides recombinant host cells transfected with the vector comprising the recombinant construct having the nucleotide encoding the polypeptide of the chimeric antigen disclosed in various embodiments herein. In an embodiment, the recombinant host cell is a prokaryotic cell, or a eukaryotic cell, preferably *E.coli* cells or yeast cells selected from *Pichia pastoris, Saccharomyces cerevisiae, Pichia, Rhodotorula* or *Yarrowia lipolytica.*

Methods

[00120] Embodiments herein further provides a method of preparing the chimeric antigen described herein. In an embodiment, the method comprises: (a) providing the host cell as disclosed herein in a culture medium; and (b) culturing the host cell for a period in the range of 48 to 72 hours at a temperature in the range of 25 to 37°C to obtain the chimeric antigen. Various techniques for preparing host cell, transformation, and culturing of host cell are known, which may be used in various embodiments herein.

[00121] Embodiments herein also provides a method for producing the feed composition. In an embodiment, the method comprises providing the chimeric antigen as described herein or the composition as described herein; the dsRNA as described herein, and optionally a carrier feed; and mixing to obtain the feed

composition. In further embodiments, said composition is coated on the carrier feed.

[00122] In another embodiment, the method comprises providing the chimeric antigen as described herein, the dsRNA as described herein, and a carrier feed; and mixing to obtain the feed composition, wherein said composition is coated on the carrier feed.

[00123] In yet another embodiment, the method comprises providing the composition as described herein; the dsRNA as described herein and a carrier feed; and mixing to obtain the feed composition, wherein said composition is coated on the carrier feed.

[00124] Embodiments herein further provides a method for treating an aquatic organism, comprising administering the chimeric antigen as described herein, or the composition as described herein, or the feed composition as described herein, to the aquatic organism. In an embodiment, said administration is oral administration. In an embodiment, said administration is by way of injection.

[00125] In one embodiment, there is provided a method for treating an aquatic organism, comprising administering the chimeric antigen, as described herein, to the aquatic organism. In another embodiment, there is provided a method for treating an aquatic organism, comprising administering the composition, as described herein, to the aquatic organism. In yet another embodiment, there is provided a method for treating an aquatic organism, comprising administering the feed composition, as described herein, to the aquatic organism. In an embodiment, the carrier feed is shrimp feed, and the aquatic organism is shrimp.

[00126] In some embodiments, said treatment is for immunizing the aquatic organism against infections caused by pathogens selected from white spot syndrome virus (WSSV), taura syndrome virus (TSV), yellow head virus (YHV), gill-associated virus, hematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV), *Enterocytozoon hepatopenaei* (EHP), *Streptococcus iniae*, *Ceratomyxa shasta*, *Ichthyophthirius multifillius*, *Cryptobia salmositica*, *Lepeophtheirus salmonis*, *Tetrahymena* species, *Trichodina* species, Epistylus

species, spring viraemia of carp (SVC), Epizootic haematopoietic necrosis, Vibrio or other non-viral species.

[00127] Description of list of sequences used in the present disclosure are listed below in Table 1 and Table 2. Table 3 depicts exemplary feed compositions.

[00128] Table 1: Description of the list of amino acid sequences

SEQ ID NOs.	Sequence reference	Description	Sequence type
SEQ ID NO 1	AR1	VP28	PEPTIDE
SEQ ID NO 2	AR2	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 3	AR3	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 4	AR4	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 5	AR5	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 6	AR6	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 7	AR7	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 8	AR8	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 9	AR9	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 10	AR10	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 11	AR11	VP 28 PEPTIDE	PEPTIDE
SEQ ID NO 12	AR12	VP 26 PEPTIDE	PEPTIDE
SEQ ID NO 13	AR13	VP 26 PEPTIDE	PEPTIDE
SEQ ID NO 14	AR14	VP 26 PEPTIDE	PEPTIDE
SEQ ID NO 15	AR15	VP 26 PEPTIDE	PEPTIDE
SEQ ID NO 16	AR16	VP 26 PEPTIDE	PEPTIDE
SEQ ID NO 17	AR17	VP 26 PEPTIDE	PEPTIDE
SEQ ID NO 18	AR18	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 19	AR19	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 20	AR20	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 21	AR21	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 22	AR22	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 23	AR23	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 24	AR24	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 25	AR25	VP 19 PEPTIDE	PEPTIDE
SEQ ID NO 26	VLP1	HBc-N terminus	PEPTIDE
SEQ ID NO 27	VLP2	HBC-C terminus	PEPTIDE

SEQ ID NO 28	VLP3	MrNV	PEPTIDE
SEQ ID NO 29	FA1	HBc-N terminus(26)-L1-VP28(1)-L1-HBC-C terminus	PEPTIDE
SEQ ID NO 30	FA2	MrNV(28)-L1-VP28(1)	PEPTIDE
SEQ ID NO 31	SP1	HBc-N terminus(26)-L1-VP28(10)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 32	SP2	HBc-N terminus(26)-L1-VP26(14)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 33	SP3	HBc-N terminus(26)-L1-VP26(15)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 34	MP1	HBc-N terminus(26)-L1-VP28(6)-L2-VP26(16)-L3-vp19(23)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 35	MP2	HBc-N terminus(26)-L1-VP26(17)-L2-VP28(6)-L3-vp19(25)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 36	MP3	HBc-N terminus(26)-L1-VP28(9)-L2-VP26(16)-L3-vp19(25)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 37	MP4	HBc-N terminus(26)-L1-VP28(9)-L2-VP26(17)-L3-vp19(25)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 38	MP5	HBc-N terminus(26)-L1-VP19(23)-L2-VP26(15)-L3-VP28(10)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 39	MP6	HBc-N terminus(26)-L1-VP28(11)-L2-VP26(14)-L3-VP19(25)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 40	MP7	HBc-N terminus(26)-L1-VP28(8)-L2-VP26(12)-L3-VP19(25)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 41	MP8	HBc-N terminus(26)-L1-VP28(8)-L2-VP26(14)-L3-VP19(24)-L4-HBC-C terminus	PEPTIDE
SEQ ID NO 42	Linker 1	Linker L1	PEPTIDE
SEQ ID NO 43	Linker 2	Linker L2	PEPTIDE
SEQ ID NO 44	Linker 3	Linker L3	PEPTIDE
SEQ ID NO 45	Linker 4	Linker L4	PEPTIDE
SEQ ID NO 56	VLP4	RGNNV Capsid protein	PEPTIDE
SEQ ID NO 57	VLP5	NoV VP1 N-terminus	PEPTIDE
SEQ ID NO 58	VLP6	NoV VP1 C-terminus	PEPTIDE
SEQ ID NO 59	VLP7	RHDV VP60 N-terminus	PEPTIDE
SEQ ID NO 60	VLP8	RHDV VP60 C-terminus	PEPTIDE
SEQ ID NO 61	MP9	RGNNV(56)-L1-VP28(9)-L2-VP26(16)-L3-VP19(25)	PEPTIDE
SEQ ID NO 62	MP10	Nov-N terminus(57)-L1-VP26(17)-L2-VP28(6)-L3-VP19(25)-L4-Nov-C terminus	PEPTIDE
SEQ ID NO 63	MP11	RHDV-N terminus(59)-L1-VP28(8)-L2-VP26(14)-L3-VP19(24)-L4-RhDV-C terminus	PEPTIDE

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[00129] Table 3: Description of the list of nucleotide sequences.

SEQ ID NOs.	Description	Sequence type
SEQ ID NO 46	dsRNA-1	NUCLEOTIDE
SEQ ID NO 47	dsRNA-2	NUCLEOTIDE
SEQ ID NO 48	dsRNA-3	NUCLEOTIDE
SEQ ID NO 49	dsRNA-4	NUCLEOTIDE
SEQ ID NO 50	nucleotide encoding FA1	NUCLEOTIDE
SEQ ID NO 51	nucleotide encoding FA2	NUCLEOTIDE
SEQ ID NO 52	nucleotide encoding MP1	NUCLEOTIDE
SEQ ID NO 53	nucleotide encoding MP2	NUCLEOTIDE
SEQ ID NO 54	nucleotide encoding MP3	NUCLEOTIDE
SEQ ID NO 55	nucleotide encoding MP4	NUCLEOTIDE

[00130] **Table 3:** depicts a list of exemplary feed compositions

Feed composition	Category	Description	Combination
Comb1	Combination of dsRNA & antigen biologics	a formulation where dsRNA coding for VP28 is mixed with HBC-VP28 antigen	dsRNA1 + FA1
Comb2	Combination of dsRNA & multi-peptide biologics	a formulation where dsRNA coding for VP28 is mixed with MP1	dsRNA1 + MP1

[00131] Although the subject matter has been described with reference to specific embodiments, this description is not meant to be construed in a limiting sense. Various modifications of the disclosed embodiments, as well as alternate embodiments of the subject matter, will become apparent to persons skilled in the art upon reference to the description of the subject matter. It is therefore contemplated that such modifications can be made without departing from the spirit or scope of the present subject matter as defined.

EXAMPLES

[00132] The disclosure will now be illustrated with working examples, which is intended to illustrate the working of disclosure and not intended to take restrictively to imply any limitations on the scope of the present disclosure. Unless

defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein. It is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary.

EXAMPLE 1: Comparative analysis of the chimeric antigen (FA1) and the wild type (WT) VP28 in priming shrimp immune systems for fighting WSSV infection.

[00133] Two chimeric antigens were synthesized, viz. *HBc-VP28* (FA1) and *MrNv-VP28* (FA2).

[00134] *HBc-VP28* (FA1): This chimeric antigen was made by fusion of WSSV VP28 (SEQ ID NO. 1) with Hepatitis B virus core antigen (HBc), i.e. VLP1 and VLP2 (SEQ ID NO. 26 and SEQ ID NO. 27, respectively).

[00135] *MrNv-VP28* (FA2): This chimeric antigen was made by fusion of WSSV VP28 (SEQ ID NO. 1) with the capsid protein of *Macrobrachium rosenbergii* nodavirus, *Mr*Nv (i.e. VLP3 of SEQ ID NO. 28).

Materials and **methods**

[00136] Specific pathogen free (SPF) shrimp (*P. vannamei*) utilized in this trial were provided by ShrimpVet Dr. Tôm® Hatchery (Ninh Thuan Province, Vietnam) obtained from the Shrimp Improvement Systems in Hawaii, U.S.A (SIS Hawaii) broodstock (Alday-Sanz et al., 2018; Moss et al. 2012; Lightner et al. 2009). Shrimp post larvae (PLs) were produced under strict bio-secure and screened for all important pathogens including White spot syndrome virus (WSSV), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Acute Hepatopancreatic Necrosis Disease (AHPND) (*OIE-Listed diseases, OIE Manual of Diagnostic Tests for Aquatic Animals, 2019*) using PCR and real-time PCR prior to shipping to ShrimpVet Laboratory. At the ShrimpVet Laboratory, the PLs were reared in primary quarantine tanks for 7 days. During the primary quarantine, the PLs were checked for the above-listed disease using PCR techniques to confirm the

disease-free status of the stock and Enterocytozoon hepatopenaei (EHP) (Tang et al, 2015). And then, the PLs which passed the primary quarantine were cultured in strict bio-secure for secondary quarantine for 30 days to reach the juveniles stage, which had an average weight range of 1,20 ± 0,00 gram. The juveniles were checked again for the listed diseases using PCR techniques.

[00137] The trial was performed in 24 plastic jars with the capacity of 10L per each. Seawater was treated accordingly to ShrimpVet's water treatment protocol in order to meet the requirements of environmental conditions for shrimp culture. Seawater was diluted with fresh water to 20 parts per thousand (ppt) of salinity (brackish water) for use in this trial. The trial was carried out in 10L plastic jars which were filled with 8L of brackish water. Each jar was outfitted with a 300 g biological filter consisting of 150 g of crushed activated coral (at least 02 months of activation) and 50 g of activated carbon; an aeration system with two air-stones and covered with a plastic cap to reduce the risk of cross-contamination. Experimental jar set-up followed the methods described by L. White et al. (2007).

[00138] WSSV samples used were collected from White Spot Disease affected P. vannamei on a shrimp farm in Loc An, Vung Tau Province, Vietnam by ShrimpVet and given the reference code number 15002. WSSV was confirmed by qPCR detection in multiple samples (Durand & Lightner, 2002).

[00139] In order to prepare inoculum for the challenge, SPF shrimp previously infected with the WSSV and stored at -80 degree C were thawed, and the cuticle was removed. The shrimp tissues were homogenized (10% weight/volume) in sterile Phosphate Buffer Saline Nilsen et al., 2017). The homogenate was centrifuged at 5000g for 5 minutes at 4°C. The clarified supernatant was then passed through a 0.45 µm filter and used to coat the feed pellets. The clarified extract was mixed with a commercial diet (without prophylactic treatment) at the ratio of 1:3 (weight/ weight) and kept at -80°C until use. The viral load on the feed was estimated and was 2.03×10^7 copies/gram coated feed.

[00140] The WSSV-positive inoculum was fed to positive control and treatments with 3 meals/day at 1-1.5% estimated shrimp body weight per meal

(corresponding to 3 - 4.5% body weight per day) for 2 consecutive days. The remaining one meal per day was normal feed. The WSSV-positive inoculums were fed to treatments with four meals/day at 5% estimated shrimp body weight for 2 consecutive days. The WSSV-negative inoculum was fed to the negative control with 04 meals/day at 5% estimated shrimp bodyweight for 2 consecutive days.

[00141] Shrimp were fed with a commercial feed diet (which did not contain any substances that could have influenced the trial) at satiation for 4 meals per day during the trial (4-5% estimated body weight). The amount of feed was adjusted depending on the estimated biomass and shrimp feeding behaviour of the jars.

[00142] In order to compare the effectiveness of WSSV VP28 (SEQ ID NO 1); and chimeric antigens, viz. ***HBc-VP28*** (represented as FA 1 and encoded by SEQ ID NO. 29) and ***MrNv-VP28*** (represented as FA 2 and encoded by SEQ ID NO. 30), in immune priming of shrimp to fight WSSV infection, these antigens were expressed in *E coli* and purified. The purified proteins were used to perform a laboratory trial in white legged shrimp, *Penaeus vannamei*. The laboratory trial comprised six groups of shrimps, and each group comprised three independent tanks each containing six shrimps. On the first day and fifth day of the trial, four of these groups were injected with the indicated dose of the indicated purified protein, the remaining two groups were not injected. Three days after the second injection, five shrimp groups—*four injected with the proteins and one not injected*– were infected with WSSV for 2 days. After the WSSV infection or challenge the mortality in shrimps was monitored at regular intervals. It was observed that ten days after the WSSV challenge the survival was 88% in the shrimp group that were not infected with WSSV. Table 4 is a schematic representation depicting the trial procedure:

[00143] Table 4

Acclimatization (2 day)	Injection first dose	4 day gap	Injection second dose	3 day gap	2 day os infection	Follow up 10 days

[00144] ***Result and Conclusions:*** In two out of the five groups that were infected with WSSV none of the shrimps survived. Shrimps survived in only three of the WSSV infected groups that were injected with VP28 or HBc-VP28 (FA 1). Highest % survival, 73%, was seen in the shrimps injected with 6 ug/gm of HBc-VP28 (FA 1). Figure 1 depicts the results. Figure 1 shows the percentage survival of the shrimps 10 days after the WSSV challenge in all the eight shrimp groups. The error bars represent the standard deviation of % survival in three independent different tanks of a given shrimp group.

[00145] Shrimps injected with chimeric antigen, HBc-VP28 (FA1), showed better survival than the shrimps injected with VP28 protein. The shrimps injected with 0.2 ug/gm and 6 ug/gm showed 50% and 77% survival, respectively, whereas the shrimps injected with 1 ug/gm of VP28 showed 27% survival (Table 5). Therefore, it was concluded that fusion of VP28 with a VLP forming peptide, which is HBc in this case (i.e. VLP1 and VLP2), is able to improve its ability to prime Shrimp's immune response.

Table 5: Tabular summary of experimental groups and results

Group (n=3 tanks, each tank with 6 shrimp)	Infection (yes/no)	Treatment	% survival after 10 days post infection
1	no	none	88.89 %
2	yes	none	0 %
3	yes	VP28 (1ug/gm)	27.78%
4	yes	VP28 (6 ug/gm)	0%
5	yes	HBc-VP28 (FA1) (0.2 ug/gm)	50%
6	yes	HBc-VP28 (FA1) (6 ug/gm)	77.78%

EXAMPLE 2: Evaluation of the effect of various dsRNA targets to provide shrimp protection against WSSV infection and analysis of the effectiveness of the oral delivery formulation (feed composition) to provide shrimp protection against WSSV infection.

[00146] dsRNA were designed that target vp28, wsv069, vp26, and rr2 genes of WSSV. The formulations (feed compositions) were prepared that can be coated on the feed of shrimp (carrier feed) to deliver these dsRNAs using proprietary technology. The formulation that delivered vp28 dsRNA (represented as dsRNA-1) was labelled as TLY140, the formulation that delivered ws069 dsRNA (represented as dsRNA-2) was labelled as TLY141, the formulation that delivered vp26 dsRNA (represented as dsRNA-3) was labelled as TLY142, the formulation that delivered rr2 dsRNA (represented as dsRNA-4) was labelled as TLY143. Another formulation that delivers chimeric antigen HBc-VP28 (represented as FA1) was also prepared and was labelled as TLY-100. Notably the chimeric antigen HBc-VP28 (FA 1) was found to provide immunity against WSSV when it was injected. This trial was also used to see if a similar level of protection was observed when delivered orally by coating on the feed.

[00147] The shrimps and WSSV innoculums were prepared in accordance with Example 1. The WSSV-coated feed had an average of 1.89E+08 WSSV genome copies per gram.

[00148] In this trial, WSSV-coated feed was fed to the shrimps as per the feeding regimen described in Example 1.

[00149] **Formulation (feed composition) preparation:** ShrimpVet basal feed (carrier feed) was weighed and put in the feed jar for top coating. Water (5%) was added to the cup and the test sample (2%) was weighed and mixed with 5% water in the cup. The mixture containing water and the test sample was poured slowly over the basal feed that was shaken by hand continuously for 5 minutes. After all the basal feed pellets (carrier feed) were thoroughly coated, they were transferred to the feed tray in the dryer. The dryer was set up at 40°C for 2 hours to let the pellets dry. Binder B (1.2%) was weighed and mixed with 5% water. The mixture containing binder and water was top-coated on the pellets that were already top-coated with test samples. Then, binder-coated pellets were dried in the dryer at 40°C for 2 hours. After drying, the top-coated feed was sealed in zipper bags and stored in cold storage for the duration of the trial.

[00150] In order to evaluate the efficacy of these formulations to provide shrimp protection against WSSV infection laboratory trials were conducted in white legged shrimp, *Penaeus* vannamei. The laboratory trial had seven groups of shrimp and each group comprised three independent tanks containing six shrimps. For the first five days five of these groups were fed with a feed that was supplemented with the indicated formulation and two groups were fed a basal diet. After the first feeding of 5 days with indicated formulation all groups were fed once a week as a booster dose. For the next three days all the groups were fed the basal diet. On the 9th day six shrimp groups—five shrimp groups that were fed with indicated formulation and one that was not fed with indicated formulation– were infected with WSSV for 2 days. After the WSSV infection or challenge the mortality in shrimps was monitored at regular intervals. Table 6 is a schematic representation depicting the trial procedure:

[00151] Table 6:

Acclimatization (1 day)	Treatment feed (5 days)	3 day gap (normal feed)	2 day os infection	Follow up (treatment feed 6th and 13 th day post challenge

[00152] ***Result and Conclusions:*** After seven days of WSSV infection in the shrimp in the group that was not treated with the indicated formulation but was infected WSSV, only half of the shrimps survived (50% survival). In the groups that were treated with the indicated formulation, more than 50% of the shrimps survived. In formulation that delivered VP28 dsRNA (Formulation TLY140), 100% of the shrimps survived, in wsv096 dsRNA treated group (Formulation TLY141) 83% of the shrimps survived, in VP26 dsRNA treated group (Formulation TLY142) 83% of the shrimps survived, in rr2 dsRNA treated group (Formulation TLY143) 77% of the shrimps survived, and in HBc-VP28 treated group (Formulation TLY-100) 94% shrimps survived after 7 days of challenge. Figure 2 shows the percentage survival of the shrimps 7 days after WSSV challenge in all the seven shrimp groups. The error bars represent the standard deviation of % survival in three independent different tanks of a given shrimp group. Hence, first,

it was concluded that the oral delivery formations in accordance with the present disclosure is effective in delivering the biologic to shrimp for providing protection against shrimp. Chimeric antigen HBc-VP28 (identified as most efficient in providing shrimp immunity against WSSV when injected into the shrimps), is also effective in providing immunity when delivered in the form of an oral delivery formulation. The reason being that 94.77 % of the shrimps survived 7 days after the WSSV challenge when treated with the oral delivery formulation, whereas no shrimp survived in the shrimps that were not treated with any compound.

[00153] Second, it was concluded that all the dsRNAs are effective in protecting shrimp against WSSV, and vp28 dsRNA (represented as dsRNA-1) is most promising. On the 7 th day of the infection, complete mortality was observed in the untreated shrimp treated with formulation TLY140 that delivered vp28 dsRNA (dsRNA-1) shows 100% survival. Where the shrimps treated with dsRNA targeting wsv069, vp26, and rr2– showed less than 100% survival (Table 7).

Table 7: Tabular summary of treatment groups and the results

Group (n=3 tanks, each tank with 6 shrimp)	Infection (yes/no)	Treatment	% survival after 7 days post infection
1	no	none	100%
2	yes	none	50%
3	yes	VP28 dsRNA (dsRNA1)	100%
4	yes	Ws069 dsRNA (dsRNA2)	83.33%
5	yes	VP26 dsRNA (dsRNA3)	77.78%
6	yes	rr2 dsRNA (dsRNA4)	83.33%
7	yes	HBc-VP28 (FA1)	94.77%

EXAMPLE 3: Evaluation of the effectiveness of combined use of protein and dsRNA in providing the protection against WSSV infection.

[00154] Samples were prepared/processed in accordance with the previous Examples. Feed supplemented with following formulations were prepared:

1. Feed supplemented with 0.5% (w/w) of VP28 ds RNA (Formulation TLY140)
2. Feed supplemented with 2% (w/w) VP28 dsRNA (Formulation TLY140)
3. Feed supplemented with 2% (w/w) MP1
4. Feed supplemented with 2% (w/w) HBc-VP28 (Formulation TLY-100).
5. Feed supplemented with 2% (w/w) VP28 dsRNA (Formulation TLY140) and MP1.
6. Feed supplemented with 2% (w/w) VP28 dsRNA (Formulation TLY140) and HBc-VP28 (Formulation TLY-100).

[00155] In order to evaluate the efficacy of the feeds supplemented with the above-mentioned formulations laboratory trials were conducted. The laboratory trial had nine groups of shrimps and each group comprised three independent tanks containing 6 shrimps. For the first five days, seven of these groups were fed with a feed that was supplemented with the indicated formulations of Example 2. After the first feeding of 5 days with formulations selected from groups 1-5 as described above, all groups were fed once a week as a booster dose. For the next three days all the eight groups were fed the basal diet. On the 9th day eight shrimp groups—six shrimp groups that were supplemented feed and Untreated two shrimp groups were infected with WSSV for 2 days. Table 8 is a schematic representation depicting the trial procedure:

Table 8:

Acclimatization (1 day)	Treatment feed (5 days)	3 day gap (normal feed)	2 day os infection	Follow up (treatment feed 6th and 13 th day post challenge

[00156] ***Result and conclusions:*** After the WSSV infection or challenge, the mortality in these groups was monitored at regular intervals. After 8 days of WSSV infections, in the Untreated two groups that were infected but not fed the supplemented diet, only 50% of shrimps survived. In the groups that were fed

supplemented diet, more than 50% of the shrimps survived. No significant difference in survival of the groups treated with PRN (commercially available solution) or indicated formulations 1/2/3/4/5 treated diet was observed. The lowest survival, 61%, was observed in the group that was fed with the feed supplemented with 0.5% of TLY140 (Table 9). Figure 3 shows the percentage survival of the shrimps 8 days after the WSSV challenge in all the shrimp groups. The error bars represent the standard deviation of % survival in three independent different tanks of a given shrimp group.

[00157] It can be concluded that shrimps fed with Formulations 1-5 (protein and dsRNA supplemented formulations) diet do have an improved survival upon WSSV infection as compared to the shrimps that were fed an unsupplemented diet.

[00158] It was observed that standard deviation in survival, or tank to tank variability, is lower in the shrimps fed with the diets supplemented with the two biologics as compared to a single biologic. Hence, it was concluded that a combination of biologic (chimeric antigen + dsRNA) may help in reducing the variability in the field outcomes.

Table 9: Tabular summary of treatment groups and the results

Group (n=3 tanks, each tank with 6 shrimp)	Infection (yes/no)	Treatment	% survival after 8 days post infection
1	no	none	100%
2	yes	none	50%
3	yes	VP28 dsRNA 0.5% (dsRNA1)	61.11%
4	yes	VP28 dsRNA 2% (dsRNA1)	83.33%
5	yes	MP1 2%	72.22%
6	yes	HBc-VP28 2% (FA1)	72.22%
7	yes	VP28 dsRNA and mp1 (2%) (comb 2)	83.33%
8	yes	VP28 dsRNA and HBc-VP28 (2%) (comb 1)	77.78%

EXAMPLE 4: Evaluation of the effect of various protein biologics which are multipeptide to provide protection to shrimp against WSSV infection and evaluation of the efficacy of MrNV as VLP-forming peptide to provide protection to shrimp against WSSV infection.

[00159] White-leg shrimp, *L. vannamei* SPF shrimp, size 12 grams (CV <10% were used for carrying out the experiments. Shrimps were fed four times per day over each 24-hour period. Shrimp were fed to satiation. MP2, MP3, MP4, and FA2 were prepared and used for evaluation.

[00160] **Preparation of the feed composition by coating on feed carrier**: 10 gm of the chimeric antigen (MP2) was mixed in 140 ml of water with 0.7 gm (0.5%) Carboxymethyl Cellulose (any commercial binder) in a juicer. Mixed thoroughly until it became a whitish creamy layer. Then, this solution was mixed with 1 kg of feed in a tray and a gentle hand was used to mix the feed. After mixing, the feed tray was kept for drying as per farm protocol. Similarly, feed compositions were prepared using MP3, MP4, and FA2 chimeric antigens.

[00161] Intramuscular injection: a WSSV inoculum (viral particles) was isolated from WSSV-infected tissues (confirmed by PCR test). The viral inoculum was diluted with Phosphate Buffer Saline to obtain different viral titer challenge doses (~10^4 copies/mL). Then, the viral inoculum was injected into the 6th abdominal segment of shrimp at a dose of 0.05 mL per shrimp. Shrimp were observed for the following 7-day post-challenge period. Normally, mortality in shrimp was detected 48-72 hours after the challenge. Table 10 is a schematic representation depicting the trial procedure:

Table 10:

10 days of treatment	11 th day WSSV injection	Follow up 7 days

[00162] ***Result and conclusions:*** The survival rates of shrimp post-injection challenge are summarized in Table 1. Statistically significant differences were observed among the groups 3-6. It was observed that the shrimp group that was not treated with any formulations as previously described formulation and was injected

with WSSV showed only 31.1% survival, whereas all groups treated with the formulations comprising the chimeric antigens (MP2, MP3, and MP4) exhibited over 80% survival eight days after intramuscular injection with WSSV. Notably, even the MrNV-VP28 (FA 2) chimeric antigen showed 82.22% survival, indicating that MrNV can also serve as a VLP-forming peptide (Table 11). Figure 4 shows the percentage survival of the shrimps 8 days after the WSSV challenge in all the shrimp groups.

Table 11: Summary of the experimental groups and results

Group (n=3 tanks, each tank with15 shrimp)	Infection (yes/no)	Treatments	% survival after 8 days post infection
1	No	none	100%
2	yes	none	31.11%
3	yes	MP2	100%
4	yes	MP3	86.67%
5	yes	MP4	100 %
6	yes	MRNV-VP28(FA2)	82.22%

[00163] Figure 5 is schematic representation illustrating the expression cassette to be integrated in yeast. The nucleotide sequence (Table 2) corresponding to the chimeric antigen was provided as part of an expression cassette and cloned in an *E. coli* vector (Figure 6).

[00164] Figure 6 is schematic representation illustrating the *E.coli* vector and yeast vector, with expression cassette. The vector was expressed in yeast cells to obtain the purified protein product of the chimeric antigen.

[00165] Figure 7 is a schematic representation illustrating chimeric antigens FA1, FA2, SP1 to SP 3, and MP1 to MP11, in accordance with the embodiments herein.

Advantages of the present disclosure

[00166] The present disclosure provides a chimeric antigen and a feed composition comprising the chimeric antigen and methods thereof with the following advantages.

a). The disclosed composition provides two mechanisms of defence to provide protection against infections.

b). There are very few purification steps involved in the manufacturing of the disclosed feed composition that makes it easy to scale up the manufacturing at an affordable cost.

c). The disclosed chimeric antigen and the feed composition provides an effective solution for the control of infections in aquatic organisms.

d). The disclosed chimeric antigen and the feed composition is multiplexable and can be adapted for several other pathogens.

I/We claims:

1. A chimeric antigen comprising a polypeptide having a polypeptide of white spot syndrome virus (WSSV) protein and a virus like particle (VLP)-forming domain, wherein the polypeptide is selected from the group consisting of:

(a) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal end to a first VLP polypeptide (VLP1) and, at the C-terminal end, to a second VLP polypeptide (VLP2) of the virus like particle (VLP)-forming domain,

wherein the first VLP polypeptide (VLP1) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 26, SEQ ID NO. 57, and SEQ ID NO. 59, and the second VLP polypeptide (VLP2) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 27, SEQ ID NO. 58, and SEQ ID NO. 60; and

(b) a polypeptide wherein the polypeptide of white spot syndrome virus (WSSV) protein is linked at the N-terminal to a third VLP polypeptide (VLP3) of the virus like particle (VLP)-forming domain,

wherein the third VLP polypeptide (VLP3) has at least 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO. 28 and SEQ ID NO. 56, and

wherein the polypeptide of white spot syndrome virus (WSSV) protein has at least 80% sequence identity to a sequence selected from SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, or combination thereof.

2. The chimeric antigen as claimed in claim 1, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of: a

VP28 peptide linked to a VP26 peptide by a linker L2, and the VP26 peptide linked to a VP19 peptide by a linker L3,
wherein the VP28 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 9, SEQ ID NO. 8, and SEQ ID NO. 11, wherein the VP26 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 16, SEQ ID NO. 17, SEQ ID NO. 14, and SEQ ID NO. 12, wherein the VP19 peptide has an amino acid sequence selected from the group consisting of SEQ ID NO. 23, SEQ ID NO. 25, and SEQ ID NO. 24, and
wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

3. The chimeric antigen as claimed in claim 1, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of:
a VP26 peptide having a sequence as set forth in SEQ ID NO. 17 linked to a VP28 peptide having a sequence as set forth in SEQ ID NO. 6 by a linker L2, and the VP28 peptide is linked to the VP19 peptide having a sequence as set forth in SEQ ID NO. 25 by a linker L3,
wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

4. The chimeric antigen as claimed in claim 1, wherein the polypeptide of white spot syndrome virus (WSSV) protein comprises a combination of:
a VP19 peptide having a sequence as set forth in SEQ ID NO. 23 linked to a VP26 peptide having a sequence as set forth in SEQ ID NO. 15 by a linker L2, and the VP26 peptide is linked to the VP28 peptide having a sequence as set forth in SEQ ID NO. 10 by a linker L3,
wherein the linker L2 has an amino acid sequence as set forth in SEQ ID NO. 43 and the linker L3 has an amino acid sequence as set forth in SEQ ID NO. 44.

5. The chimeric antigen as claimed in claim 1, wherein the polypeptide of WSSV protein is linked to VLP1 by a linker L1 having an amino acid sequence as set forth in SEQ ID NO. 42, and wherein the polypeptide of WSSV protein is linked to VLP2 by a linker L1 or L4, wherein the linker L1 has an amino acid sequence as set forth in SEQ ID NO. 42, and the linker L4 has an amino acid sequence as set forth in SEQ ID NO. 45.

6. The chimeric antigen as claimed in anyone of claims 1 to 5, wherein the chimeric antigen further comprises a His-tag, wherein the His-tag is attached at the N-terminal or C-terminal end.

7. The chimeric antigen as claimed in anyone of claims 1 to 5, wherein the chimeric antigen comprises a polypeptide having an amino acid sequence selected from a group consisting of SEQ ID NO. 29, SEQ ID NO. 30, SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 33, SEQ ID NO. 34, SEQ ID NO. 35, SEQ ID NO. 36, SEQ ID NO. 37, SEQ ID NO. 38, SEQ ID NO. 39, SEQ ID NO. 40, SEQ ID NO. 41, SEQ ID NO. 61, SEQ ID NO. 62, and SEQ ID NO. 63.

8. A composition comprising the chimeric antigen as claimed in any one of claims 1 to 7.

9. A feed composition for an aquatic organism, comprising:
   (a) the chimeric antigen as claimed in any one of claims 1 to 7, or the composition as claimed in claim 8; and
   (b) a double stranded ribonucleotide (dsRNA).

10. The feed composition as claimed in claim 9, wherein the dsRNA comprises a nucleotide having at least 80% sequence identity to a sequence selected from a group consisting of SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, and SEQ ID NO. 49.

11. The feed composition as claimed in claim 9, further comprising a carrier feed, wherein said feed composition is coated on said carrier feed.

12. The feed composition as claimed in claim 9, wherein the dsRNA targets a gene selected from the group consisting of VP28 gene, VP26 gene, VP19 gene, wsv069 gene and rr2 gene, of white spot syndrome virus.

13. The feed composition as claimed in claim 9, wherein the aquatic organism is selected from fish, shrimp, prawn, crab, snail, krill, lobster, squid, clam, mussel, or scallop.

14. The feed composition as claimed in claim 11, wherein the carrier feed is selected from a protein, a lipid, a pH modifier, a bulking agent, a polymer, an antioxidant, a preservative, a chelating agent, a vitamin, a mineral, an amino acid, or combinations thereof.

15. A nucleotide encoding the chimeric antigen as claimed in any one of claims 1 to 7.

16. The nucleotide as claimed in claim 15, wherein the nucleotide has a sequence selected from a group consisting of SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 56, SEQ ID NO. 57, SEQ ID NO. 58, and SEQ ID NO. 59.

17. A recombinant construct comprising the nucleotide as claimed in claim 15 or 16, operably linked to a promoter.

18. An expression vector comprising the recombinant construct as claimed in claim 17.

19. A recombinant host cell comprising the vector as claimed in claim 18.

20. A method for preparing the chimeric antigen as claimed in anyone of claims 1 to 7, wherein the method comprises:

(a) providing the host cell as claimed in claim 19 in a culture medium; and

(b) culturing the host cell in the culture medium for a period in the range of 48 to 72 hours at a temperature in the range of 25 to 37°C to express the chimeric antigen.

21. A method for producing the feed composition as claimed in claim 9, wherein the method comprises providing the chimeric antigen, or the composition; and mixing with a carrier feed for aquatic organism, to obtain the feed composition.

22. The method for producing the feed composition as claimed in claim 9, wherein said feed composition is coated on the carrier feed for aquatic organism.

23. A method for treating an aquatic organism, comprising administering the chimeric antigen as claimed in any one of claims 1 to 7, or the composition as claimed in claim 8, or the feed composition as claimed in claim 9, to the aquatic organism.

24. The method as claimed in claim 23, wherein said treatment is for immunizing the aquatic organism against infections caused by a pathogen selected from the group consisting of white spot syndrome virus, taura syndrome virus (TSV), yellow head virus (YHV), gill-associated virus, hematopoietic necrosis virus (IHHNV), infectious myonecrosis virus (IMNV), *Enterocytozoon hepatopenaei* (EHP), *S. iniae*, *Ceratomyxa shasta*, *Ichthyophthirius multifillius*, *Cryptobia salmositica*, *Lepeophtheirus salmonis*, *Tetrahymena* species, *Trichodina* species, Epistylus species, spring viraemia of carp (SVC), Vibrio, Epizootic haematopoietic necrosis (EHNV), and other non-viral species.